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REVIEW

Biological significance of agmatine, an endogenous ligand at imidazoline binding sites

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Abbreviations: ADC, arginine decarboxylase; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AP5, (±)-2-amino-5-phosphonopentanoic acid; APR, absolute proximal reabsorption; cCDS, classical CDS; CDS, Clonidine Displacing Substance; DAO, diamine oxidase; IFN-γ, interferon-gamma; IL-10, Interleukin-10; irCDS, immunoreactive CDS; LPS, lipopolysaccharide; MAO, monoamine oxidase; MAP-kinases, mitogen-activated protein kinases; MTC cells, mouse kidney proximal tubule cells; NMDA, N-methyl-D-aspartate; NO, nitric oxide; NOS, NO synthase; ODC, ornithine decarboxylase; PDGF, platelet derived growth factor; PKC, protein kinase C; RVLM, rostroventrolateral medulla; SAMDC, *S*-adenosylmethionine decarboxylase; SHR, spontaneously hypertensive rats; SNGFR, single nephron glomerular filtration rate; SSAO, semicarbazide-sensitive aminoxidase; SSAT, spermidine/spermine acetyltransferase; TGF-β, transforming growth factor-β

Imidazolin binding sites

Many publications have shown that imidazoline derivatives such as clonidine, moxonidine or rilmenidine reduce sympathetic tone *via* a central mechanism and that as a result they reduce plasma catecholamines and blood pressure (Reid *et al*., 1995). This reduction in blood pressure appears not to be regulated *via* peripheral, presynaptically localized receptors, since neither catecholamine depletion by reserpine, or destruction of the nerve endings with 6-hydroxydopamine produces a notable weakening of the clonidine-induced blood pressure reduction (Haeusler, 1974a,b; Kobinger & Pichler, 1976; Finch *et al*., 1975). In contrast, selective α_2-adrenoceptor antagonists such as rauwolscine dose-dependently block hypotension induced by intravertebral application of clonidine. While some classical α_2-adrenoceptor antagonists such as SKF86466 (in contrast to the imidazoline derivatives efaroxan and idazoxan) did not inhibit the clonidine-induced hypotension in the CNS (Ernsberger *et al*., 1988b; 1994; Haxhiu *et al*., 1994), this was discussed to due to underdosage of the antagonist (Bock *et al*., 1999). Hence the effects of clonidine are due to a central α_2-adrenoceptor-mediated mechanism. The first signs that imidazoline derivatives might also work *via* non-adrenergic binding sites stem from Ruffolo (1977); the imadazoline derivative tetrahydrozoline was able to antagonize oxymetazoline-induced contraction, but not the contractile response induced by phenylethylamine derivatives such as noradrenaline, methoxamine or phenylephrine. Clear indications for a novel receptor type came from Bousquet *et al*. (1984), who reported hypotension after microinjection of clonidine into the rostroventrolateral medulla (RVLM). α-methylnoradrenaline showed no blood pressure reducing effect in the same model. The authors therefore assumed that binding sites must be present in the RVLM which preferentially bind imidazolines. Radioligand binding studies on RVLM membranes showed selective binding sites for imidazolines (Ernsberger *et al*., 1987). These data confirmed the assumptions of Ernsberger *et al*. (1988a; 1992); Buccafusco *et al*. (1995) and Bousquet *et al*. (1984) that the C_1 region of the RVLM appeared to be the decisive area involved (Reis *et al*., 1989).

Since then, two different imidazoline binding site subtypes have been identified (Michel & Insel, 1989; Michel & Ernsberger, 1992; Ernsberger *et al*., 1992). The I_1-binding site, which shows a high affinity binding to [^{3}H]-clonidine, is localized in the frontal cortex and the ventrolateral medulla (Bricca *et al*., 1989; Ernsberger *et al*., 1990a; 1992; Gomez *et al*., 1991), an area associated with central blood pressure regulation. Functionally, the I_1-binding site seems to be involved in central blood pressure regulation (Ernsberger *et al*., 1988b; 1994; Haxhiu *et al*., 1994; Hamilton, 1992a,b; Hamilton *et al*., 1992; Hieble & Ruffolo, 1992), even though its importance remains largely unclarified, since in functional α_{2A}-adrenoceptor 'knock out' mice (D79N; Macmillan *et al*., 1996) no evidence of I_1-imidazoline binding site-mediated effects was revealed (Zhu *et al*., 1999). Its amino acid sequence and the DNA coding for it also remain to be determined, although an imidazoline binding site antisera cDNA has been isolated and characterized as encoding a 1504 amino acid protein (IRAS-1) showing properties of an I_1-binding site (Piletz *et al*., 2000). I_1-binding sites have also

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been demonstrated in the spinal cord, kidney and pancreas (Regunathan *et al*., 1993; Ernsberger *et al*., 1995; Schulz & Hasselblatt, 1989a), but not in the left ventricle (Raasch *et al*., 2000).

Unlike the I_1-binding site, the role of the I_2-binding sites has been better characterized, whereby I_2-binding sites can be further differentiated amongst I_{2A}- and I_{2B}-binding sites depending on their amiloride sensitivity. I_2 binding sites have been demonstrated in various tissues such as brain (Brown *et al*., 1990), liver (Tesson & Parini, 1991) and kidney (Michel *et al*., 1989), whereby studies by Tesson *et al*. (1991) concluded that they are associated with mitochondria. Further studies focused their localization to the outer mitochondrial membrane. Functionally, the I_2-binding site has been characterized as a regulatory subunit of monoamine oxidase (MAO, Figure 1), and later on it became clear that both MAO A and B share the same I_2-binding site as a novel domain on the protein (Limon *et al*., 1992; Olmos *et al*., 1993). Moreover, studies on MAO A- and MAO B-deficient mice indicate that (1) the I_2 binding sites identified by [^{3}H]-idazoxan reside solely on MAO B, and (2) the binding sites on MAO A and a 28-kDa protein identified in livers of MAO A- and MAO B-deficient mice by photolabelling with 2-[3-azido-4-[(125)I]iodophenoxyl]methylimidazoline ([^{125}I]-AZIPI) may represent additional subtypes of the imidazoline-binding site family (Remaury *et al*., 2000). *In vitro* studies have shown that selective ligands of the I_2-binding site reduce MAO activity (Carpene *et al*., 1995; Tesson *et al*., 1995; Raasch *et al*., 1996; 1999). Further on, no correlation with a reduced oxygen utilization could be shown *in vitro* (Raasch *et al*., 1999). After protein molecular studies showed that I_2-binding sites could be found on various MAO isoenzymes with varying molecular weights (Escriba *et al*., 1996), and that a binding domain (Escriba *et al*., 1999) for imadazoline derivatives could be identified in the MAO-B (Raddatz & Lanier, 1997; Raddatz *et al*., 1997; 1999; 2000), the functional role of the I_2-binding site, unlike the I_1-binding site, could be considered as established. Chronic treatment of rats with specific I_2-ligands reduces MAO activity in various organs and catecholamines increase as a consequence (Raasch *et al*., 1999). In pathophysiological states such as heroin dependency or neurodegenerative disorders such as Alzheimer's disease or Huntington's chorea, the I_2-binding site density is reduced (Garcia-Sevilla *et al*., 1999), which indicates that I_2-binding sites might also be of clinical significance. In addition to this correlation to MAO, the I_2-binding site has also been suggested to be involved in cell growth (Regunathan *et al*., 1996a) or analgesic effects (Olmos *et al*., 1994; Sastre *et al*., 1996a). However, both are isolated findings and require confirmation with more detailed investigations. In this view, the 28-kDa protein identified in livers of MAO A- and MAO B-deficient mice (Olmos *et al*., 1994; Remaury *et al*., 2000) may be of some importance (see Figure 1; for detailed information see corresponding section of this review).

Furthermore, some authors have attributed specific functions such as noradrenaline release (Fuder & Schwarz, 1993; Molderings & Göthert, 1995; Likungu *et al*., 1996; Molderings *et al*., 1999b), secretion of gastric acid and pepsin (Molderings *et al*., 1998a; 1999a) and insulin from β-cells (Chan, 1998) to binding sites for which the affinity profile is not consistent with those of I_1- or I_2-binding sites (nonI_1/non I_2-binding sites see Figure 1).

Clonidine displacing substance

Apart from a specific, saturable, high affinity and reversible binding, the corresponding anatomical, histological and subcellular distribution of the putative binding sites as well as the identification of a physiological function, the establishment of the protein sequence, DNA structure, signal transduction and the identification of endogenous ligands are decisive criteria for the establishment of a new receptor system (Ernsberger, 1999).

Isolation, chemical characterization and receptor specificity of CDS

On the basis of the fact that the phenylethyl derivative noradrenaline and the imadazoline derivative clonidine influence blood pressure *via* central α_2-adrenoceptors and/or imidazoline binding site-mediated mechanisms, it had to be

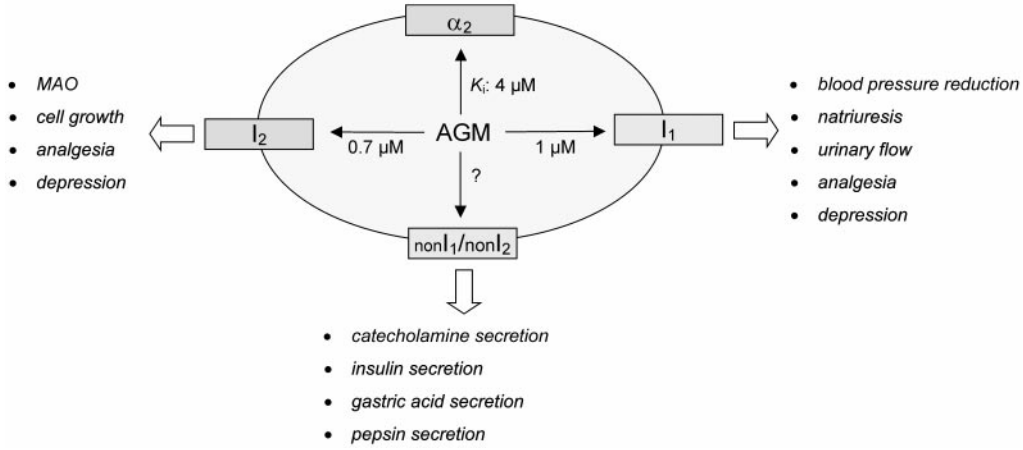

Figure 1 Imidazoline binding sites and their suggested functions. Agmatine binds with a moderate affinity (K_i values are taken from Li *et al*.) to α_2-adrenoceptors as well as to I_1 and I_2 binding sites. Some authors (Chan, 1998; Molderings *et al*., 1998a; 1999a) attributed functions to binding sites (nonI_1/non I_2-binding sites) the affinity profile of which is consistent with neither the I_1- nor the I_2-binding sites.

asked whether other non-catecholaminergic and until now unidentified substances participate in the regulation of blood pressure and heart rate. Atlas (Atlas & Burstein, 1984a,b; Atlas *et al*., 1987) isolated a substance from rat and calf brain by ion exchange chromatography, electrophoresis and HPLC. This isolate bound specifically to α_2-adrenoceptors and displaced clonidine, but not the α_1-ligand prazosin or the β-ligand cyanopindolol. Because of this property, the substance was named 'Clonidine Displacing Substance' (CDS). Occasionally, this CDS is referred to as 'classical CDS' (cCDS) to emphasize its detection by radioligand binding studies. Even though Atlas (Atlas & Burstein, 1984a,b; Atlas *et al*., 1987) did not clarify its structure, CDS was characterized as a hydrophobic substance with a molecular weight of 587 Da[1] stable to heat, acid hydrolysis and proteolytic enzymes such as trypsin, chymotrypsin, pronase, papain and pyroglutamase. Moreover it was postulated that CDS was not an amino acid and that it possessed no amino groups as shown by a negative ninhydrin and fluorecamine reaction. Due to its electrophoretic properties it was claimed that CDS was positively charged. Wavelengths of 224 and 276 nm represented its absorption maxima, which suggested the presence of aromatic residues in the molecule (see also Table 1; Atlas & Burstein, 1984a,b; Meeley *et al*., 1988a,b). Meeley *et al*. (1988a,b) developed a specific antibody directed against the clonidine analogue p-aminoclonidine. Since this antibody revealed a cross-reactivity with CDS, the authors concluded that there were structural similarities between clonidine and CDS and claimed that a phenyl and imidazole ring were mandatory structural characteristics for CDS, which confirmed the findings of Atlas & Burstein (1984a,b) regarding the relative hydrophobic character and the positive charge at neutral pH. CDS determined by radioimmunoassays is referred to in some studies as 'immunoreactive CDS' (irCDS) in order to emphasize its mode of determination. Since the distribution of irCDS in various organs of rats is directly correlated with biological activity attributed to cCDS, both cCDS and irCDS have been suggested to be similar (Meeley *et al*., 1988a). For this reason we have not distinguished between cCDS and irCDS in later sections of this review; only the term CDS is used irrespective of the way in which it was determined.

Later on, CDS could be characterized by radioligand binding studies as being 30 fold selective for imidazoline binding sites compared to α_2-adrenoceptors (Table 1), which strengthened the hypothesis that CDS might be an endogenous ligand for the imidazoline binding site (Ernsberger *et al*., 1988a; 1990b). Studies showing that CDS has only a weak affinity towards the inhibitory G-protein also fit in with this idea (Atlas, 1991). Unlike clonidine, CDS was incapable of influencing basal adenylate cyclase activity in human platelets or the noradrenaline-induced inhibition of adenylate cyclase at a concentration able to displace clonidine binding, findings which certainly do not support an α_2-adrenoceptor-mediated mechanism of action for CDS.

Distribution of CDS in central and peripheral tissues

After CDS had initially been isolated from the brains of several species (Atlas & Burstein, 1984a,b; Meeley *et al*., 1986; Ernsberger *et al*., 1988a; Regunathan *et al*., 1991a), CDS was also shown in various peripheral tissues by a specific antiserum directed against CDS (see Table 1; Meeley *et al*., 1988b; 1992; Dontenwill *et al*., 1988; Hensley *et al*., 1989). The existence of peripheral CDS was confirmed by a specific bioassay, which is based on the ability of CDS to induce contraction of the vas deferens or gastric fundus (Diamant & Atlas, 1986; Felsen *et al*., 1987). CDS, which has been isolated from brain, gastric fundus, heart, small intestine, kidney, liver, skeletal muscle and serum, contracted the gastric funds in a manner completely or at least partially antagonizable by verapamil. CDS isolated from the adrenal glands, however, relaxed the gastric fundus. The different extent of antagonism as well as the relaxation by adrenal CDS was explained by the possible co-extraction of other, effect-masking substances. Finally, Synetos *et al*. (1991) isolated CDS from human plasma and showed its biological effectiveness through the contraction of rat aortal vascular rings. Because of the significantly reduced plasma concentrations of CDS in adrenalectomized rats compared to sham-operated animals, Meeley *et al*. (1992) suggested the adrenals as the possible source of CDS circulating in the blood.

Biological function of CDS

Cardiovascular effects of CDS Since CDS and clonidine compete for a common binding site, it was questioned whether CDS would have agonistic or antagonistic activity in a functional test. After central application of CDS, arterial blood pressure increases significantly without any change in heart rate both in the cat and the rat (Bousquet *et al*., 1986; 1987), which in this way is opposite to the effects seen after central dosing of clonidine (see Table 2; Bousquet *et al*., 1984). Intracisternal application of CDS produces no change

Table 1 Comparison of physicochemical and physiological properties of CDS and agmatine (according to Atlas (1994; 1995); Meeley *et al*. (1992); Raasch *et al*. (1995a)). The adrenal gland was identified as the organ with the highest CDS, but low agmatine tissue levels, whereas the small intestine contains high agmatine but very low CDS levels

Property	*CDS*	*Agmatine*
Quantity in brain (cow)	3–4 ng g^{-1}	200–400 ng g^{-1}
Quantity in rat brain	9 ± 2 units $g_{wet\ weight^{-1}}$	2.4 ± 0.6 ng $g_{wet\ weight^{-1}}$
Quantity in rat adrenal gland	114 ± 16 units $g_{wet\ weight^{-1}}$	6.9 ± 3.3 ng $g_{wet\ weight^{-1}}$
Quantity in rat small intestine	3 ± 1 units $g_{wet\ weight^{-1}}$	55.4 ± 9.4 ng $g_{wet\ weight^{-1}}$
Absorption	224, 276 nm (aromatic)	200 nm (aliphatic)
Ninhydrin reaction	Negative	Positive
Molecular weight	587.8 ± 2 daltons	130 daltons
Affinity to α_2-adrenoceptors	10–12 nM	4 μM
Affinity to imidazoline binding sites	20–40 nM	1 μM
Retention time (RP18)	26 min	not retained

Table 2 Biological functions of CDS, agmatine and imidazoline derivatives on various effector systems

Effects on	*CDS*	*Agmatine*	*Imidazoline derivative (moxonidine)*
Vascular contraction	increase	no effect	increase
Blood pressure	increase after icv. application; antagonizes clonidine induced hypotension	no – moderate increase after icv.; synergistic effect to clonidine action	decrease after icv. application
Heart rate after icv. application	no effect	increase, decrease and no effects	decrease
Catecholamine release	increase (chromaffin cells)	increase (chromaffin cells) and decrease in isolated vascular preparations and pithed SHR	decrease in chromaffin cells, isolated vascular preparations and pithed SHR
Insulin secretion	glucose-induced insulin secretion is potentiated similar to efaroxan, effect is mediated via IBS	glucose-induced secretion is moderately increased not comparable to efaroxan effects: effect is not mediated via IBS	decrease (moxonidine) and increase (efaroxan) of insulin release
NO		competitive inhibitor of NOS	
Cell growth		increase	increase (idazoxan)
Analgesia		potentiate analgesic activity of opiates; improves development of morphine tolerance	potentiate analgesic activity of opiates; improves development of morphine tolerance
Renal function		increase of urinary flow and natriuresis	increase of urinary flow and natriuresis
Gastric fundus	increase of contraction	increase of gastric acid and pepsin secretion	increase of gastric acid and pepsin secretion (moxonidine)

in blood pressure in anaesthetized rabbits (Bousquet *et al*., 1987). Furthermore, CDS can antagonize clonidine-stimulated hypotension directly, i.e. the blood pressure reduction is reduced (Bousquet *et al*., 1986) and the dose-response curve for clonidine is clearly shifted to the right by CDS. This finding completely contradicts the results of Meeley *et al*. (1986), who observed a clear drop in blood pressure and heart rate after injection of CDS into the C1-region of the rat RVLM. Combination experiments with clonidine were not performed in this study. The reasons underlying these discrepant results may lie in the various solvents used for isolating and purifying the CDS (Bousquet *et al*., 1987). Alternatively, effects from CDS-extract impurities such as aminoacids, CDS-fragments, catecholamines, histamine or potassium might also have lead to these inconsistencies (Reis *et al*., 1992; Szabo *et al*., 1995; Singh *et al*., 1995).

CDS stimulates catecholamine release Apart from the above described property of CDS to contract various organ preparations, CDS has also been identified as a catecholamine releasing substance (Table 2). CDS binds with a high affinity to membranes of bovine chromaffin cells, whereby the displacement of [^{3}H]-idazoxan by CDS was not impeded by guanosine 5′-(β,γ-imido)triphosphate, indicating that the corresponding imidazoline binding site was not coupled to a GTP binding protein (Regunathan *et al*., 1991a). It should be noted that this was an I_2 like site labelled by [^{3}H]-idazoxan and that I_2-binding sites are consistently unaffected by guanine nucleotides. The concentration-dependent adrenaline release from chromaffin cells in response to CDS was comparable to that in response to nicotine, while the CDS-stimulated noradrenaline release was only about a quarter of the noradrenaline release induced by nicotine. Unlike the nicotine response, the release of either catecholamine following CDS can not be blocked by hexamethonium, which suggests a nicotine receptor-independent mechanism (Regunathan *et al*., 1991a). Since the catecholamine release is also not inhibited by the specific α_2-antagonist SKF-86466, which shows no affinity towards imidazoline binding sites (Ernsberger *et al*., 1990b), but is influenced by cobalt (Regunathan *et al*., 1991a), a specific imidazoline binding site-dependent and calcium-dependent release mechanism induced by CDS is suggested.

Insulinotropic effects of CDS For the further functional characterization of CDS, its influence on glucose stimulated insulin release was investigated in isolated Langerhans cells (Table 2 and Figure 2). The existence of imidazoline binding sites was shown in the pancreas (Schulz & Hasselblatt, 1989a,b), but the imidazoline binding sites of the β-cells of the pancreas appear to differ from the known I_1- and I_2-binding sites (Figures 1 and 2; Brown *et al*., 1993a; Chan *et al*., 1994; 1995; Morgan *et al*., 1995), and are also not identical with the binding site for sulphonylurea derivatives (Brown *et al*., 1993b; Rustenbeck *et al*., 1997). For this reason Morgan *et al*. (1999) have speculated about the existence of a pancreas specific I_3-binding site. Moreover, it could be shown that imadazoline derivatives such as efaroxan or phentolamine increase the release of insulin by influencing the K-ATP channel (Figure 2; Chan & Morgan, 1990; Dunne *et al*., 1995; Plant & Henquin, 1990). CDS isolated from rat brain potentiated the glucose (6 mM) induced secretory insulin response concentration-dependently to a similar extent as efaroxan, and reversed the inhibitory effects of diazoxide on glucose-stimulated insulin release just as other similar imadazoline derivatives do. That this CDS effect is possibly mediated *via* imidazoline binding sites can be concluded from the observation that imadazoline derivatives such as RX801080 and KU14R antagonize the insulin-releasing effect of CDS. In addition, the effects of CDS on insulin secretion were not altered by pretreatment of the CDS extract (protease incubation as well 3000 Da molecular filtration centrifugation; Chan *et al*., 1997), which confirms the structural properties of CDS postulated by Atlas & Burstein (1984a,b), i.e. that CDS is not a peptide, but rather a low molecular weight substance. In closing, efaroxan-pretreated

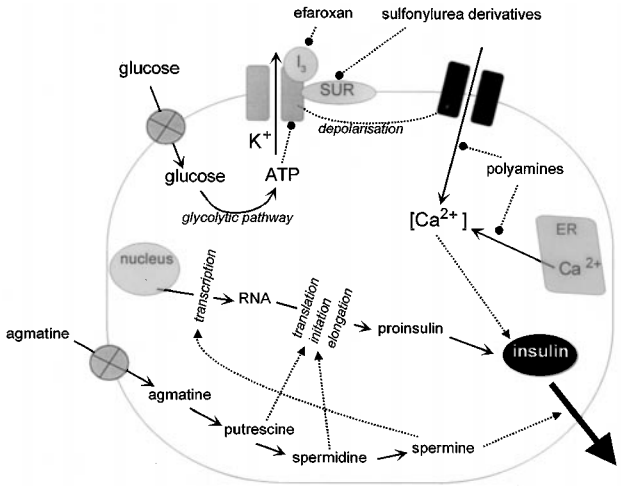

Figure 2 Current working hypothesis depicting mechanisms regulating insulin secretion from pancreatic β-cells and proposed sites for interference by agmatine. ATP generated by glucose metabolism shuts down K^+-channels, resulting in depolarization and subsequent influx of Ca^{2+} through voltage-activated Ca^{2+}-channels. This influx of Ca^{2+} increases cytosolic Ca^{2+} concentration, which is accompanied by mobilization of Ca^{2+} from the endoplasmic reticulum (ER), an event triggering secretory granule translocation and exocytotic release of insulin. The respective binding of imidazolines (such as efaroxan) and sulphonylurea derivatives (such as glibenclamide) to I_3-binding sites and the sulphonylurea receptor, also closes the K^+-channels. Agmatine does not bind to I_3-binding sites. However, agmatine may enhance insulin secretion *via* its metabolites, after it is taken up by specific transporters. Putrescine, spermidine are necessary for proinsulin biosynthesis, whereas spermine may exert a stimulatory or permissive role in RNA transcription and long-term insulin release. Polyamines are also probably involved in regulation of cytosolic Ca^{2+}-concentration by blocking Ca^{2+}-influx and its release from intracellular stores. Abbreviations: ····▶ : stimulation; ····●: inhibition.

islet cells appear to be desensitized to CDS concerning insulin release, which correlates with observations obtained for efaroxan itself (Chan, 1998).

Agmatine

Synthesis and metabolism of agmatine

Biosynthesis of agmatine Li *et al*. (1994) succeeded in identifying and characterizing mammalian agmatine by ion and molecular weight exclusion chromatography, high pressure liquid chromatography and mass spectroscopy, as a candidate for CDS. Agmatine, the decarboxylation product of the amino acid arginine, was first identified in 1910 by Kossel in herring sperm and is known as an intermediate in the polyamine metabolism of various bacteria, fungi, parasites and marine fauna (Tabor & Tabor, 1984; Yamamoto *et al*., 1988; Ramakrishna & Adiga, 1975), where polyamines have been attributed an important function in cellular growth. Agmatine is chemically characterized as follows (Table 1): its molecular mass is 130 Da, the UV absorption maximum of 200 nm suggests an aliphatic structure and a ninhydrin positive reaction confirms the existence of an amino group. Radioligand binding studies on membranes of bovine cerebral cortex, the ventrolateral medulla and on chromaffin cells have revealed K_ds towards α_2-adrenoceptors, I_1- and I_2-binding sites of 4, 0.7 and 1 μM, respectively (Figures 1 and 3; Li *et al*., 1994a). It had low affinity for the α_1- and β-adrenoceptors, $5-HT_3$ serotonin and D_2 dopamine binding sites (Li *et al*., 1994a), or the κ opioid and adenosine A_1-receptors (Szabo *et al*., 1995). An interaction with the sigma3 binding site has been shown on murine neuroblastoma cells (Molderings *et al*., 1996). As a functional correlate to CDS, agmatine concentration-dependently releases adrenaline and noradrenaline from chromaffin cells (Table 2). Since chromaffin cells express imidazoline binding sites, but not α_2-adrenoceptors (Regunathan *et al*., 1993), this can be considered as an indication for an agonistic function of agmatine at these binding sites. However, since there is a lack of proof that agmatine-induced catecholamine release can be blocked by antagonists of the I_1-binding site, it is not certain whether these binding sites mediate this effect. Moreover, data showing an inhibitory potency or no effect of agmatine on noradrenaline release (Häuser & Dominiak, 1995; Häuser *et al*., 1995; Molderings & Göthert, 1995; Molderings *et al*., 1997; 2000; Schäfer *et al*., 1999b) fuels doubts as to whether this catecholamine releasing effect is really mediated *via* a direct mechanism whereby imidazoline binding sites are involved. On the other hand, it was suggested that agmatine influences noradrenaline release *via* a dual interaction, namely a competitive antagonism and an allosteric activation of the rat α_{2D}-adrenoceptor, since (1); noradrenaline, moxonidine- or clonidine-induced noradrenaline release in segments of rat vena cava was dose-dependently enhanced or inhibited by agmatine, and (2); binding of clonidine and rauwolscine was inhibited, the rate of association and dissociation of clonidine was altered, and [^{14}C]-agmatine was inhibited from binding to its specific recognition site by agmatine (Molderings *et al*., 2000).

Agmatine arises enzymatically from the activity of arginine decarboxylase (ADC) on arginine (Figures 3 and 4) and is not supplied from nutritional components or bacterial colonization. ADC isolated from rat brain differs from plant or bacteria-derived ADC concerning localization, since ADC is associated with the mitochondria rather than the cytoplasm (as is typical for bacteria). The second difference concerns its substrate specificity. In contrast to bacterial ADC, mammalian ADC uses ornithine in addition to arginine, whereby it is not a typical ornithine decarboxylase, since it is neither cytosolic nor inhibited by diflouromethylornithine, a universal and irreversible inhibitor of all isoforms of ornithine decarboxylase (ODC) (Hunter *et al*., 1991). Finally, the optimum temperature of mammalian ADC is 30°C. At the bacterial temperature optimum of 37°C the enzyme activity of the mammalian ADC is only one third as active as it is at 30°C. Only the pH optimum (8.25) is similar between mammalian and bacterial ADC (Li *et al*., 1994a; 1995; Regunathan & Reis, 2000). Inhibition experiments in macrophages with lipopolysaccharides (LPS), transforming growth factor-β (TGF-β) and Interleukin-10 (IL-10) showed that ADC activity is subject to physiological control (Sastre *et al*., 1998). The co-localization of I_2-binding sites and ADC on mitochondria has been discussed as a potential intracellular receptor-controlled regulatory loop for endogenous biosynthesis (Figure 3; Li *et al*., 1995). However, the organ specific distribution of ADC in rats (Regunathan & Reis, 2000) differs from that of agmatine (Raasch *et al*., 1995a),

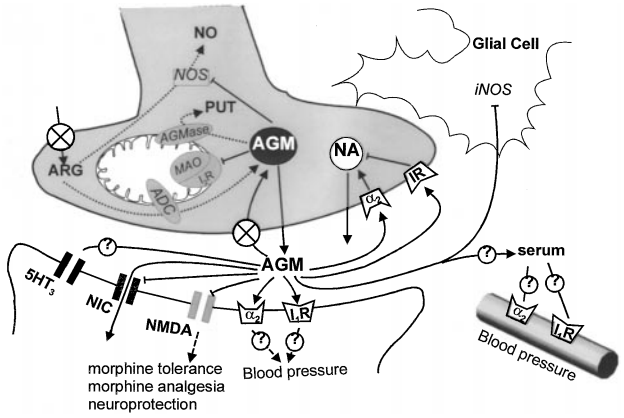

Figure 3 Schematic representation of an agmatinergic synapse: L-arginine enters the nerve ending *via* a transporter and is decarboxylated by the mitochondrial arginine decarboxylase (ADC) to agmatine (AGM), which is stored in vesicles and metabolized to putrescine (PUT) by agmatinase (AGMase). Agmatine inhibits NO synthase (NOS) as well as monoamine oxidase (MAO) since it was demonstrated that I_2-binding site (I_2-BS) is a regulative binding site of MAO. After agmatine is released from the neuron it is subject for a specific uptake or it interacts with various pre- and postsynaptic receptors including the I_1-binding site (I_1-BS), α_2 adrenoceptor (α_2-R), NMDA, nicotinic cholineric (NIC), $5-HT_3$ (*via* the sigma-2 binding site) receptor. Furthermore, agmatine enters postsynaptic neurons *via* nicotinic and possibly NMDA ion channels. Whether such released agmatine represents a source for serum agmatine has not yet been determined. Peripheral effects of agmatine on blood pressure and cell growth are also a matter of debate. Released agmatine binds to presynaptic imidazoline binding sites and α_2 adrenoceptors and in this way is involved in the regulation of catecholamines. Agmatine penetrates glial cells where it also modulates the expression and activity of iNOS.

revealing some doubt that there is a close correlation between agmatine and its biosynthetic enzyme.

Organ-specific, cellular and subcellular distribution of agmatine Using high pressure liquid chromatography, agmatine has been demonstrated in nearly all organs of the rat (Table 1), whereby the highest concentrations are found in the stomach (71 ng g^{-1} wet weight), followed by the aorta, small and large intestine, and spleen; it is found in lower concentrations (<10 ng g^{-1} wet weight) in the lungs, vas deferens, adrenals, kidneys, heart, liver, skeletal muscle, brain and testes (Raasch *et al*., 1995a,b). Gas chromatography studies by Stickle *et al*. (1996) confirmed an organ specific distribution of agmatine. This distribution pattern of agmatine in various organs (Raasch *et al*., 1995a) differs widely from that of CDS (Table 1; Meeley *et al*., 1992). As an example, high concentrations of CDS but only low concentrations of agmatine are found in the adrenal gland. Moreover, the low correlation ($r=0.2193$) between agmatine and CDS tissue levels in both studies indicates clearly that agmatine can not exclusively represent CDS, but that, if at all, it only represents a member of a whole CDS family.

The concentration of agmatine in rat plasma is only 0.45 ng ml^{-1} (Raasch *et al*., 1995a,b), which renders it doubtful that agmatine acts as a circulating hormone since the K_d values for the I_1- (0.7 μM) and I_2-binding sites (1 μM; (Li *et al*., 1994a) are approximately 200–300 fold higher compared to rat plasma concentrations. In addition, the source for circulating agmatine remains unidentified, since (1) ADC has not been detected in plasma until now, and (2) the adrenals, which were identified as sources for CDS (Meeley *et al*., 1992), contain only minimal amounts of agmatine (see Table 1; Raasch *et al*., 1995a). Stimulation experiments on

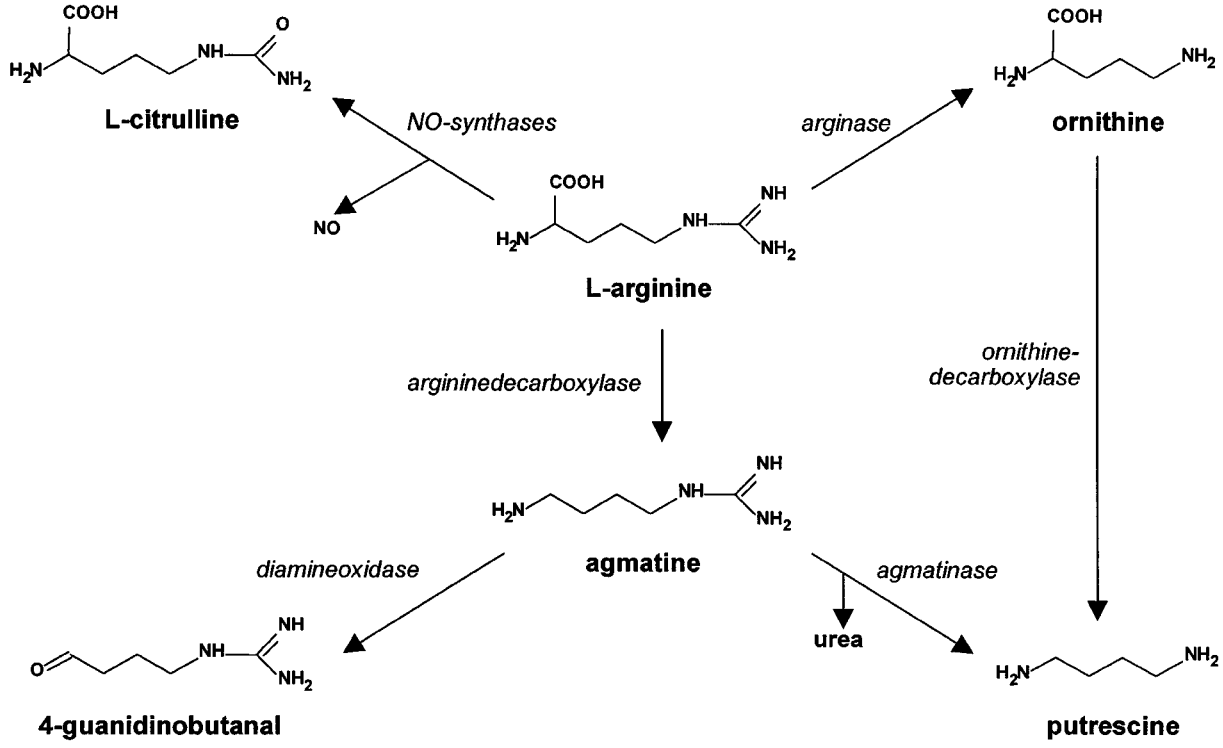

Figure 4 Metabolism of L-arginine in the mammalian organism.

animals designed to investigate this question have not been performed until now. In humans, substantially higher plasma concentrations (47 ng ml^{-1}) were determined when compared to rats (Feng *et al*., 1997). The reasons underlying this large difference remain to be clarified. An age-dependency for agmatine tissue concentrations could not be established, with the exception of the cerebral cortex, where concentrations declined by nearly 50% with age (Raasch *et al*., 1995a).

Using immunohistochemistry with specific antibodies against agmatine (Wang *et al*., 1995), agmatine was found to be regionally distributed in the cerebral cortex, the lower brain stem, the midbrain, frontal brain, thalamus and the hypothalamus in rat brain (Otake *et al*., 1998). In this way the distribution of agmatine-containing neurones correlates with the distribution pattern of α_2-adrenoceptors and imidazoline binding sites to the extent that (1) in most agmatine containing regions, α_2-adrenoceptors and imidazoline binding sites are also expressed (Kamisaki *et al*., 1990; de vos *et al*., 1991; 1994; Bricca *et al*., 1993; Nicholas *et al*., 1993; 1996; King *et al*., 1995; Ruggiero *et al*., 1995) and (2) agmatinergic neurones are concentrated in brain regions (e.g. cerebral cortex), which project to areas (e.g. striatum and midline thalamus), which contain α_2-adrenoceptors and imidazoline binding sites (Berendse & Groenewegen, 1991; Jones & Yang, 1985; Saper *et al*., 1986). There also appear to be a multitude of interactions between agmatinergic cells and receptor areas in the corticothalamostriatal regulatory loops.

At the cellular level, agmatine could be shown in smooth muscle and endothelial cells. Since ADC is expressed only in endothelial cells and not in smooth vascular muscle cells, it was concluded that agmatine either originates in the serum or is taken up from the endothelial cells and stored in the smooth vascular muscle cells, although a corresponding transporter for agmatine has not yet been identified in the vasculature (Regunathan *et al*., 1996a). There are, however, reports on an agmatine-transport system of bacterial (Kashiwagi *et al*., 1986; Driessen *et al*., 1988) and neuronal origin (Sastre *et al*., 1997). Glial cells not only express imidazoline binding sites, they also synthesize agmatine (Regunathan *et al*., 1995a), whereby the agmatine concentration and ADC activity in the cultivated cells are substantially higher than the concentrations in brain, which might indicate that glial cells represent the main site for synthesis and storage. It is well known that cells under culture conditions can undergo alterations in distinct features such as receptor population, enzyme activity and this might also alter agmatine content. This could be why there are differences in agmatine content between neuronal and glial cells. In this respect, interferon-gamma (IFN-γ) was able to increase ADC activity without inducing nitric oxide synthase (iNOS) activity significantly in astrocytes, whereas LPS stimulated iNOS but not ADC activity. These data suggest that the ADC activity in neuronal tissue is subject to regulation, and that two different stimuli influence two pathways of arginine metabolism in entirely different ways.

At the subcellular level, agmatine was shown by immuncytochemistry to be localized mainly in large dense-core vesicles in the cytoplasm and in the immediate vicinity of the endoplasmic reticulum and the mitochondria (Figure 3; Otake *et al*., 1998; Reis *et al*., 1998), which correlates well with the demonstration of a mitochondria-associated ADC (Li *et al*., 1995). Moreover, an association of agmatine with small synaptic vesicles was shown in the rat hippocampus within the nerve endings, whereby the function of these endings has not yet been clarified. The co-transmitter function to L-glutamate has been speculated, since inhibitory effects at the N-methyl-D-aspartate (NMDA) receptor have been attributed to agmatine (Yang & Reis, 1999), which would suggest a regulation of the excitatory effect of L-glutamate *via* co-transmission.

Release of agmatine In rat brain slices and synaptosomes, a release of agmatine, but not of putrescine could be shown in response to depolarization with 55 mM KCl (Figure 3; Regunathan *et al*., 1996b; Reis & Regunathan, 1998). In the absence of Ca^{2+}, the release of agmatine was significantly reduced, suggesting a calcium dependent mechanism. Significant quantities of radiolabelled agmatine could be released from bovine chromaffin cells by 55 mM KCl and 10 pM nicotine induced depolarization (Tabor & Tabor, 1984). Immunocytochemical studies on the storage of agmatine support the findings of a stimulation-receptive agmatine release from neuronal tissue, since agmatine-like immunoreactivity is primarily associated with small synaptic vesicles (20–30 nm diameter) in nerve endings that form asymmetric contacts to the spines of pyramidal cell dendrites. While the adrenals were identified as a source of circulating CDS (Meeley *et al*., 1992), no data exists about the release of endogenous agmatine into the circulation.

Inactivation of agmatine The existence of a specific transporter system working against a concentration gradient (Kashiwagi *et al*., 1986) as well as an agmatine-putrescine antiporter (Driessen *et al*., 1988) have been shown in prokaryotes. In synaptosomes from rat brain, a selective ATP- and temperature dependent as well as Na-independent agmatine transporter could be shown (Figure 3; Sastre *et al*., 1997). The affinity of agmatine to this transporter is extremely low with a K_m of 18.8 mM. Other polyamine transporters, however, have also shown K_m values in the millimolar range (Seiler & Dezeure, 1990). The fact that agmatine uptake can not be inhibited by amino acids, catecholamines or polyamines at concentrations (1 mM) which can be judged as high (with the exception of amino acids) underscores the specificity of the transporter. Combination experiments with carbachol and nicotine have excluded the possibility that the agmatine-transporter is a nicotine-associated ion channel; earlier studies showed that [^{3}H]-agmatine can act as a marker for ion flux through nicotinergic ion channels (Quik, 1985) and that agmatine acts as an antagonist of the retinal nicotine receptor (Loring, 1990). The transporter is also no K^+_{ATP}- channel. However, agmatine-uptake can be suppressed by $CoCl_2$, $CdCl_2$ or verapamil and doubled by application of a calcium-free medium, an indication that agmatine is transported through a calcium channel, although it could be excluded that the calcium channels were of an L- or T-type (Sastre *et al*., 1997). Finally, agmatine-uptake into synaptosomes is blocked by the imidazoline derivatives idazoxan and phentolamine, but not by clonidine, moxonidine, rilmenidine or p-aminoclonidine, so that apart from a guanidine or imidazoline partial structure, other structural properties also contribute to the inhibitory effect. Recently another agmatine uptake system was identified and pharmacologically characterized in human

glioma cells; it was energy dependent, and saturable with a K_m of 8.6 μM and a V_{max} of 64.3 nmol min^{-1} mg protein^{-1} but distinct from the putrescine transporter and known amino acid or monoamine carriers and not associated with a calcium- or 5-HT$_3$ receptor channel or an organic cation transporter (Molderings *et al*., 2001). Those features, especially the much higher affinity ($\sim$2200 fold), clearly indicate its difference from the agmatine transporter identified by Sastre *et al*. (1997). From all these findings it seems reasonable to suspect that this transporter might be involved in the regulation of extracellular agmatine concentrations.

From bacterial polyamine metabolism it is well known that agmatine can be degraded enzymatically by agmatinase (Satishchandran & Boyle, 1986; Panagiotidis *et al*., 1987) or agmatine deaminase (Mercenier *et al*., 1980). The question is now whether the same metabolization pattern can be demonstrated in mammals, and whether an alternative means for putrescine biosynthesis apart from decarboxylation of ornithine (Tabor & Tabor, 1984) exists. The first evidence for a breakdown of agmatine to putrescine with cleavage of urea (Figures 3 and 4) in rat brain was obtained by Gilad *et al*. (1996a). Later, Sastre *et al*. (1996b) identified a mitochondrial agmatinase in rat brain with a low affinity for agmatine (K_m=5.3 mM), and a V_{max} of 530 nmol h^{-1} mg^{-1} protein. These enzyme kinetic parameters are comparable with those of the bacterial agmatinase (Satishchandran & Boyle, 1986). When one considers the relatively low concentrations of agmatine in rat brain (Raasch *et al*., 1995a) and the very low affinity of agmatine to the enzyme, one can reasonably question the extent to which agmatinase is of any physiological relevance. Since agmatinase is co-localized with ADC (Li *et al*., 1995; Regunathan & Reis, 2000), however, one can not rule out the presence of mM agmatine concentrations in intracellular compartments, which would then represent an adequate substrate concentration for the agmatinase. The high V_{max} might also compensate for the lower affinity. A regional heterogeneous distribution of agmatinase has indeed been shown in rat brain (Sastre *et al*., 1996b), with the highest activity in the hypothalamus, followed by the medulla oblongata and hippocampus, and the lowest activity in the striatum and cerebral cortex. This distribution pattern is largely consistent with the imidazoline binding sites (Mallard *et al*., 1992), but less so with the α_2-adrenoceptors (Ruggiero *et al*., 1995). Moreover, the distribution of agmatinase seems not to be consistent with the regional pattern of agmatinergic neurones in the CNS as determined by immunocytochemistry (Wang *et al*., 1995).

Outside of the CNS, agmatinase could also be demonstrated in macrophages (Sastre *et al*., 1998), where its activity can be stimulated by LPS, but inhibited by TGF-β and IL-10. Since ADC in macrophages is inhibited by LPS, TGF-β, and IL-10, and the activity of iNOS is also regulated by these substances (Wang *et al*., 1995), it seems reasonable to suggest that arginine might be metabolized by these three enzymes, and possibly other enzymes, to agmatine, nitric oxide (NO) and polyamines. Aside from agmatinase-induced degradation, agmatine can also be metabolized in the kidney by diamine oxidase (DAO; Figure 4; Holt & Baker, 1995; Lortie *et al*., 1996), where the affinity of agmatine to this enzyme is in the micromolar range and therefore substantially higher than that of agmatinase. Studies with specific DAO inhibitors underscore the importance of DAO in agmatine metabolism. Indeed, it has been speculated that specific inhibition of DAO by the antidepressant drug phenelzine might lead to an increase in serum and tissue concentrations of agmatine and in this way contribute to its antidepressive efficacy (Holt & Baker, 1995). However, this hypothesis is weakened by the fact that (1) no changes in plasma or tissue levels of agmatine were found during chronic DAO blockade; and (2) no DAO activity could be demonstrated in the brain (Lortie *et al*., 1996), suggesting an organ specific metabolism of agmatine, i.e. degradation by DAO in the kidney and agmatinase in central tissues. Moreover, it seems feasible that an increase in histamine due to DAO blockade might participate in antidepressive effects, since an antidepressant-like effect, *via* activation of H$_1$-receptors, has been suggested elsewhere (Lamberti *et al*., 1998).

In studies by Holt & Baker (1995), agmatine (50 μM) had no influence on either the semicarbazide-sensitive aminoxidase (SSAO) or MAO. By using specific substrates it could be shown that agmatine does not modulate either MAO-A or MAO-B; hardly surprising to many, given that other polyamines such as putrescine are not substrates for MAO (Blaschko, 1974). These results are somewhat inconsistent with the fact that the I$_2$-binding site has been characterized as a regulatory subunit of MAO (Tesson & Parini, 1991; Tesson *et al*., 1991; 1995; Carpene *et al*., 1995; Raasch *et al*., 1996; Raddatz *et al*., 1995; 1997; Escriba *et al*., 1996; Raddatz & Lanier, 1997) and that agmatine has been identified as a ligand at imidazoline binding sites (Li *et al*., 1994a; Piletz *et al*., 1995; 1996a). In this respect, the fact that agmatine is able, just as other I$_2$-ligands, to inhibit MAO isolated from rat liver, does not seem completely surprising (Figure 3; Raasch *et al*., 1996; 1999). The IC$_{50}$ value of 167 μM reported by Raasch *et al*. (1999) is about 3 fold higher than the maximal concentration used by Holt & Baker (1995) in their study. At 50 μM only a minor inhibition of MAO could be seen, even in Raasch *et al*.'s (1999) study. However, it seems more than doubtful that the agmatine-induced inhibition of MAO *in vitro* should have some relevance *in vivo*, since plasma and tissue concentrations of agmatine are approximately 500–40,000 fold lower than the EC$_{50}$ values in the *in vitro* assays. Furthermore, an interaction between endogenous agmatine and MAO inhibitors is unlikely because of their different sites of action.

Biological functions of agmatine

Effects of agmatine on the central nervous system (Antinociceptive effects of agmatine) Since the mid 80's it has been known (Gossop, 1988) that clonidine potentiates the analgesic activity of opiates, an effect which is purportedly mediated *via* α_2-adrenoceptors (Maze *et al*., 1988; Quan *et al*., 1993). Studies on α_{2A}-knock-out mice have clearly revealed the participation of α_{2A}-adrenoceptors in analgesia (Hein *et al*., 1999; Hein, 2001). In this context, Fairbanks & Wilcox (1999) demonstrated that the centrally acting antihypertensive drug moxonidine produces antinociception in mice with dysfunctional α_{2A}-adrenoceptors (α_{2A}-D79N not a null mutation; Macmillan *et al*., 1996), whereby the selective α_{2A}-adrenoceptor antagonist SK&F86466 (Hieble *et al*., 1986) and the I$_1$-binding site/α_{2A}-adrenoceptor mixed antagonist efaroxan (Haxhiu *et al*., 1994) antagonizes the moxonidine effects. The following mechanisms may underlie these results:

(1) other substypes of α_{2A}-adrenoceptor – either α_{2B} or α_{2C} – may participate in analgesia (Fairbanks & Wilcox, 1999); (2) since gene expression of the α_{2A}-adrenoceptor is reduced by 80% *via* targeted mutation in the α_{2A}-D79N mice (Macmillan *et al*., 1996), there is still the possibility of a residual activity of the α_{2A}-adrenoceptor. This conclusion is authoritatively confirmed by the observation of different physiological effects between mice with fully disrupted α_{2A}-adrenoceptors (α_{2A}-adrenoceptor knock out mice) and those with gene-targeted mutation receptors (α_{2A}-D79N, Altman *et al*., 1999); (3) imidazoline binding sites may induce antinociceptive effects (Figure 1), which raises the question of the biological relevance of agmatine mediating such effects, since agmatine was shown to be synthesized, stored, released and metabolized either by uptake or by enzymatic breakdown in tissue of neuronal origin (for detailed information see sections above).

Agmatine alone ($0.1-10$ mg kg^{-1}) was ineffective in the mouse tailflick assay, but after intravenous or intrathecal application it potentiated the analgesic efficacy of morphine dose-dependently by factors of five or nine, respectively, without affecting morphine-induced gastrointestinal transit (Kolesnikov *et al*., 1996). Such results were confirmed by using slightly modified study protocols of Bradley & Headley (1997) and Horváth *et al*. (1999). The potentiation of morphine activity by agmatine seems to be mediated *via* a δ- rather than a κ_1- or κ_3 opiate receptor mechanism. Moreover, chronic studies also showed that agmatine, at a dose (0.1 mg kg^{-1}) which did not potentiate morphine activity, reduced the development of tolerance during a 10-day morphine regime. It is not unlikely that this effect is mediated *via* I_2-binding sites, since selective I_2-ligands such as idazoxan or 2-benzofuranylimidazoline also suppressed the morphine-induced development of tolerance, while selective I_1-ligands or α_2-adrenoceptor antagonists did not (Boronat *et al*., 1998). How this binding site mediates suppression of the development of tolerance is still not completely understood, even considering the fact that I_2-binding sites have been implicated as a regulatory binding site on MAO. Moreover, results using idazoxan, showing increases in cerebral levels of 3-methoxy-4-hydroxyphenylethyleneglycol (MOPEG) and 3,4,dihydroxy-phenylacetic acid (DOPAC) in morphine-withdrawn mice and an enhancement of morphine's elevating effects on MAO (Airio & Ahtee, 1999) suggests rather an interaction with MAO than an increase in cerebral noradrenaline turnover and release as a mechanism underlying idazoxan's overcoming of morphine tolerance. However, such a noradrenaline release can not be mediated classically *via* presynaptic α_2-adrenoceptor, since it has repeatedly been shown that idazoxan inhibits noradrenaline release by this mechanism (Molderings *et al*., 1997). Another I_2-mediated mechanism, possibly attributed to the 28-kDa protein which is present in MAO deficient mice (Remaury *et al*., 2000), was speculated to be involved in the reduction of morphine tolerance, since an astrocyte hyperplasia following chronic dosing of I_2-selective imadazoline derivatives (Olmos *et al*., 1994; Alemany *et al*., 1995) can antagonize the morphine-induced suppression of astrocyte growth (Stiene-Martin *et al*., 1991; Stiene-Martin & Hauser, 1993). Since astrocytes play an important role in the regulation of synaptic density (Meshul *et al*., 1987), the astrocyte growth can modulate synaptic plasticity and appears to be associated with chronic morphine dosing (Nestler *et al*., 1996). Recently, a functional interaction between opioid- receptors and I_2-binding sites could be shown. The first results on a so-called Gi-Go transducer protein have also been obtained, a protein which appears to play some role in this interaction (Sanchez-Blazquez *et al*., 2000). However, the low affinity of agmatine to the I_2-binding sites compared to the selective I_2 ligands provides reason to doubt this concept. Moreover, the concept of an I_2-mediated mechanism for agmatine's antinociceptive effects contradicts the conclusion derived from the findings using α_{2A}-adrenoceptor knock out mice, in which if anything a participation of I_1-binding sites was suggested. Overall, it must be determined whether endogenous agmatine would participate in such an antinociceptive effect. To answer this, physiological or pathophysiological conditions have to be identified whereby agmatine levels (e.g. depression; Halaris *et al*., 1999) as well as morphine's effects are altered.

Aside from improvements in development of tolerance and morphine's analgesic action, agmatine also dose-dependently improved ($20-40$ mg kg^{-1}) acute morphine withdrawal symptoms under naloxone in rats, such as jumping, wet dog shakes, writhing, defecation, ptosis, teeth chattering and diarrhoea (Aricioglu-Kartal & Uzbay, 1997). However, this behavioural pattern could not be induced by giving agmatine alone. A lacking impediment of locomotor activity under agmatine alone and in combination with naloxone suggests that the observed agmatine effects were not due to sedation or muscle relaxation. The authors of this study also associated the effects observed less not so much with an interaction at α_2-adrenoceptors, but much more with an interaction at imidazoline binding sites or NOS.

(Effects of agmatine on the NMDA receptor) After it was recognized that NMDA receptors participate in the development of opiate dependency and development of tolerance (Elliott *et al*., 1995; Trujillo, 1995), it had to be asked whether ligands of imidazoline binding sites modulate the above discussed morphine tolerance *via* an NMDA receptor-mediated mechanism. The binding of the NMDA ligand [H]-(+)-MK-801 to cerebral cortex membranes can be reduced by various imidazolines with weak potencies (K_i) of 37–190 μM, whereby the potencies of the I_1-ligand (moxonidine), the I_2-ligand (idazoxan) and the α_2 agonist (RX821002) were not different (Boronat *et al*., 1998). This suggests a lack of a correlation between the potency at NMDA receptors and the ability to prevent opiate tolerance. Compared to the other substances, agmatine had the lowest affinity for the I_2-binding site, but the highest affinity for the NMDA receptor (Boronat *et al*., 1998). Moreover, agmatine differed from the other substances in this study since it had a significantly shallower Hill-Slope and the curve-fitting characteristics were different. All these signs could mean that agmatine differs from other imadazoline derivatives in its binding behaviour to NMDA receptors, and that even if this hypothesis were to be rejected for the imadazoline derivatives, the agmatine effects on morphine tolerance and potentiation could be mediated *via* NMDA receptors (Figure 3).

Against this background, whole cell patch clamp studies on cultivated hippocampal neurones showed that agmatine specifically induces a voltage and concentration-dependent block of the NMDA current, but not the AMPA- (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) or kainate flux

(Yang & Reis, 1999). This inhibition was reversible and most potent at hyperpolarizing membrane potentials, and less effective at positive potentials. Even in the presence of 10 μM glycine, agmatine (100 μM) showed no measurable whole-cell flux, which does not support an NMDA-agonistic effect of agmatine. As a NMDA antagonist, (±)-2-amino-5-phosphonopentanoic acid (AP5) inhibits both, the inward and outward directed NMDA current. In the presence of agmatine there was an additional inhibition of the AP5-inhibited NMDA current, comparable to the inhibition under agmatine alone, making it reasonable to presume that agmatine is not a competitive NMDA antagonist. It could be shown, however, that agmatine interacts with the NMDA-pore directly at a site part-way across the membrane electric field (Yang & Reis, 1999). By investigating various agmatine structural analogues (arcaine, putrescine, spermine, arginine) regarding their ability to alter NMDA current, it could be shown that the guanidine structure of agmatine appears to be essential for suppressing NMDA current. A similar structure-activity relationship was also reported concerning the MAO inhibitory activity of agmatine (Raasch *et al*., 1999). Since the agmatine concentration (100 μM) required for an effective NMDA receptor blockade is relatively high, one has to question the physiological significance of this effect, especially against the background of published agmatine tissue concentrations (Raasch *et al*., 1995a; Feng *et al*., 1997; Stickle *et al*., 1996). However, since agmatine is not uniformly distributed in the CNS, but preferentially distributed in certain areas and subcellular structures (Otake *et al*., 1998; Reis *et al*., 1998), a concentration adequate to block NMDA might be reached, providing that the agmatine becomes released.

Glutamate, the most important excitatory neurotransmitter in the brain, also is potentially neurotoxic (Choi *et al*., 1988). Glutamate exposure to primary cultures of cerebellar granule cells has been characterized as a model for neurotoxic effects, which correlates with effects at NMDA receptors (Lysko *et al*., 1989). NMDA antagonists are consequently able to suppress such neurotoxic glutamate effects. In this context, imadazoline derivatives revealed some neuroprotective efficacy (Gustafson *et al*., 1990; Maiese *et al*., 1992; Olmos *et al*., 1996; 1999; Degregorio-Rocasolano *et al*., 1999). It therefore seemed natural to study whether agmatine, as an endogenous ligand of imidazoline binding sites with NMDA antagonistic activity (see above), possesses any neuroprotective activity. In cultivated cerebellar neurones, agmatine suppresses the NMDA-induced neurotoxic effects at concentrations of between 10–100 μM, but at higher concentrations it becomes toxic itself (LD_{50}: 700 μM; Gilad *et al*., 1996b), perhaps by inhibiting growth effects as discussed later. Similar neuroprotective effects in cell cultures were shown by Olmos *et al*. (1999), who associated this effect of agmatine directly with an antagonistic activity at the NMDA receptor. *In vivo* studies on gerbils revealed neuroprotection after a global frontal brain ischaemia following an intraperitoneal application of agmatine (10–100 mg kg^{-1}). These results occurred in line with a complete (after agmatine treatment) or partial (controls) recovery of the neurological deficit within 72 h, as revealed by the motor performance of the animals (Gilad *et al*., 1996b). The importance of endogenous agmatine during cerebral ischaemia is also stressed by the fact that the activity of ADC is increased transiently and in parallel to ODC by about 7 fold (Gilad *et al*., 1996c). This study seems to be a very important study, since it was shown that due to pathophysiological events the rate limiting enzyme of agmatine's biosynthesis is enhanced, suggesting a biological function for agmatine. As in many other functional studies, however, the applied dose of agmatine was indeed very high. Also, the extent of the agmatine transport is unknown in the brain and might indeed be limited under normal conditions. However, it is known that the blood-brain barrier after an ischaemic insult is damaged very early on and in a long-lasting manner (Dietrich *et al*., 1991), which could allow better access to the brain for exogenous substances. Nevertheless, circulating agmatine concentrations are very low (Raasch *et al*., 1995a), so that it is not very likely that endogenous agmatine from the periphery will contribute to neuroprotective effects. Apart from this it appears questionable, especially considering the relatively low affinity of agmatine to the NMDA receptor (K_i = 219 μM, Olmos *et al*., 1999) and the endogenous tissue concentrations, whether endogenous agmatine could be held accountable for the survival-promoting effects observed *in vivo* (Gilad *et al*., 1996b). But at this point we must refer once again to the non-uniform distribution of agmatine in the CNS (Otake *et al*., 1998) which could theoretically approach efficacious concentrations at the NMDA receptor, provided of course that it is released.

(Agmatine's significance in depression) Various findings indicate a potential pathophysiological role of imidazoline binding sites in the development of depression (Figure 1): the density of I_1-binding sites in human platelet plasma membranes is upregulated in depressive patients and normalized by antidepressive therapy (Piletz & Halaris, 1995; Piletz *et al*., 1996b,c). A similar increase in I_1-binding sites was observed in untreated women with dysphoric premenstrual syndrome (Halbreich *et al*., 1993). Alterations in various imidazoline binding site proteins (45-kDa, 29/30-kDa) could be shown by Western blotting in membranes from platelets and brains of unipolar depressive patients, and from cortical autopsy samples from suicidal individuals (Sastre *et al*., 1995; Garcia-Sevilla *et al*., 1996; 1998a). The concentration of agmatine was raised in the plasma of depressed patients compared to a control group (70.6 ± 6.5 vs 38.5 ± 5.4 ng ml^{-1}, $P < 0.05$). After treatment with the antidepressant bupropion, agmatine plasma concentrations normalized to 57.0 ± 6.7 ng ml^{-1}, but no correlation between the bupropion and agmatine plasma concentrations was found (Halaris *et al*., 1999). At the same time the density of I_1-binding sites and the immunodensity of a 33-kDa band in platelet membranes from depressive patients increased compared to controls. Both parameters fell to control levels with bupropion treatment, whereby the number (B_{max}) of I_1-binding sites correlated well with the agmatine plasma concentration ($r = 0.800$, $P = 0.005$). The finding regarding elevated levels of agmatine and I_1-binding sites is surprising, since upregulation of an endogenous ligand normally occurs in line with a downregulation of its receptor, and vice versa. Whatever causes this uniform elevation of those parameters remains unclear, but the course of disease might play a significant role. Hence longer-lasting, disease-following studies on plasma agmatine concentrations and receptor

densities in platelets are necessary to provide a satisfactory and plausible explanation. For other neurodegenerative disorders (e.g. Parkinson's disease, Alzheimer's disease, glial tumours), alterations in the expression of imidazoline binding sites in the brain or platelets could also be shown (Garcia-Sevilla *et al*., 1998b; 1999; Ulibarri *et al*., 1999). However, until today no studies have been performed, analogous to the depression studies, which have pursued the question of changes in agmatine plasma concentrations and their potential role in those diseases.

Cardiovascular properties of agmatine (Effects of agmatine on isolated vessels and atria (for overview see Table 3)) Agmatine exerted no effect of its own and failed to alter the concentration-dependent contractile effects of the α_2-adrenoceptor agonist UK14304 on the KCI-precontracted porcine palmar lateral vein, and unlike clonidine, agmatine did not increase contractility in the endothelium-denuded thoracic artery of the rat (Pinthong *et al*., 1995a). These results were confirmed both in intact and endothelium-denuded thoracic aortal segments (Gonzalez *et al*., 1996; Schäfer *et al*., 1999a). Agmatine also did not influence the contractile responses either to clonidine or phenylephrine (Schäfer *et al*., 1999a). Similarly, agmatine failed to impart any direct inotropic activity in the isolated, electrically stimulated atrium. In contrast, the imidazoline derivatives cirazoline and moxonidine produced significant increases in contractility, which were found to be due to an α_1-adrenoceptor-mediated mechanism (Raasch *et al*., 2000). Such negative results on contraction were confirmed in studies where agmatine (0.1 – 100 μM) was ineffective at inhibiting electrically stimulated contraction of the rat isolated vas deferens and isolated guinea-pig ileum (Pinthong *et al*., 1995a). This is inconsistent with the effects of CDS on these preparations (Diamant & Atlas, 1986; Felsen *et al*., 1987; Meeley *et al*., 1992), creating some doubt that agmatine might be CDS. Another explanation may be that the positive effects of the functional tests were due to other substances co-purified during the CDS isolation. Only Jurkiewicz *et al*. (1996) and Gonzáles *et al*. (1996) observed both a clear agmatine-induced potentiation of electrically stimulated contraction and a competitive antagonism between agmatine and clonidine. Those inconsistent observations of Pinthong *et al*. (1995a), Jurkiewicz *et al*. (1996), Gonzáles *et al*. (1996) and Schäfer *et al*. (1999a) can presumably be explained by the use of different tissue preparations, and less so by the use of different agmatine concentrations, since all authors used agmatine concentrations up to the millimolar range. However, the biological relevance of positive effects of agmatine on isolated organ preparations using such high concentrations seems more than dubious, especially when considering that endogenous agmatine concentrations in vessels and plasma are in the nanomolar range (Raasch *et al*., 1995a), and that K_d values are 0.7 and 1 μM for the I_1-/I_2-binding sites (Li *et al*., 1994a). Furthermore, one has to determine which receptor or binding site mediates agmatine's effects on isolated veins as observed by Jurkiewicz *et al*. (1996), and Gonzáles *et al*. (1996) since it was demonstrated that agmatine was able to compete with [^{3}H]-clonidine binding in these preparations (Pinthong *et al*., 1995a), suggesting the existence of α_2-adrenoceptors and/or imidazoline binding sites. However, the imidazoline binding sites in endothelium and vascular smooth muscles were characterized as I_2- and less I_1-binding sites (Regunathan *et al*., 1996a). Since the I_2-binding site is implicated in MAO regulation (Carpene *et al*., 1995), the observed effects are probably mediated *via* α_2-adrenoceptors. However, the affinity of agmatine at α_2-adrenoceptors (K_d: 4 μM) is less than it is at the imidazoline binding site, a fact which weakens this hypothesis. Forskolin-induced accumulation of [^{3}H]-cyclic AMP in the isolated palmar lateral vein of the pig is another model for investigating α_2-adrenoceptor effects. This phenomenon can be concentration-dependently antagonized by α_2-adrenoceptor antagonists, while agmatine has no influence on basal [^{3}H]-cyclic AMP concentration or forskolin-stimulated [^{3}H]-cyclic AMP accumulation. The clonidine- or UK-14304-induced inhibition of forskolin-stimulated [^{3}H]-cyclic AMP accumulation was also uninfluenced by agmatine. This

Table 3 Effects of agmatine on cardiovascular functions

Effect on		*Tissue/species*	*Concentration/dose*	*Reference*
Vascular contraction	→	rat thoric aorta, porcine palmar lateral vein, rat tail aorta, rat thoric aorta	$10^{-9}-10^{-3}$ M	(Pinthong *et al*., 1995a; Gonzalez *et al*., 1996; Schäfer *et al*., 1999a)
Inotropy	→	rat left atrium	$10^{-9}-10^{-4}$ M	(Raasch *et al*., 2000)
Clonidine's CRC on vascular contraction	rightward and no shift	rat tail aorta, rat thoric aorta	1, 5 mM	(Gonzalez *et al*., 1996; Schäfer *et al*., 1999a)
Blood pressure	↓	anaesthetized Sprague Dawley rats and anaesthetized SHR	0.1 – 100 mg kg^{-1}	(Sun *et al*., 1995; Gao *et al*., 1995; Häuser & Dominiak, 1995; Schäfer *et al*., 1999a,b)
Heart rate	↓	anaesthetized SHR	33 – 100 mg kg^{-1}	(Schäfer *et al*., 1999a)
Blood pressure	↓	pithed SHR	10 – 100 mg kg^{-1}	(Schäfer *et al*., 1999a)
Heart rate	↓	pithed SHR	33 – 100 mg kg^{-1}	(Schäfer *et al*., 1999a)
Clonidine's DRC on vascular contraction	no shift	pithed SHR	10 mg kg^{-1}	(Schäfer *et al*., 1999a)
Clonidine induced BP-reduction	↑	anaesthetized SHR	0.3 – 33 mg $^{-1}$	(Schäfer *et al*., 1999a)

Abbreviations: BP: blood pressure; CBC: concentration response curve; DRC: dose response curve; SHR: spontaneously hypertensive rat; →: no change; ↓: decrease; ↑: increase.

confirmed at the subcellular level that agmatine exerts neither agonistic nor antagonistic activity at the α_2-adrenoceptor (Pinthong *et al*., 1995a). This overall conclusion was contradicted by Molderings *et al*. (2000), who demonstrated both a competitive antagonism and an allosteric activation of agmatine with the rat α_{2D}-adrenoceptor, which seemed to be concentration-dependent. However, it should be mentioned that the α_{2D}-adrenoceptor is an autoreceptor modulating noradrenaline release from sympathetic nerves and is different from the postsynaptic α_2-adrenoceptors investigated by Pinthong *et al*. (1995a).

(Interaction of agmatine with other neurohumoral systems (NO, sympathetic nerve system)) The question arises as to why agmatine has no effect on its own on isolated vessels and why it only minimally (at most) antagonizes the contraction response to clonidine, which is actually what should be expected from a 'clonidine displacing substance', especially since the existence of α_2-adrenoceptors and imidazoline binding sites within the vascular wall have been confirmed (Pinthong *et al*., 1995a; Regunathan *et al*., 1995b; 1996b), and agmatine induces a fall in blood pressure in the pithed spontaneously hypertensive rats (SHR; Häuser & Dominiak, 1995; Häuser *et al*., 1995; Schäfer *et al*., 1999a). The agmatine doses required to cause a significant blood pressure reduction ($10-100$ mg kg^{-1}) were, however, extremely high ($\sim$10% of the lethal dose in the experimental model), which contradicts the hypothesis of a specific vasodilatation, and can be considered much more as a non-specific toxic reaction. When one compares agmatine with other vasoactive substances such as bradykinin, noradrenaline or angiotensin II, a 1000–10,000 fold higher dosing is required to bring about the same blood pressure effect (Gao *et al*., 1995). This also speaks against a direct effectiveness of agmatine as a vascular neuromodulator.

It is also plausible, but rather unlikely, that a null effect of agmatine on isolated vessels may represent the sum of a contracting and a dilating effect. Regarding potential interactions with relaxing neurotransmitters, an interaction with NO (Auguet *et al*., 1995; Galea *et al*., 1996; Schwartz *et al*., 1997) could be shown, but not with acetylcholine (Colucci *et al*., 1998) or bradykinin (Gao *et al*., 1995). Concerning interactions with vasoconstrictive neurotransmitters, some evidence exists for angiotensin II (Regunathan & Reis, 1997) and catecholamines (Gonzalez *et al*., 1996; Schäfer *et al*., 1999b; Molderings *et al*., 2000).

At first it was speculated whether agmatine might function as an alternative substrate for endothelial NOS (Ishikawa *et al*., 1995), and in this way contribute towards vasodilatation. This hypothesis was confirmed by the fact that agmatine provokes no relaxation of isolated vessel rings denuded of endothelium or pretreated with L-NAME. Agmatine itself is not a precurser for NO-synthase, but is rather a weak competitive inhibitor of various NOS isoenzymes (Figure 3; Auguet *et al*., 1995; Galea *et al*., 1996). The clearest effect was an inhibition of iNOS, whereby eNOS was maximally inhibited by 60% with 10 mM agmatine (Galea *et al*., 1996). However, even though agmatine has been detected in endothelium (Regunathan *et al*., 1996a), such high concentrations in the millimolar range are rather unlikely *in vivo* (Raasch *et al*., 1995a). Therefore, the relevance of this *in vitro* finding for the *in vivo* situation remains dubious.

The stimulation of presynaptic α_2-adrenoceptors leads to vasodilatation, mediated *via* inhibition of noradrenaline release from sympathetic varicosities (Langer & Hicks, 1984). Agmatine suppresses noradrenergic neurotransmission in rat tail arteries, since it inhibits contraction after transmural nerve stimulation for about 10 min. However, contraction by exogenous noradrenaline was not inhibited, so that the authors concluded a presynaptic effect of agmatine (Gonzalez *et al*., 1996). This was confirmed by dosing with idazoxan or rauwolscine which antagonized the agmatine-mediated effect and resulted even in a delayed potentiation of the contraction response. The unchanged [^{3}H]-noradrenaline uptake into chromaffin cells showed that this hypothetical presynaptic effect was not mediated by an inhibition of noradrenaline reuptake into the sympathetic nerve endings (Gonzalez *et al*., 1996). An inhibition of noradrenaline release by presynaptic imidazoline binding sites with a subsequent vasodilatation could be demonstrated in various vascular preparations and in the pithed SHR (Figure 3; Göthert & Molderings, 1991; Molderings *et al*., 1991; Göthert *et al*., 1995; Molderings & Göthert, 1995; Häuser *et al*., 1995; Raasch *et al*., 1999; Schäfer *et al*., 1999b). Nevertheless, the mechanism by which agmatine modulates noradrenaline release remains unclear: Firstly, it was demonstrated (Schäfer *et al*., 1999b) that agmatine failed to reduce noradrenaline release when α_2-adrenoceptors were blocked reversibly and irreversibly by rauwolscine and phenoxybenzamine, respectively, but not under selective blockade of I_1-binding sites with AGN192403 (Munk *et al*., 1996). These data contradict at least in part (Schäfer *et al*., 1998) the only sparse findings published until now regarding AGN192403 (Munk *et al*., 1996), which have characterized this agent as a high affinity I_1-ligand with no affinity for α_2-adrenoceptors, showing neither agonistic nor antagonistic effects on blood pressure and the sympathetic nervous system. Similar effects of agmatine on noradrenaline release modulated by presynaptic imidazoline binding sites were also demonstrated in various vascular preparations (Molderings & Göthert, 1995). This study suggested that the presynaptic activity of agmatine is probably related to a regulation of noradrenaline release by presynaptic imidazoline binding sites. However, a ganglionic mechanism can not be excluded since (1) I_1-binding sites are present at the cell bodies of sympathetic ganglia and adrenal medulla (Molderings *et al*., 1993); and (2) agmatine blocks nicotinic-cholinergic transmission in sympathetic ganglia (Loring, 1990). Furthermore, since activation of I_1-binding sites releases prostaglandins (Ernsberger *et al*., 1995) and histamine (Molderings *et al*., 1999a), which were both shown to diminish noradrenaline release (Wennmalm & Junstad, 1976; Göthert *et al*., 1999), it also seems likely that agmatine reduces noradrenaline overflow *via* such an indirect mechanism. Secondly, agmatine was suggested to act as an antagonist at the ligand recognition site of the α_{2D}-adrenoceptor and enhances the effects of the α_2-adrenoceptor agonist moxonidine probably by binding to an allosteric binding site on the α_{2D}-adrenoceptor (Molderings *et al*., 2000). However, all those effects (Schäfer *et al*., 1999b; Molderings *et al*., 2000) were obtained with agmatine concentrations in the millimolar range, which generates serious doubts of any *in vivo* relevance. This dual interaction of agmatine at α_{2D}-adrenoceptors probably explains the inconsistent effects of agmatine on catecholamine release,

since data also exists concerning a prosecretory effect on adrenaline and noradrenaline release from chromaffin cells (Li *et al*., 1994a), adrenal medulla (Häuser *et al*., 1995) and vas deferens (Jurkiewicz *et al*., 1996). It is conspicuous, however, that divergent results have also been published for other imidazoline derivatives concerning a catecholamine releasing effect (Regunathan *et al*., 1991a,b; Reis *et al*., 1992; Ohara-Imaizumi & Kumakura, 1992; Steffen & Dominiak, 1996; Jurkiewicz *et al*., 1996). Overall, the inconsistent literature emphasizes that the effects of imidazoline derivatives and especially agmatine on the sympathetic nervous system are complex.

(Effects of agmatine on blood pressure and heart rate *in vivo* (for overview see Tables 3 and 4 and Figure 3)) After intravenous administration, agmatine reduces the blood pressure of anaesthetized SHR or Sprague Dawley rats at a dose of 100 $\mu g\ kg^{-1}$ (Sun *et al*., 1995; Gao *et al*., 1995; Häuser & Dominiak, 1995; Schäfer *et al*., 1999a); although doses of 10 $mg\ kg^{-1}$ and more showed long-lasting blood pressure effects (until 25 min), no reflex tachycardia but rather a significantly reduced heart rate were observed. Compared to pithed SHR, agmatine clearly exerts a higher blood pressure reducing potency in anaesthetized animals (Schäfer *et al*., 1999a). After intravenous application, agmatine exerted no influence on the pressor activity of clonidine. Even though clonidine has an approximately 700 fold higher affinity for I_1-binding sites and a 115–246 fold higher affinity for all three α_2-adrenoceptor subtypes, agmatine even at a 10,000 fold higher dose compared to clonidine had no effect on clonidine-induced blood pressure increases in pithed rats. Hence, antagonistic or CDS-like effects at the α_2-adrenoceptors and I_1-binding sites in the vessel musculature can be excluded. In complete contrast, an additive blood pressure and heart rate reduction could be seen in anaesthetized SHR during continuous clonidine infusion with agmatine doses as low as 100 $\mu g\ kg^{-1}$, which suggests in fact a synergistic effect to clonidine (Schäfer *et al*., 1999a,b).

The lack of agmatine effect on isolated vessels (Gonzalez *et al*., 1996; Pinthong *et al*., 1995a; Schäfer *et al*., 1999a), as well as the markedly lower dose required to produce a comparable reduction of blood pressure in anaesthetized compared to pithed SHR (Schäfer *et al*., 1999a), suggests a central regulation of blood pressure (see Table 4 for an overview). However, no effects of an intracerebroventricular application of agmatine (1–1000 nmol 5 μl^{-1}) on blood pressure and heart rate were observed (Penner & Smyth, 1996). Also, Head *et al*. (1997) saw no increase in heart rate in conscious rabbits, except at high doses (100 $\mu g\ kg^{-1}$) that caused agitation, tachypnoea, increase in blood pressure and a reversal of the dose-dependent bradycardia effect (0.01–10 $\mu g\ kg^{-1}$). These negative findings on blood pressure and heart rate were also confirmed by Sun *et al*. (1995) after an injection of agmatine into the RVLM of anaesthetized rats. In contrast, when agmatine was injected into the greater cisterna of anaesthetized rats (Sun *et al*., 1995) and conscious rabbits (Szabo *et al*., 1995), blood pressure and sympathetic nerve activity increased, indicating a site dependency of central application which might be due to differences in the pattern of imidazoline binding sites and α_2-adrenoceptors with different species or different modes of application. This hypothesis is confirmed by findings of Schäfer *et al*. (1999a), whereby agmatine caused a dose-dependent, significant increase in blood pressure without changing the heart rate after its intracerebroventricular injection to SHR, whereas no change in blood pressure but an increase in heart rate was observed after its injection into the IV ventricle. A comparison between moxonidine and agmatine after injection into the IV-ventricle of conscious rabbits revealed that both substances caused a similar bradycardial effect (Head *et al*., 1997). These effects were attenuated by efaroxan (I_1- and α_2-adrenoceptor antagonist) and 2-methoxy-idazoxan (α_2-adrenoceptor antagonist). From these results Head *et al*. (1997) concluded that agmatine might be an α_2-adrenoceptor agonist. The fact that agmatine exerts no hypotensive effect as other agonists for central α_2-adrenoceptors contradicts this. The authors saw an indication that agmatine possessed a simultaneous hypertensive activity which might mask an α_2-adrenoceptor-induced hypotension. The dual interaction of agmatine on noradrenaline release *via* an allosteric activation or a competitive antagonism at α_{2D}-adrenoceptors (Molderings *et al*., 2000) could probably contribute to this effect and strengthen the hypothesis of Head *et al*. (1997). Both in conscious rabbits and anaesthetized SHR, ventricularly applied agmatine shows no effects on blood pressure after induction of hypotension by clonidine or moxonidine (Head *et al*., 1997; Schäfer *et al*., 1999a), while bradycardia is potentiated by agmatine (Schäfer *et al*., 1999a). If the functional data for CDS and agmatine after central administration are compared, there appears to be absolutely no correlation between the two substances.

Agmatine and its potency on cell growth Since polyamines participate in DNA replication and cellular proliferation (Pegg & McCann, 1982), it was a plausible hypothesis that

Table 4 Blood pressure and heart rate changes after central agmatine-dosing

Species	*Injection site*	*Dose* ($nmol\ kg^{-1}$)	*BP*	*HR*	*Reference*
Conscious rabbit	4. ventricle	0.04–44	→	↘	(Head *et al*., 1997)
Conscious rabbit	4. ventricle	440	↑	↑	(Head *et al*., 1997)
Anaesthetized SDR	greater cistern	132–1320	↗	→	(Szabo *et al*., 1995)
Anaesthetized SDR	intracisternal	300–1200	↑		(Sun *et al*., 1995)
Anaesthetized SDR	RVLM	300–1200	→		(Sun *et al*., 1995)
Anaesthetized SDR	cerebroventricular	1–1000	→		(Penner & Smyth, 1998)
Anaesthetized SHR	cerebroventricular	10–1000	↗	→	(Schäfer *et al*., 1999a)
Anaesthetized SHR	4. ventricle	10–1000	→	↗	(Schäfer *et al*., 1999a)

Abbreviations: BP, blood pressure; HR, heart rate; →: no change; ↗: moderate increase; ↑: increase, ↘: moderate decrease.

agmatine might also be involved in cellular growth processes. Regunathan *et al*. (1996; 1997; 1999) demonstrated a partial inhibition of agmatine (100–1000 μM) on foetal calf serum-stimulated thymidine incorporation in endothelial cells, vascular smooth muscle cells and asterocytes, whereby measurements of lactate dehydrogenase release and morphological examination ruled out a cytotoxic effect being responsible for the inhibition of growth (Regunathan *et al*., 1999). The low potency of agmatine compared to idazoxan regarding proliferation inhibition might be due to its lower affinity towards I_2-binding sites compared to other imadazoline derivatives. However, the manner by which I_2-binding sites (which are associated with MAO and located in mitochondria) should mediate growth effects has not yet been clarified. Due to its rapid metabolism to putrescine (Gilad *et al*., 1996a), it might be speculated whether the antiproliferative effects of agmatine can be attributed to other polyamines. However, putrescine acts as a proliferation promoter, since it doubled thymidine incorporation (Regunathan *et al*., 1996a; Regunathan & Reis, 1997), and would therefore act antagonistically to agmatine. The antiproliferative effects of agmatine and idazoxan in vascular smooth muscle cells could also be observed following other stimulatory conditions such as noradrenaline, angiotensin II or platelet derived growth factor (PDGF; Regunathan & Reis, 1997). Growth factors, especially PDGF, activate primarily membrane receptor-associated tyrosine kinases, which trigger intracellular processes such as activation of protein kinase C (PKC) and various mitogen-activated protein kinases (MAP-kinases), which ultimately result in expression of the immediate-early genes c-*fos* and c-*myc* are involved in DNA-synthesis and cell division (Somlyo & Somlyo, 1994; van Biesen *et al*., 1995). Since both angiotensin II and noradrenaline activate PKC and MAP kinases *via* a G-protein coupled mechanism, it has to be assumed that the antiproliferative effect of agmatine is a mechanism occurring further downstream (Figure 5) since the effects of both G-protein-coupled agents and PDGF (tyrosine kinase mediated) could not be inhibited. It is not known whether this interaction occurs within the cytosolic signal transduction cascade (e.g. Ca^{2+}, PKC, MAP kinase) or at a transcriptional level. Satriano *et al*. (1998) also showed that agmatine suppresses growth of MTC-cells (mouse kidney proximal tubule cells) by thymidine incorporation experiments, an effect that could be antagonized by putrescine. The authors, however, pursued a completely different hypothesis to explain the agmatine-mediated cell growth, which was based on the following findings: (1) Agmatine time-dependently reduced the intracellular content of putrescine and spermidine. (2) Agmatine was not converted intracellularly to polyamines (in MTC cells). (3) Exogenous agmatine reduced ODC activity dose- and time-dependently in MTC cells, where even high agmatine concentrations (1 mM) exert no cytotoxic effects; the specificity of this effect could also be seen with other cell lines, and such findings were also confirmed by Vargiu *et al*. (1999). (4) Agmatine (1 mM) time-dependently suppressed the polyamine transporter in MTC cells, and this results in a reduction of the intracellular content (c. 15% of control values) of exogenously applied [^{3}H]-putrescine. This result could also be confirmed by others, where it was shown that intracellular spermidine content in rat hepatocytes can be reduced by an agmatine-induced inhibition of uptake (Vargiu

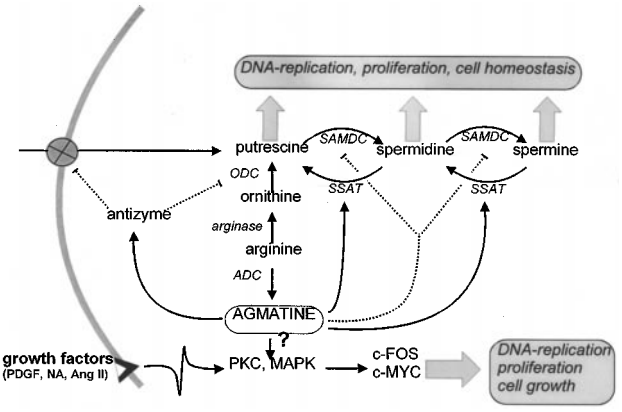

Figure 5 Influence of agmatine on cell growth: Control of cell growth can be attributed to two different pathways, namely a membrane receptor controlled pathway, and a pathway dependent on cellular polyamine content. Putrescine content is regulated by an active tranport mechanism as well as by arginine metabolism. Agmatine acts by stimulating an antizyme that inhibits both processes, so that a reduction in putrescine content occurs which contributes to an antiproliferative activity for agmatine. Agmatine also stimulates the spermidine/spermine acetyltransferase (SSAT), the key rate-limiting enzyme for polyamine intraconversion, and simultaneously inhibits S-adenosylmethionine decarboxylase (SAMDC), an enzyme which also has a modulating effect on intracellular polyamine content. Growth factors also stimulate membrane-located receptors. Following stimulation of protein kinase C (PKC) and MAP kinase (MAPK), expression of c-FOS and c-MYC occurs which eventually leads to DNA replication, cell proliferation and cell growth. Whether agmatine exerts an influence on this signal cascade has not yet been established.

et al., 1999). (5) Agmatine induced antizyme which regulates the synthesis and transport of polyamines. This was established by (a) an agmatine-dependent translational frame-shift of antizyme mRNA to produce a full-length protein and (b) a suppression of agmatine-dependent inhibitory activity by either anti-antizyme IgG or antizyme inhibitor. Satriano *et al*. (1998) therefore deduced the following hypothesis (Figure 4): the intracellular polyamine content, which plays an important role in DNA-replication and cell division, is regulated either by endogenous cellular biosynthesis from arginine to ornithine and further on under ODC catalysis to putrescine, or by the uptake of exogenous polyamines. Both the membrane-located polyamine transporter as well as ODC are subject to regulation by antizyme, whereby this protein exerts an inhibitory effect on both proteins. Agmatine acts as a regulator of antizyme, which means that agmatine is able to control synthesis.

A third hypothesis for agmatine's antiproliferative effects is that agmatine regulates the interconversion pathway of polyamine metabolism at the level of the rate-limiting enzyme (Figure 5). In hepatocyte cultures agmatine (0.5 mM) inhibits cell growth and increases the activity of the spermidine/spermine acetyltransferase (SSAT) about 10–25 fold in a manner dependent on oxygen saturation (5 vs 21%). This result was confirmed at the protein level, i.e. the increase in activity was due to an increased expression of SSAT (Vargiu *et al*., 1999). The content of putrescine simultaneously increases in line with reductions in spermine and spermidine, whereby putrescine-accumulation was considered to be responsible for the reduced ODC activity, since it is also known that the biosynthesis of putrescine is regulated *via* a

negative feedback mechanism (Persson *et al*., 1986). The observed increase in activity of *S*-adenosylmethionine decarboxylase (SAMDC), which catalyses the breakdown of putrescine to spermidine and spermine, appears to be a consequence of the increased intracellular putrescine content. The authors attributed the observed inhibition of cell growth mainly to the depletion of spermidine and spermine. However, since cellular putrescine levels increase concomitantly, it seems somehow unclear whether cell growth should be inhibited, since putrescine causes the opposite effects (Regunathan *et al*., 1996a; Regunathan & Reis, 1997). Moreover, Vargiu *et al*. (1999) attributed the inhibition of cell growth solely to an agmatine-induced modification of putrescine metabolism. However, they ignored the observation that total polyamine content in cells increased by 40% depending on the agmatine concentration, which would indicate an alteration in uptake or biosynthesis, although both were in fact shown to be reduced (Satriano *et al*., 1998; Vargiu *et al*., 1999). Some questions, therefore, remain to be answered.

Taken together, all three hypotheses failed more or less to explain whether agmatine's mediated growth effects would be mediated *via* imidazoline binding sites, a fact which would strengthen the hypothesis of an endogenous ligand. In case of a growth control of agmatine *via* a feedback regulation of enzyme activity, as shown for ODC (Vargiu *et al*., 1999), agmatine would indeed be biologically active, possibly as an intermediate in the metabolism of arginine and ornithine to the polyamines, but it would not be a compulsory endogenous ligand for imidazoline binding sites. Another limitation of all three hypotheses is the fact that agmatine inhibits growth at concentrations (0.1 – 1 mM) which although non-cytotoxic (Vargiu *et al*., 1999), are much higher than circulating agmatine and even tissue concentrations. Therefore, the biological significance of this agmatine effect remains uncertain.

Effects of agmatine on renal function Renal sodium regulation is important both under normal and pathological conditions for circulatory regulation. A range of different central and peripheral parameters influence sodium excretion and reabsorption. The role of α-adrenoceptors in this context has been demonstrated (Michel & Rump, 1996). Radioligand binding studies succeeded in demonstrating both α_2-adrenoceptors as well as imidazoline binding sites, where different distributions could be shown within the kidney (Coupry *et al*., 1990; Evans & Haynes, 1995; Ernsberger *et al*., 1995). Moxonidine studies suggested a functional participation of imidazoline binding sites in natriuresis (Allan *et al*., 1993; 1996), but it is not yet clear whether moxonidine influences sodium excretion and urinary flow *via* a central and/or peripheral mechanism (reviewed by Smyth & Penner, 1999). In this context the question arises as to the renal function of agmatine, especially considering its demonstration in both brain and kidney (Raasch *et al*., 1995a; Lortie *et al*., 1996).

At concentrations that induce no changes in haemodynamics or creatinine clearance, agmatine as well as the I_1-ligands moxonidine, clonidine and 2,6-dimethyl-clonidine increase urinary flow-rate, whereby agmatine first shows significant changes at ~10 fold higher doses (Allan *et al*., 1993; Li *et al*., 1994b; Ernsberger *et al*., 1995; Penner & Smyth, 1996). The increase in urinary flow under moxonidine, 2,6-dimethylclonidine and agmatine results from an increased osmotic clearance, while under clonidine it is due to an increased clearance of free water, consistent with the imidazoline-mediated inhibition of the Na^+/H^+-exchange in isolated renal tubular cells (Bidet *et al*., 1990). This suggests that the osmotic clearance is possibly an imidazoline binding site-mediated effect, but that free water clearance might be due to an α_2-receptor-related mechanism (Smyth & Penner, 1995), since compared to clonidine the relative affinities of agmatine, moxonidine and dimethyl clonidine are higher for imidazoline binding sites than they are for α_2-adrenoceptors (Ernsberger *et al*., 1995).

In the perfused isolated kidneys of Wistar-Frömter rats, agmatine dose-dependently and significantly increases the single nephron glomerular filtration rate (SNGFR) and the absolute proximal reabsorption (APR), after application into the renal interstitium and the urinary space of surface glomeruli. In denervated kidneys microperfused in exactly the same way, the SNGFR, but not the APR, was increased after agmatine application. The agmatine effects were transient, possibly due to metabolization by DAO (Lortie *et al*., 1996). While the I_2-ligand BU-224 exerts a similar effect to agmatine, moxonidine was ineffective (Lortie *et al*., 1996). The effects of the synthetic and DAO-stable I_2-ligand BU-224 were, however, longer lasting, which corresponds well with the reversible activity of agmatine and its degradation in the kidney. Because of the application of agmatine into the urinary space, one can assume that agmatine increases the SNGFR due to an effect on the glomerulus as well as the associated vessels. The negative results of agmatine on isolated vessels (Pinthong *et al*., 1995a; Schäfer *et al*., 1999a), however, contradict such a participation. The simultaneous increase in APR could be directly mediated, a supposition supported by the finding that agmatine increases the Na^+/K^+ ATPase in renal membranes (Bidet *et al*., 1990). The fact that agmatine increases SNGFR after denervation to the same extent as that seen in innervated kidneys, excludes the possibility of a presynaptic mechanism. In contrast to innervated kidneys, the APR in denervated kidneys was not raised after agmatine, which allows us to conclude either (1) that the glomerular and tubular effects of agmatine are mediated by different mechanisms; or (2) that from denervation and the resulting removal of presynaptic α_2-adrenoceptors, the glomerular balance as well as the APR response to agmatine are destroyed. It has also been shown that the agmatine-induced increase in glomerular filtration might be mediated *via* a NO-synthase-dependent mechanism (Schwartz *et al*., 1997).

Metabolic effects of agmatine It has long been known that agmatine increases insulin release from rat pancreatic islets of Langerhans cells (Alberti *et al*., 1973; Sener *et al*., 1989) glucose uptake into the isolated rat respiratory diaphragm (Frank, 1927; Weitzel *et al*., 1971; 1972a,b), glucose oxidation in isolated fat cells, and the glycogen content in the respiratory diaphragm (Weitzel *et al*., 1971; 1972a,b). In all these cases the agmatine effect appeared to be receptor-independent. Since those studies, however, the existence of a specific imidazoline binding site has been demonstrated in the pancreas (Schulz & Hasselblatt, 1989a,b), where the imidazoline binding sites located on the pancreatic *β*-cells is not identical to either of the known I_1- and I_2 binding sites

(Figures 1 and 2; Chan *et al*., 1994; 1995; Morgan *et al*., 1995; Tsoli *et al*., 1995) or the binding site for sulphonylurea derivatives (Brown *et al*., 1993b; Ishida-Takahashi *et al*., 1996; Rustenbeck *et al*., 1997), even though I_2-binding sites are expressed abundantly (Brown *et al*., 1993a). However, the archetypal I_2-ligand idazoxan is not an insulin secretagogue and displays negligible antagonist activity towards other imidazoline secretagogues. Equally, there is no convincing evidence that I_1-binding sites are involved in insulin secretion, especially since few if any I_1-binding sites were detected on pancreatic β-cells (Brown *et al*., 1993a; Rustenbeck *et al*., 1997). For this reason, the existence of a pancreas specific I_3-binding site was postulated (Morgan *et al*., 1999). It could also be shown that imadazoline derivatives such as clonidine or moxonidine inhibit insulin release from isolated pancreatic cells (Langer *et al*., 1983; Hillaire-Buys *et al*., 1985; Skoglund *et al*., 1988; Tsoli *et al*., 1995), although efaroxan or phentolamine increase insulin release by influencing the K-ATP channels (Figure 2; Chan & Morgan, 1990; Chan *et al*., 1991; Plant & Henquin, 1990; Jonas *et al*., 1992; Berdeu *et al*., 1994) and exert vasoconstrictive effects on isolated perfused rat pancreas (Berdeu *et al*., 1994). Considering that CDS and even imadazoline derivatives seem to possess insulin pro-secretory activity, one might ask whether agmatine possesses insulin regulatory activity.

Under a slightly stimulating glucose concentration (8.3 mM) and unchanged pancreatic flow rate, agmatine (0.1 – 3 mM) induced a moderate increase in insulin secretion. In comparable studies, efaroxan was substantially more effective, also with combined stimulation by arginine (10 mM) and glucose (5 mM; Berdeu *et al*., 1996). Unlike efaroxan (0.1 mM), even the highest concentration of agmatine (3 mM) was without effect on the vascular flow rate in isolated pancreas. The lacking insulin secretion under glucose/arginine stimulation and the moderate release of insulin under higher glucose concentrations allow us to presume different mechanisms of release governed by agmatine and efaroxan. Hence, the hypothesis was forwarded that the secretolytic effect of agmatine (unlike efaroxan) is not mediated *via* imidazoline binding sites, as previously suggested by Weitzel *et al*. (1971; 1972), but rather by metabolic products. Polyamines in particular are suggested to mediate agmatine-mediated insulin secretion, assuming that agmatine is subject to uptake into β-cells as insinuated by Sastre *et al*. (1997) and Molderings *et al*. (2001) for neuronal tissue. The polyamines themselves possess no affinity towards imidazoline binding sites but were shown to be involved in regulation and proliferation of pancreatic β-cells and hormone production by insulin-secreting cells (Figure 2; Sjoholm, 1993). From the lack of secretolytic and vasopressive activities of agmatine, consistent with the findings of other authors (Piletz *et al*., 1995), Berdeu *et al*. (1996) concluded that agmatine cannot function as the endogenous ligand at the imidazoline binding sites of the pancreas or blood vessels.

Agmatine and its function in the gastrointestinal tract The following facts suggest a potential role of agmatine in the gastrointestinal tract: (1) Imidazoline binding sites have been demonstrated in the stomachs of various animal species, identified as I_2- or non I_1-/non I_2 binding sites (Houi *et al*., 1987; Tesson *et al*., 1992; Molderings *et al*., 1995; 1998b; 1999c); (2) Clonidine and clonidine-like substances at high concentrations stimulate gastric acid secretion (Houi *et al*., 1987; Medgett & McCulloch, 1979; del tacca *et al*., 1982a,b; Bhandare *et al*., 1991). However, clonidine may exert this effect by modulating vagal cholinergic stimulation *via* α_2-adrenoceptors (Blandizzi *et al*., 1990a,b; 1995); (3) In isolated organ preparations of the gastric fundus, CDS leads to a concentration-dependent contraction, due to an interaction with imidazoline binding sites (Felsen *et al*., 1987); (4) Stomach, small and large intestine have been characterized as the organs with the highest agmatine tissue concentrations (Raasch *et al*., 1995a,b). However, since ADC has not been yet been demonstrated in gastric fundus or the intestine, there remain some doubts as to whether agmatine in fact originates from arginine metabolism. Indeed, it may originate from bacterial colonization or food intake after adsorption by a transporter (which also remains to be identified in gastric tissue).

Glavin *et al*. (1995) were the first to show that agmatine possesses pro-secretory and ulcerogenic activities, since it increases the secretion of gastric acid and pepsin, reduces mucus thickness, and exacerbates stress-induced mucosal lesions (Table 5). Hence, it appears that agmatine-mediated effects are contrary to those mediated by moxonidine (Glavin & Smyth, 1995a,b), consistent with the differing activities of moxonidine and agmatine on peristalsis (Table 5; Liu & Coupar, 1997). Since agmatine has been characterized as an endogenous ligand for imidazoline binding sites, one should actually expect agonistic effects such as those seen for moxonidine. Since this is not the case, different mechanisms are probably inferred. For this reason, an inverse agonism for agmatine at the imidazoline binding

Table 5 Effects of agmatine on gastric function

Effects on		*Dosage*	*Species/tissue*	*Reference*
Gastric acid secretion	↑	1 – 20 mg kg^{-1} i.p.	pylorus ligated rat	(Glavin *et al*., 1995)
Gastric acid secretion	↑	0.5 – 10 kg^{-1} i.p	conscious rat	(Glavin *et al*., 1995)
Secretion volume	↑	0.1 – 20 kg^{-1} i.p.	pylorus ligated rat	(Glavin *et al*., 1995)
Pepsin secretion	↑	0.1 – 20 kg^{-1} i.p.	pylorus ligated rat	(Glavin *et al*., 1995)
Adherent mucus	↓	10 – 20 kg^{-1} i.p.	restraint stress rat	(Glavin *et al*., 1995)
Stress-induced gastric mucosal injury	↑	10 – 20 kg^{-1} i.p.	conscious rat	(Glavin *et al*., 1995)
Peristalsis	→	0.01 – 100 μM	rat ileum	(Liu & Coupar, 1997)
Fluid transport rate	↓	0.3 – 1 μmol min^{-1}	rat ileum and jejunum	(Liu & Coupar, 1997)
Histamine release from ELC	↑	100 – 1000 μM	rat gastric strips	(Molderings *et al*., 1999a,d)
Agmatine content in gastric juice	↑		*H. pylori* pos. patients	(Molderings *et al*., 1999c)

Abbreviations: ELC: enterochromaffin like cells; *H. pylori*: *Helicobacter pylori*; →: no change; ↑: increase; ↓ decrease.

sites has been suggested (Glavin *et al*., 1995). Secondly, it seems feasible that moxonidine-like substances stimulate gastric acid secretion *via* α_2-adrenoceptors (Daly, 1984), whereas agmatine acts at imidazoline binding sites of the non I_1-/non I_2 subtype (Molderings *et al*., 1998b; 1999c). Against this background it has been purported that imadazoline derivatives do not stimulate acid secretion directly, but rather indirectly, i.e. by altering endogenous histamine release from enterochromaffin-like cells (Table 5; Molderings *et al*., 1999a). This is further supported by the facts that imidazoline-induced acid secretion (Molderings *et al*., 1999c) can be blocked by an H_2-receptor antagonist (Houi *et al*., 1987), and that polyamines can regulate histamine release (Purcell *et al*., 1994). That prostaglandins contribute to this indirect agmatine effect on gastric function seems rather unlikely, since (1) an activation of I_1-binding sites releases prostaglandins (Ernsberger *et al*., 1995); (2) I_1-binding sites were not determined in stomach (Molderings *et al*., 1999c); and (3) prostaglandins decrease gastric acid release and enhance mucus thickness, which are opposite to agmatine's effects (Glavin *et al*., 1995).

Whether agmatine reveals gastric functions as an endogenous neurotransmitter is uncertain, especially since *Helicobacter pylori* has been shown to generate and release agmatine (Molderings *et al*., 1999d). Moreover, *H. pylori* expresses ADC and contains adequate arginine (Tomb *et al*., 1997). Therefore, it seems reasonable to hypothesize that agmatine may be responsible for stimulating stomach acid release during gastrointestinal *H. pylori* colonization, especially since *H. pylori*-induced acid release is not yet completely understood (el Omar *et al*., 1995; Courillon-Mallet *et al*., 1995; Sobhani *et al*., 1996). More recently it was shown that the growth of *H. pylori* is dependent on agmatine concentrations in the culture medium and that cell lines from different stomach biopsies synthesize different quantities of agmatine (Molderings *et al*., 1999d). Furthermore, the agmatine content in the gastric juice of *H. pylori* positive patients (83.5 ± 18.7 ng ml^{-1}) is double that of gastric juice isolated from *H. pylori* negative patients (46.8 ± 18.7 ng ml^{-1}; Molderings *et al*., 1999d, Table 5). Considering the mechanism of an agmatine-induced secretion of gastric acid, a route *via* the stimulated release of histamine from enterochromaffin cells seems the most probable. It can not, however, be ruled out that an increased gastrin secretion leads to the increased quantity of acid, particularly since *H. pylori* infection is associated with a raised plasma concentration of gastrin (Peterson, 1996; Molderings *et al*., 1999a). However, since there is no correlation between plasma gastrin concentration and agmatine concentration in gastric juice, this hypothesis appears doubtful according to our current state of knowledge. The significance of agmatine in the gastric juice of *H. pylori* negative patients remains unclear. Whether agmatine is subject to degradation to putrescine in both the gastric juice and fundus itself has not yet been demonstrated. In *H. pylori* positive patients, however, an increased putrescine and spermidine content could be shown in biopsies, supporting the hypothesis that agmatine can contribute to the carcinogenic actions of *H. pylori* by this route, since polyamines have been attributed an important role in cell proliferation and carcinogenesis (Berdinskikh *et al*., 1991; Leveque *et al*., 1998; Blachier *et al*., 1995).

Is agmatine a CDS?

As the preceding paragraphs have described, agmatine can be characterized as a substance synthesized by an ADC in mammals (Li *et al*., 1994a), which is heterogeneously distributed in various organs (Raasch *et al*., 1995a) and subcellular compartments (Otake *et al*., 1998), released (Reis & Regunathan, 1998) and inactivated by reuptake (Sastre *et al*., 1997; Molderings *et al*., 2001) or metabolism *via* agmatinase (Sastre *et al*., 1996b) or DAO (Holt & Baker, 1995). Moreover, effects on various organ systems have been shown, even though very high agmatine concentrations were needed to provoke various biological effects. Now we are faced with the question as to whether agmatine represents the active principle in the CDS isolated by Atlas (Atlas & Burstein, 1984a,b; Atlas *et al*., 1987).

Comparison between CDS and agmatine regarding their molecular and physiological properties (overview in Tables 1 and 2)

A molecular mass of 587 daltons has been determined for CDS (Atlas & Burstein, 1984a,b; Atlas *et al*., 1987). Its UV spectrum shows two absorption maxima at 224 and 276 nm, which suggests an aromatic structure. A ninhydrin-negative reaction excludes the possibility that it contains an amino group. The physicochemical properties of agmatine on the other hand seem to contradict those of CDS completely, i.e. a molecular weight of 130 daltons, an absorption maximum of 200 nm (suggesting an aliphatic structure), as well as a ninhydrin-positive reaction confirming an amine structure. These chemical differences alone, combined with the different abundance in the brain and the widely differing affinities for imidazoline binding sites and α_2-adrenoceptors, have already lead Atlas (1994; 1995) to declare that agmatine and CDS are not identical. Piletz *et al*. (1995) and Pinthong *et al*. (1995b) made similar arguments, when they demonstrated differing affinity profiles for agmatine and CDS to imidazoline binding sites and α_2-adrenoceptors by radioligand binding studies and contradicted the findings of Li *et al*. (1994a). In the response from Reis *et al*. (1994) published in Science to Atlas's publication (Atlas, 1994), the authors quoted various, primarily methodological and preparative reasons to explain the physicochemical inconsistencies. In addition, the authors emphasized once again that agmatine does not represent CDS exclusively, but that CDS might represent a family of substances, of which agmatine is only one member. This was quite deliberately expressed in the title of their publication, in which the authors talked of agmatine as 'one' and not 'the' CDS.

Since 1994, numerous papers have been published which have concerned themselves with the physiological and/or pathophysiological functions of agmatine and discussed the hypothesis that agmatine might be a neurotransmitter or neuromodulator (see Table 2, Figure 3). However, the following findings and considerations contradict this hypothesis: (1) Findings with agmatine against CDS are in some cases completely contradictory to those published for CDS. This applies particularly to the cardiovascular system with respect to agmatine's effects on isolated vessels (Table 4) and after its i.c.v. application (Table 5). After central application,

CDS shows clear, blood pressure-increasing effects and acts antagonistically to clonidine (Bousquet *et al*., 1986; 1987; Pinthong *et al*., 1995b). Central agmatine dosing leads on the other hand to hypertensive effects, whereby individual findings from different publications appear to be inconsistent, and as mentioned above, such discrepancies are usually attributed to the use of different species and/or sites of application (Sun *et al*., 1995; Szabo *et al*., 1995; Penner & Smyth, 1996; Head *et al*., 1997; Schäfer *et al*., 1999a). Unlike clonidine and CDS (Synetos *et al*., 1991), at most weak and usually no effects could be shown in isolated organs (Pinthong *et al*., 1995a; Gonzalez *et al*., 1996; Schäfer *et al*., 1999a). A clonidine-antagonistic activity could not be demonstrated. After peripheral agmatine-application, a hypotensive effect was even observed, albeit at extremely high and non-physiological concentrations (Gao *et al*., 1995; Schäfer *et al*., 1999a). In addition to the cardiovascular actions, contrary effects between CDS and agmatine have been determined for catecholamine and insulin secretion which predispose a negation of a CDS-like activity for agmatine. Clearer effects of agmatine could be shown for gastrointestinal (Table 5) and renal functions, analgesia, as well as cell growth (Figure 5), whereby CDS was not investigated for its ability to modulate all these functions. (2) From a mechanistic viewpoint there is inconsistency regarding the correlation between agmatine's function on various biological systems (e.g. growth effects, insulin release; see Figures 2 and 5) and its mediation *via* imidazoline binding sites. Although biological effects were indeed observed, they were rather attributed to polyamines derived from the metabolic breakdown of agmatine. This contradicts a function as an endogenous ligand for imidazoline binding sites. (3) Another important objection is the distribution pattern of agmatine in various organs (Raasch *et al*., 1995a) which differs widely from that of CDS (Meeley *et al*., 1992, see Table 1). As an example, the highest concentrations of CDS are found in the adrenal gland where only low concentrations of agmatine exist. Moreover, the low correlation ($r=0.2193$) between agmatine and CDS tissue levels in both studies clearly indicates that agmatine can not exclusively represent CDS, but that it represents rather a member of a whole CDS family. In addition, since agmatine is not recognized by the CDS-antibody (Wang *et al*., 1997), irCDS can not be agmatine. If one considers that cCDS is similar to irCDS (Meeley *et al*., 1992), one therefore has to conclude that CDS is not agmatine. Whether antibodies against agmatine (Wang *et al*., 1995) recognize CDS has not been investigated until now. (4) The most serious doubt as to whether agmatine is a CDS is that all *in vitro* and *in vivo* reactions of agmatine, as described in preceding sections, only occurred at heroic concentrations, usually much higher than those endogenously present in the corresponding tissues. For example, the agmatine doses (i.v.) required to cause a significant blood pressure reduction were approximately 10% of the lethal dose in the experimental model. Furthermore, when one compares agmatine with other vasoactive substances such as bradykinin, noradrenaline or angiotensin II, only a 1000–10,000 fold higher dosage brings about the same blood pressure effect (Gao *et al*., 1995), which excludes agmatine as being an endogenous neuromodulator or neurotransmitter. The extremely high concentrations of agmatine are probably necessary because of its moderate affinity in the micromolar range towards I_1- and I_2-binding sites as well as towards α_2-adrenoceptors. This differs grossly (25–330 fold) from the affinities of CDS observed at the same binding sites and receptors.

Identification of other CDS's from the CDS family

Because of the fact that agmatine appears in the majority of publications, and that it is seen as a substance within a whole CDS family, we should now ask whether other substances with CDS similar properties have been identified.

Arginine is known to be metabolized by NOS to NO and citrulline, whereby NO acts as a potent vasodilator. As an alternative to this route it could be shown that arginine can be degraded to hydroxyarginine, and that this can also be degraded to NO in a second step, whereby hydroxyarginine itself has relaxant properties (Zembowicz *et al*., 1991; 1992a,b,c). As an analogue to this metabolic path, it has also been shown that the hydroxylation product of agmatine, just like agmatine itself, is able to dilate aortal rings, whereby no relaxing effect could be seen following preincubation with a NOS inhibitor (L-NAME) or denudation of the epithelium (Ishikawa *et al*., 1995). Such vasoactive properties of agmatine, however, contradict studies where no relaxing effects for agmatine have been shown. From these observations the authors concluded that agmatine and hydroxyagmatine represent substrates for NOS and in this way possess endothelium-dependent relaxing activity. However, until now neither the existence of this substance nor the metabolic path itself have been confirmed in the tissue. These findings also contradict those of others (Galea *et al*., 1996) who characterized agmatine as an inhibitor rather than a substrate for NOS.

Modified protocols for CDS preparations have resulted in the isolation of another 'clonidine-displacing substance', for which it has not yet been definitely clarified whether it is different or identical to the substance described by Atlas (Atlas & Burstein, 1984a,b; Atlas *et al*., 1987). Spectroscopic studies and HPLC have shown that this isolated CDS is not noradrenaline, adrenaline, histamine, agmatine, guanosine, GMP, GDP or GDP, but that it represents a guanosine derivative (Grigg *et al*., 1998; Parker *et al*., 1999a,b). Functional studies and binding data, however, remain to be performed.

To this extent there is some evidence that CDS might comprise a family of structurally and functionally similar substances, of which agmatine is one, but specific evidence, especially at a functional level, is outstanding for other CDSs.

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References

AIRIO, J. & AHTEE, L. (1999). The involvement of noradrenergic transmission in the morphine-induced locomotor hyperactivity in mice withdrawn from repeated morphine treatment. *Br. J. Pharmacol.,* **126,** 1609 – 1619.

ALBERTI, K., WOODS, H. & WHALLEY, M. (1973). Mechanism of action of the monoguanidine hypoglaemic agents, galegine and agmatine. *Eur. J. Clin. Invest.,* **3,** 208.

ALEMANY, R., OLMOS, G., ESCRIBA, P.V., MENARGUES, A., OBACH, R. & GARCIA-SEVILLA, J.A. (1995). LSL 60101, a selective ligand for imidazoline I2 receptors, on glial fibrillary acidic protein concentration. *Eur. J. Pharmacol.,* **280,** 205 – 210.

ALLAN, D.R., PENNER, S.B. & SMYTH, D.D. (1993). Renal imidazoline preferring sites and solute excretion in the rat. *Br. J. Pharmacol.,* **108,** 870 – 875.

ALLAN, D.R., PENNER, S.B. & SMYTH, D.D. (1996). Antagonism by idazoxan at low dose but not high dose, of the natriuretic action of moxonidine. *Br. J. Pharmacol.,* **117,** 29 – 34.

ALTMAN, J.D., TRENDELENBURG, A.U., MACMILLAN, L., BERNSTEIN, D., LIMBIRD, L., STARKE, K., KOBILKA, B.K. & HEIN, L. (1999). Abnormal regulation of the sympathetic nervous system in alpha2A- adrenergic receptor knockout mice. *Mol. Pharmacol.,* **56,** 154 – 161.

ARICIOGLU-KARTAL, F. & UZBAY, I.T. (1997). Inhibitory effect of agmatine on naloxone-precipitated abstinence syndrome in morphine dependent rats. *Life Sci.,* **61,** 1775 – 1781.

ATLAS, D. (1991). Clonidine-displacing substance (CDS) and its putative imidazoline receptor. New leads for further divergence of alpha 2-adrenergic receptor activity. *Biochem. Pharmacol.,* **41,** 1541 – 1549.

ATLAS, D. (1994). Identifying clonidine-displacing substance. *Science,* **266,** 462 – 464.

ATLAS, D. (1995). Molecular and physiological properties of clonidine-displacing substance. *Ann. N.Y. Acad. Sci.,* **763,** 314 – 324.

ATLAS, D. & BURSTEIN, Y. (1984a). Isolation and partial purification of a clonidine-displacing endogenous brain substance. *Eur. J. Biochem.,* **144,** 287 – 293.

ATLAS, D. & BURSTEIN, Y. (1984b). Isolation of an endogenous clonidine-displacing substance from rat brain. *FEBS Lett.,* **170,** 387 – 390.

ATLAS, D., DIAMANT, S., FALES, H.M. & PANNELL, L. (1987). The brain's own clonidine: purification and characterization of endogenous clonidine displacing substance from brain. *J. Cardiovasc. Pharmacol.,* **10** (Suppl 12): S122 – S127.

AUGUET, M., VIOSSAT, I., MARIN, J.G. & CHABRIER, P.E. (1995). Selective inhibition of inducible nitric oxide synthase by agmatine. *Jpn. J. Pharmacol.,* **69,** 285 – 287.

BERDEU, D., GROSS, R., RIBES, G., LOUBATIERES-MARIANI, M.M. & BERTRAND, G. (1994). Effects of imidazolines and derivatives on insulin secretion and vascular resistance in perfused rat pancreas. *Eur. J. Pharmacol.,* **254,** 119 – 125.

BERDEU, D., PUECH, R., LOUBATIERES-MARIANI, M.M. & BERTRAND, G. (1996). Agmatine is not a good candidate as endogenous ligand for imidazoline sites of pancreatic B cells and vascular bed. *Eur. J. Pharmacol.,* **308,** 301 – 304.

BERDINSKIKH, N.K., IGNATENKO, N.A., ZALETOK, S.P., GANINA, K.P. & CHORNIY, V.A. (1991). Ornithine decarboxylase activity and polyamine content in adenocarcinomas of human stomach and large intestine. *Int. J. Cancer,* **47,** 496 – 498.

BERENDSE, H.W. & GROENEWEGEN, H.J. (1991). Restricted cortical termination fields of the midline and intralaminar thalamic nuclei in the rat. *Neuroscience,* **42,** 73 – 102.

BHANDARE, P.N., RATABOLI, P.V. & D'SOUZA, R.S. (1991). Dual action of clonidine on ethanol-induced gastric lesions: is the imidazoline-preferring receptor involved? *Eur. J. Pharmacol.,* **199,** 243 – 245.

BIDET, M., TAUC, M., KOECHLIN, N. & POUJEOL, P. (1990). Video microscopy of intracellular pH in primary cultures of rabbit proximal and early distal tubules. *Pflugers Arch.,* **416,** 270 – 280.

BLACHIER, F., SELAMNIA, M., ROBERT, V., M'RABET-TOUIL, H. & DUEE, P.H. (1995). Metabolism of L-arginine through polyamine and nitric oxide synthase pathways in proliferative or differentiated human colon carcinoma cells. *Biochim. Biophys. Acta.,* **1268,** 255 – 262.

BLANDIZZI, C., BERNARDINI, M.C., NATALE, G. & DEL TACCA, M. (1990a). Alpha 2-adrenoceptor-mediated inhibitory and excitatory effects of detomidine on rat gastric acid secretion. *J. Pharm. Pharmacol.,* **42,** 685 – 688.

BLANDIZZI, C., BERNARDINI, M.C., VIZI, E.S. & DEL TACCA, M. (1990b). Modulation of gastric acid secretion by peripheral presynaptic alpha 2- adrenoceptors at both sympathetic and parasympathetic pathways. *J. Auton. Pharmacol.,* **10,** 305 – 312.

BLANDIZZI, C., NATALE, G., COLUCCI, R., CARIGNANI, D., LAZZERI, G. & DEL TACCA, M. (1995). Characterization of alpha 2-adrenoceptor subtypes involved in the modulation of gastric acid secretion. *Eur. J. Pharmacol.,* **278,** 179 – 182.

BLASCHKO, H. (1974). The natural history of amine oxidases. *Rev. Physiol. Biochem. Pharmacol.,* **70,** 83 – 148.

BOCK, C., NIEDERHOFFER, N. & SZABO, B. (1999). Analysis of the receptor involved in the central hypotensive effect of rilmenidine and moxonidine. *Naunyn Schmiedebergs Arch. Pharmacol.,* **359,** 262 – 271.

BORONAT, M.A., OLMOS, G. & GARCIA-SEVILLA, J.A. (1998). Attenuation of tolerance to opioid-induced antinociception and protection against morphine-induced decrease of neurofilament proteins by idazoxan and other 12-imidazoline ligands. *Br. J. Pharmacol.,* **125,** 175 – 185.

BOUSQUET, P., FELDMAN, J. & ATLAS, D. (1986). An endogenous, non-catecholamine clonidine antagonist increases mean arterial blood pressure. *Eur. J. Pharmacol.,* **124,** 167 – 170.

BOUSQUET, P., FELDMAN, J. & ATLAS, D. (1987). Central cardiovascular effects of a noncatecholamine endogenous ligand for clonidine receptors. *J. Cardiovasc. Pharmacol.,* **10** (Suppl 12): S167 – S171.

BOUSQUET, P., FELDMAN, J. & SCHWARTZ, J. (1984). Central cardiovascular effects of alpha adrenergic drugs: differences between catecholamines and imidazolines. *J. Pharmacol. Exp. Ther.,* **230,** 232 – 236.

BRADLEY, K.J. & HEADLEY, P.M. (1997). Effect of agmatine on spinal nociceptive reflexes: lack of interaction with alpha2-adrenoceptor or mu-opioid receptor mechanisms. *Eur. J. Pharmacol.,* **331,** 133 – 138.

BRICCA, G., DONTENWILL, M., MOLINES, A., FELDMAN, J., BELCOURT, A. & BOUSQUET, P. (1989). The imidazoline preferring receptor: binding studies in bovine, rat and human brainstem. *Eur. J. Pharmacol.,* **162,** 1 – 9.

BRICCA, G., ZHANG, J., GRENEY, H., DONTENWILL, M., STUTZMANN, J., BELCOURT, A. & BOUSQUET, P. (1993). Relevance of the use of [3H]-clonidine to identify imidazoline receptors in the rabbit brainstem. *Br. J. Pharmacol.,* **110,** 1537 – 1543.

BROWN, C.A., CHAN, S.L., STILLINGS, M.R., SMITH, S.A. & MORGAN, N.G. (1993b). Antagonism of the stimulatory effects of efaroxan and glibenclamide in rat pancreatic islets by the imidazoline, RX801080. *Br. J. Pharmacol.,* **110,** 1017 – 1022.

BROWN, C.A., LOWETH, A.C., SMITH, S.A. & MORGAN, N.G. (1993a). Stimulation of insulin secretion by imidazoline compounds is not due to interaction with non-adrenoceptor idazoxan binding sites. *Br. J. Pharmacol.,* **108,** 312 – 317.

BROWN, C.M., MACKINNON, A.C., MCGRATH, J.C., SPEDDING, M. & KILPATRICK, A.T. (1990). Alpha 2-adrenoceptor subtypes and imidazoline-like binding sites in the rat brain. *Br. J. Pharmacol.,* **99,** 803 – 809.

BUCCAFUSCO, J.J., LAPP, C.A., WESTBROOKS, K.L. & ERNSBERGER, P. (1995). Role of medullary I1-imidazoline and alpha 2-adrenergic receptors in the antihypertensive responses evoked by central administration of clonidine analogs in conscious spontaneously hypertensive rats. *J. Pharmacol. Exp. Ther.,* **273,** 1162 – 1171.

CARPENE, C., COLLON, P., REMAURY, A., CORDI, A., HUDSON, A., NUTT, D. & LAFONTAN, M. (1995). Inhibition of amine oxidase activity by derivatives that recognize imidazoline 12 sites. *J. Pharmacol. Exp. Ther.,* **272,** 681–688.

CHAN, S.L. (1998). Clonidine-displacing substance and its putative role in control of insulin secretion: a minireview. *Gen. Pharmacol.,* **31,** 525–529.

CHAN, S.L., ATLAS, D., JAMES, R.F. & MORGAN, N.G. (1997). The effect of the putative endogenous imidazoline receptor ligand, clonidine-displacing substance, on insulin secretion from rat and human islets of Langerhans. *Br. J. Pharmacol.,* **120,** 926–932.

CHAN, S.L., BROWN, C.A., SCARPELLO, K.E. & MORGAN, N.G. (1994). The imidazoline site involved in control of insulin secretion: characteristics that distinguish it from I1- and I2-sites. *Br. J. Pharmacol,* **112,** 1065–1070.

CHAN, S.L., BROWN, C.A., SCARPELLO, K.E. & MORGAN, N.G. (1995). Pancreatic beta-cells express an imidazoline binding site that is distinct from I1 and I2 sites. *Ann. N.Y. Acad. Sci.,* **763,** 153–156.

CHAN, S.L., DUNNE, M.J., STILLINGS, M.R. & MORGAN, N.G. (1991). The alpha 2-adrenoceptor antagonist efaroxan modulates K + ATP channels in insulin-secreting cells. *Eur. J. Pharmacol,* **204,** 41–48.

CHAN, S.L. & MORGAN, N.G. (1990). Stimulation of insulin secretion by efaroxan may involve interaction with potassium channels. *Eur. J. Pharmacol,* **176,** 97–101.

CHOI, D.W., KOH, J.Y. & PETERS, S. (1988). Pharmacology of glutamate neurotoxicity in cortical cell culture: attenuation by NMDA antagonists. *J. Neurosci.,* **8,** 185–196.

COLUCCI, R., BLANDIZZI, C., CARIGNANI, D., PLACANICA, G., LAZZERI, G. & DEL TACCA, M. (1998). Effects of imidazoline derivatives on cholinergic motility in guinea-pig ileum: involvement of presynaptic alpha2-adrenoceptors or imidazoline receptors? *Naunyn Schmiedebergs Arch. Pharmacol.,* **357,** 682–691.

COUPRY, I., ATLAS, D., PODEVIN, R.A., UZIELLI, I. & PARINI, A. (1990). Imidazoline-guanidinium receptive site in renal proximal tubule: asymmetric distribution, regulation by cations and interaction with an endogenous clonidine displacing substance. *J. Pharmacol.. Exp. Ther.,* **252,** 293–299.

COURILLON-MALLET, A., LAUNAY, J.M., ROUCAYROL, A.M., CALLEBERT, J., EMOND, J.P., TABUTEAU, F. & CATTAN, D. (1995). Helicobacter pylori infection: physiopathologic implication of N alpha-methyl histamine. *Gastroenterology,* **108,** 959–966.

DALY, M.J. (1984). The classification of adrenoceptors and their effects on gastric acid secretion. *Scand. J. Gastroenterol.,* **89** (Suppl.): 3–9.

DE VOS, H., BRICCA, G., DE KEYSER, J., DE BACKER, J.P., BOUSQUET, P. & VAUQUELIN, G. (1994). Imidazoline receptors, non-adrenergic idazoxan binding sites and alpha 2-adrenoceptors in the human central nervous system. *Neuroscience,* **59,** 589–598.

DE VOS, H., CONVENTS, A., DE KEYSER, J., DE BACKER, J.P., VAN MEGEN, I.J., EBINGER, G. & VAUQUELIN, G. (1991). Autoradiographic distribution of alpha 2 adrenoceptors, NAIBS, and 5-HT1A receptors in human brain using [3H]idazoxan and [3H]rauwolscine. *Brain Res.,* **566,** 13–20.

DEGREGORIO-ROCASOLANO, N., OLMOS, G., GASULL, T., BORONAT, M.A., TRULLAS, R. & GARCIA-SEVILLA, J.A. (1999). Protection by imidazol(ine) compounds of L-glutamate neurotoxicity through NMDA receptor blockade. *Ann. N.Y. Acad. Sci.,* **881,** 452.

DEL TACCA, M., SOLDANI, G., BERNARDINI, C., MARTINOTTI, E. & IMPICCIATORE, M. (1982a). Pharmacological studies on the mechanisms underlying the inhibitory and excitatory effects of clonidine on gastric acid secretion. *Eur. J. Pharmacol.,* **81,** 255–261.

DEL TACCA, M., SOLDANI, G., POLLONI, A., BERNARDINI, C. & MARTINOTTI, E. (1982b). The effect of pirenzepine on gastric acid secretion and gastrin release induced by bombesin and meal in the dog. *Scand. J. Gastroenterol.,* **72** (Suppl.): 105–110.

DIAMANT, S. & ATLAS, D. (1986). An endogenous brain substance, CDS (clonidine-displacing-substance), inhibits the twitch response of rat vas deferens. *Biochem. Biophys. Res. Commun.,* **134,** 184–190.

DIETRICH, W.D., HALLEY, M., VALDES, I. & BUSTO, R. (1991). Interrelationships between increased vascular permeability and acute neuronal damage following temperature-controlled brain ischemia in rats. *Acta Neuropathol.,* **81,** 615–625.

DONTENWILL, M., BRICCA, G., MOLINES, A., BOUSQUET, P. & BELCOURT, A. (1988). Production and characterization of anti-clonidine antibodies not cross-reacting with catecholamines. *Eur. J. Pharmacol.,* **149,** 249–255.

DRIESSEN, A.J., SMID, E.J. & KONINGS, W.N. (1988). Transport of diamines by Enterococcus faecalis is mediated by an agmatine-putrescine antiporter. *J. Bacteriol.,* **170,** 4522–4527.

DUNNE, M.J., HARDING, E.A., JAGGAR, J.H., SQUIRES, P.E., LIANG, R., KANE, C., JAMES, R.F. & LONDON, N.J. (1995). Potassium channels, imidazolines, and insulin-secreting cells. *Ann. N.Y. Acad. Sci.,* **763,** 243–261.

EL OMAR, E.M., PENMAN, I.D., ARDILL, J.E., CHITTAJALLU, R.S., HOWIE, C. & MCCOLL, K.E. (1995). Helicobacter pylori infection and abnormalities of acid secretion in patients with duodenal ulcer disease. *Gastroenterology,* **109,** 681–691.

ELLIOTT, K., KEST, B., MAN, A., KAO, B. & INTURRISI, C.E. (1995). N-methyl-D-aspartate (NMDA) receptors, mu and kappa opioid tolerance, and perspectives on new analgesic drug development. *Neuropsychopharmacology,* **13,** 347–356.

ERNSBERGER, P. (1999). The I1-imidazoline receptor and its cellular signaling pathways. *Ann. N.Y. Acad. Sci.,* **881,** 35–53.

ERNSBERGER, P., FEINLAND, G., MEELEY, M.P. & REIS, D.J. (1990b). Characterization and visualization of clonidine-sensitive imidazole sites in rat kidney which recognize clonidine-displacing substance. *Am. J. Hypertens.,* **3,** 90–97.

ERNSBERGER, P., GIULIANO, R., WILLETTE, R.N., GRANATA, A.R. & REIS, D.J. (1988b). Hypotensive action of clonidine analogues correlates with binding affinity at imidazole and not alpha-2-adrenergic receptors in the rostral ventrolateral medulla. *J. Hypertens.,* **6** (Suppl.): S554–S557.

ERNSBERGER, P., GIULIANO, R., WILLETTE, R.N. & REIS, D.J. (1990a). Role of imidazole receptors in the vasodepressor response to clonidine analogs in the rostral ventrolateral medulla. *J. Pharmacol. Exp. Ther.,* **253,** 408–418.

ERNSBERGER, P., GRAVES, M.E., GRAFF, L.M., ZAKIEH, N., NGUYEN, P., COLLINS, L.A., WESTBROOKS, K.L. & JOHNSON, G.G. (1995). I1-imidazoline receptors. Definition, characterization, distribution, and transmembrane signaling. *Ann. N.Y. Acad. Sci.,* **763,** 22–42.

ERNSBERGER, P., HAXHIU, M.A., GRAFF, L.M., COLLINS, L.A., DRESHAJ, I., GROVE, D.L., GRAVES, M.E., SCHÄFER, S.G. & CHRISTEN, M.O. (1994). A novel mechanism of action for hypertension control: moxonidine as a selective I1-imidazoline agonist. *Cardiovasc. Drugs Ther.,* **8** (Suppl.1): 27–41.

ERNSBERGER, P., MEELEY, M.P., MANN, J.J. & REIS, D.J. (1987). Clonidine binds to imidazole binding sites as well as alpha 2-adrenoceptors in the ventrolateral medulla. *Eur. J. Pharmacol.,* **134,** 1–13.

ERNSBERGER, P., MEELEY, M.P. & REIS, D.J. (1988a). An endogenous substance with clonidine-like properties: selective binding to imidazole sites in the ventrolateral medulla. *Brain Res.,* **441,** 309–318.

ERNSBERGER, P., WESTBROOKS, K.L., CHRISTEN, M.O. & SCHÄFER, S.G. (1992). A second generation of centrally acting antihypertensive agents on putative I_1-imidazoline receptors. *J. Cardiovasc. Pharmacol.,* **20** (Suppl.4): S1–S10.

ESCRIBA, P.V., ALEMANY, R., SASTRE, M., OLMOS, G., OZAITA, A. & GARCIA-SEVILLA, J.A. (1996). Pharmacological modulation of immunoreactive imidazoline receptor proteins in rat brain: relationship with non-adrenoceptor [3H]-idazoxan binding sites. *Br. J. Pharmacol.,* **118,** 2029–2036.

ESCRIBA, P.V., OZAITA, A. & GARCIA-SEVILLA, J.A. (1999). Pharmacologic characterization of imidazoline receptor proteins identified by immunologic techniques and other methods. *Ann. N.Y. Acad. Sci.,* **881,** 8–25.

EVANS, R.G. & HAYNES, J.M. (1995). Alpha 2 adrenoceptor- and Imidazoline-prefering binding sites in the dog kidney. *Ann. N.Y. Acad. Sci.,* **763,** 357–360.

FAIRBANKS, C.A. & WILCOX, G.L. (1999). Moxonidine, a selective alpha2-adrenergic and imidazoline receptor agonist, produces spinal antinociception in mice. *J. Pharmacol. Exp. Ther.,* **290,** 403–412.

FELSEN, D., ERNSBERGER, P., MEELEY, M.P. & REIS, D.J. (1987). Clonidine displacing substance is biologically active on smooth muscle. *Eur. J. Pharmacol.,* **142,** 453–455.

FENG, Y., HALARIS, A.E. & PILETZ, J.E. (1997). Determination of agmatine in brain and plasma using high-performance liquid chromatography with fluorescence detection. *J. Chromatogr. B Biomed. Sci. Appl.,* **691,** 277–286.

FINCH, L., BUCKINGHAM, R.E., MOORE, R.A. & BUCHER, T.J. (1975). Evidence for a central alpha-sympathomimetic action of clonidine in the rat. *J. Pharm. Pharmacol.,* **27,** 181–186.

FRANK, E. (1927). Über eine synthetische Substanz (Synthalin) mit insulinartiger Wirkung. *Die Naturwissenschaften,* **9,** 213–215.

FUDER, H. & SCHWARZ, P. (1993). Desensitization of inhibitory prejunctional alpha 2-adrenoceptors and putative imidazoline receptors on rabbit heart sympathetic nerves. *Naunyn Schmiedebergs Arch. Pharmacol.,* **348,** 127–133.

GALEA, E., REGUNATHAN, S., ELIOPOULOS, V., FEINSTEIN, D.L. & REIS, D.J. (1996). Inhibition of mammalian nitric oxide synthases by agmatine, an endogenous polyamine formed by decarboxylation of arginine. *Biochem. J.,* **316,** 247–249.

GAO, Y., GUMUSEL, B., KOVES, G., PRASAD, A., HAO, Q., HYMAN, A. & LIPPTON, H. (1995). Agmatine: a novel endogenous vasodilator substance. *Life Sci.,* **57,** L83–L86.

GARCIA-SEVILLA, J.A., ESCRIBA, P.V., BUSQUETS, X., WALZER, C. & GUIMON, J. (1996). Platelet imidazoline receptors and regulatory G proteins in patients with major depression. *Neuroreport,* **8,** 169–172.

GARCIA-SEVILLA, J.A., ESCRIBA, P.V. & GUIMON, J. (1999). Imidazoline receptors and human brain disorders. *Ann. N.Y. Acad. Sci.,* **881,** 392–409.

GARCIA-SEVILLA, J.A., ESCRIBA, P.V., OZAITA, A., WALZER, C. & GUIMON, J. (1998a). Density of imidazoline receptors in platelets of euthymic patients with bipolar affective disorder and in brains of lithium-treated rats. *Biol. Psychiatry,* **43,** 616–618.

GARCIA-SEVILLA, J.A., ESCRIBA, P.V., WALZER, C., BOURAS, C. & GUIMON, J. (1998b). Imidazoline receptor proteins in brains of patients with Alzheimer's disease. *Neurosci. Lett.,* **247,** 95–98.

GILAD, G.M., GILAD, V.H. & RABEY, J.M. (1996c). Arginine and ornithine decarboxylation in rodent brain: coincidental changes during development and after ischemia. *Neurosci. Lett.,* **216,** 33–36.

GILAD, G.M., SALAME, K., RABEY, J.M. & GILAD, V.H. (1996b). Agmatine treatment is neuroprotective in rodent brain injury models. *Life Sci.,* **58,** L-6.

GILAD, G.M., WOLLAM, Y., IAINA, A., RABEY, J.M., CHERNIHOVSKY, T. & GILAD, V.H. (1996a). Metabolism of agmatine into urea but not into nitric oxide in rat brain. *Neuroreport,* **7,** 1730–1732.

GLAVIN, G.B., CARLISLE, M.A. & SMYTH, D.D. (1995). Agmatine, an endogenous imidazoline receptor agonist, increases gastric secretion and worsens experimental gastric mucosal injury in rats. *J. Pharmacol. Exp. Ther.,* **274,** 741–744.

GLAVIN, G.B. & SMYTH, D.D. (1995a). Moxonidine decreases gastric secretion and gastric mucosal injury in rats. *Ann. N.Y. Acad. Sci.,* **763,** 433–435.

GLAVIN, G.B. & SMYTH, D.D. (1995b). Effects of the selective I1 imidazoline receptor agonist, moxonidine, on gastric secretion and gastric mucosal injury in rats. *Br. J. Pharmacol.,* **114,** 751–754.

GOMEZ, R.E., ERNSBERGER, P., FEINLAND, G. & REIS, D.J. (1991). Rilmenidine lowers arterial pressure via imidazole receptors in brainstem C1 area. *Eur. J. Pharmacol.,* **195,** 181–191.

GONZALEZ, C., REGUNATHAN, S., REIS, D.J. & ESTRADA, C. (1996). Agmatine, an endogenous modulator of noradrenergic neurotransmission in the rat tail artery. *Br. J. Pharmacol.,* **119,** 677–684.

GOSSOP, M. (1988). Clonidine and the treatment of the opiate withdrawal syndrome. *Drug Alcohol Depend.,* **21,** 253–259.

GÖTHERT, M., BRÜSS, M., BÖNISCH, H. & MOLDERINGS, G.J. (1999). Presynaptic imidazoline receptors. New developments in characterization and classification. *Ann. N.Y. Acad. Sci.,* **881,** 171–184.

GÖTHERT, M. & MOLDERINGS, G.J. (1991). Involvement of presynaptic imidazoline receptors in the alpha 2- adrenoceptor-independent inhibition of noradrenaline release by imidazoline derivatives. *Naunyn Schmiedebergs Arch. Pharmacol.,* **343,** 271–282.

GÖTHERT, M., MOLDERINGS, G.J., FINK, K. & SCHLICKER, E. (1995). Alpha 2-adrenoceptor-independent inhibition by imidazolines and guanidines of noradrenaline release from peripheral, but not central noradrenergic neurons. *Ann. N.Y. Acad. Sci.,* **763,** 405–419.

GRIGG, M., MUSGRAVE, I.F. & BARROW, C.J. (1998). Isolation and partial structure determination of a clonidine-displacing substance from bovine lung and brain. *J. Auton. Nerv. Syst.,* **72,** 86–93.

GUSTAFSON, I., WESTERBERG, E. & WIELOCH, T. (1990). Protection against ischemia-induced neuronal damage by the alpha 2-adrenoceptor antagonist idazoxan: influence of time of administration and possible mechanisms of action. *J. Cereb. Blood Flow Metab.,* **10,** 885–894.

HÄUSER, W. & DOMINIAK, P. (1995). Effects of agmatine on catecholamine release and blood pressure of spontaneously hypertensive rats. A possible role for imidazoline receptors? *Pharm. Pharmacol. Lett.,* **5,** 87–89.

HÄUSER, W., GÜTTING, J., NGUYEN, T. & DOMINIAK, P. (1995). Influence of imidazolines on catecholamine release in pithed spontaneously hypertensive rats. *Ann. N.Y. Acad. Sci.,* **763,** 573–579.

HAEUSLER, G. (1974a). Proceedings: Sympathetic nerve activity after noradrenaline depletion and its alteration by clonidine. *Naunyn Schmiedebergs Arch. Pharmacol.,* **282** (Suppl.): R29.

HAEUSLER, G. (1974b). Clonidine-induced inhibition of sympathetic nerve activity: no indication for a central presynaptic or an indirect sympathomimetic mode of action. *Naunyn Schmiedebergs Arch. Pharmacol.,* **286,** 107–111.

HALARIS, A., ZHU, H., FENG, Y. & PILETZ, J.E. (1999). Plasma agmatine and platelet imidazoline receptors in depression. *Ann. N.Y. Acad. Sci.,* **881,** 445–451.

HALBREICH, U., PILETZ, J.E., CARSON, S., HALARIS, A. & ROJANSKY, N. (1993). Increased imidazoline and alpha 2 adrenergic binding in platelets of women with dysphoric premenstrual syndromes. *Biol. Psychiatry,* **34,** 676–686.

HAMILTON, C.A. (1992a). Adrenergic and nonadrenergic effects of imidazoline and related antihypertensive drugs in the brain and periphery. *Am. J. Hypertens.,* **5,** 58S–63S.

HAMILTON, C.A. (1992b). The role of imidazoline receptors in blood pressure regulation. *Pharmacol. Ther.,* **54,** 231–248.

HAMILTON, C.A., YAKUBU, M.A., HOWIE, C.A. & REID, J.L. (1992). Do centrally-acting antihypertensive drugs act at non-adrenergic as well as alpha-2 adrenoceptor sites? *Clin. Exp. Hypertens. [A],* **14,** 815–835.

HAXHIU, M.A., DRESHAJ, I., SCHÄFER, S.G. & ERNSBERGER, P. (1994). Selective antihypertensive action of moxonidine is mediated mainly by I1-imidazoline receptors in the rostral ventrolateral medulla. *J. Cardiovasc. Pharmacol.,* **24** (Suppl.1): S1–S8.

HEAD, G.A., CHAN, C.K. & GODWIN, S.J. (1997). Central cardiovascular actions of agmatine, a putative clonidine-displacing substance, in conscious rabbits. *Neurochem. Int.,* **30,** 37–45.

HEIN, L. (2001). Transgenic models of alpha 2-adrenergic receptor subtype function. *Rev. Physiol. Biochem. Pharmacol.,* **142,** 161–185.

HEIN, L., LIMBIRD, L.E., EGLEN, R.M. & KOBILKA, B.K. (1999). Gene substitution/knockout to delineate the role of alpha 2-adrenoceptor subtypes in mediating central effects of catecholamines and imidazolines. *Ann. N.Y. Acad. Sci.,* **881,** 265–271.

HENSLEY, M.L., MEELEY, M.P., MCCAULEY, P.M., ERNSBERGER, P. & REIS, D.J. (1989). Clonidine-displacing substance is present in peripheral tissues of the rat. *Am. J. Hypertens.,* **2,** 917–919.

HIEBLE, J.P., DEMARINIS, R.M., FOWLER, P.J. & MATTHEWS, W.D. (1986). Selective alpha-2 adrenoceptor blockade by SK&F 86466: in vitro characterization of receptor selectivity. *J. Pharmacol. Exp. Ther.,* **236,** 90 – 96.

HIEBLE, J.P. & RUFFOLO, R.R., JR. (1992). Imidazoline receptors: historical perspective. *Fundam. Clin. Pharmacol.,* **6** (Suppl.1): 7S – 13S.

HILLAIRE-BUYS, D., GROSS, R., BLAYAC, J.P., RIBES, G. & LOUBATIERES-MARIANI, M.M. (1985). Effects of alpha-adrenoceptor agonists and antagonists on insulin secreting cells and pancreatic blood vessels: comparative study. *Eur. J. Pharmacol.,* **117,** 253 – 257.

HOLT, A. & BAKER, G.B. (1995). Metabolism of agmatine (clonidine-displacing substance) by diamine oxidase and the possible implications for studies of imidazoline receptors. *Prog. Brain Res.,* **106,** 187 – 197.

HORVATH, G., KEKESI, G., DOBOS, I., SZIKSZAY, M., KLIMSCHA, W. & BENEDEK, G. (1999). Effect of intrathecal agmatine on inflammation-induced thermal hyperalgesia in rats. *Eur. J. Pharmacol.,* **368,** 197 – 204.

HOUI, N., KAMISAKI, Y. & ITOH, T. (1987). Effects of histamine H2 receptor antagonists on acid secretion stimulated by imidazoline derivatives in isolated parietal cells. *Eur. J. Pharmacol.,* **144,** 67 – 76.

HUNTER, K.J., STROBOS, C.A. & FAIRLAMB, A.H. (1991). Inhibition of polyamine biosynthesis in Crithidia fasciculata by D,L-alpha-difluoromethylornithine and D,L-alpha-difluoromethylarginine. *Mol. Biochem. Parasitol.,* **46,** 35 – 43.

ISHIDA-TAKAHASHI, A., HORIE, M., TSUURA, Y., ISHIDA, H., AI, T. & SASAYAMA, S. (1996). Block of pancreatic ATP-sensitive K + channels and insulinotrophic action by the antiarrhythmic agent, cibenzoline. *Br. J. Pharmacol.,* **117,** 1749 – 1755.

ISHIKAWA, T., MISONOU, T., IKENO, M., SATO, K. & SAKAMAKI, T. (1995). N omega-hydroxyagmatine: a novel substance causing endothelium- dependent vasorelaxation. *Biochem. Biophys. Res. Commun.,* **214,** 145 – 151.

JONAS, J.C., PLANT, T.D. & HENQUIN, J.C. (1992). Imidazoline antagonists of alpha 2-adrenoceptors increase insulin release in vitro by inhibiting ATP-sensitive K + channels in pancreatic beta-cells. *Br. J. Pharmacol.,* **107,** 8 – 14.

JONES, B.E. & YANG, T.Z. (1985). The efferent projections from the reticular formation and the locus coeruleus studied by anterograde and retrograde axonal transport in the rat. *J. Comp Neurol.,* **242,** 56 – 92.

JURKIEWICZ, N.H., GARCEZ DO, C.L., HIRATA, H., DA COSTA, S.W. & JURKIEWICZ, A. (1996). Functional properties of agmatine in rat vas deferens. *Eur. J. Pharmacol.,* **307,** 299 – 304.

KAMISAKI, Y., ISHIKAWA, T., TAKAO, Y., OMODANI, H., KUNO, N. & ITOH, T. (1990). Binding of [3H]p-aminoclonidine to two sites, alpha 2-adrenoceptors and imidazoline binding sites: distribution of imidazoline binding sites in rat brain. *Brain Res.,* **514,** 15 – 21.

KASHIWAGI, K., KOBAYASHI, H. & IGARASHI, K. (1986). Apparently unidirectional polyamine transport by proton motive force in polyamine-deficient Escherichia coli. *J. Bacteriol.,* **165,** 972 – 977.

KING, P.R., GUNDLACH, A.L. & LOUIS, W.J. (1995). Quantitative autoradiographic localization in rat brain of alpha 2-adrenergic and non-adrenergic I-receptor binding sites labelled by [3H]rilmenidine. *Brain Res.,* **675,** 264 – 278.

KOBINGER, W. & PICHLER, L. (1976). Centrally induced reduction in sympathetic tone-a postsynaptic alpha- adrenoceptor-stimulating action of imidazolines. *Eur. J. Pharmacol.,* **40,** 311 – 320.

KOLESNIKOV, Y., JAIN, S. & PASTERNAK, G.W. (1996). Modulation of opioid analgesia by agmatine. *Eur. J. Pharmacol.,* **296,** 17 – 22.

KOSSEL, A. (1910). Über das Agmatin. *Hoppe Seylers. Z. Physiol. Chem.,* **66,** 257 – 261.

LAMBERTI, C., IPPONI, A., BARTOLINI, A., SCHUNACK, W. & MALMBERG-AIELLO, P. (1998). Antidepressant-like effects of endogenous histamine and of two histamine H1 receptor agonists in the mouse forced swim test. *Br. J. Pharmacol.,* **123,** 1331 – 1336.

LANGER, J., PANTEN, U. & ZIELMANN, S. (1983). Effects of alpha-adrenoceptor antagonists on clonidine-induced inhibition of insulin secretion by isolated pancreatic islets. *Br. J. Pharmacol.,* **79,** 415 – 420.

LANGER, S.Z. & HICKS, P.E. (1984). Alpha-adrenoreceptor subtypes in blood vessels: physiology and pharmacology. *J. Cardiovasc. Pharmacol.,* **6** (Suppl 4): S547 – S558.

LEVEQUE, J., BURTIN, F., CATROS-QUEMENER, V., HAVOUIS, R. & MOULINOUX, J.P. (1998). The gastrointestinal polyamine source depletion enhances DFMO induced polyamine depletion in MCF-7 human breast cancer cells in vivo. *Anticancer Res.,* **18,** 2663 – 2668.

LI, G., REGUNATHAN, S., BARROW, C.J., ESHRAGHI, J., COOPER, R. & REIS, D.J. (1994a). Agmatine: an endogenous clonidine-displacing substance in the brain. *Science,* **263,** 966 – 969.

LI, G., REGUNATHAN, S. & REIS, D.J. (1995). Agmatine is synthesized by a mitochondrial arginine decarboxylase in rat brain. *Ann. N.Y. Acad. Sci.,* **763,** 325 – 329.

LI, P., PENNER, S.B. & SMYTH, D.D. (1994b). Attenuated renal response to moxonidine and rilmenidine in one kidney- one clip hypertensive rats. *Br. J. Pharmacol.,* **112,** 200 – 206.

LIKUNGU, J., MOLDERINGS, G.J. & GÖTHERT, M. (1996). Presynaptic imidazoline receptors and alpha 2-adrenoceptors in the human heart: discrimination by clonidine and moxonidine. *Naunyn Schmiedebergs Arch. Pharmacol.,* **354,** 689 – 692.

LIMON, I., COUPRY, I., LANIER, S.M. & PARINI, A. (1992). Purification and characterization of mitochondrial imidazoline-guanidinium receptive site from rabbit kidney. *J. Biol. Chem.,* **267,** 21645 – 21649.

LIU, L. & COUPAR, I.M. (1997). Involvement of alpha-2 adrenoceptors in the effects of moxonidine on intestinal motility and fluid transport. *J. Pharmacol. Exp. Ther.,* **283,** 1367 – 1374.

LORING, R.H. (1990). Agmatine acts as an antagonist of neuronal nicotinic receptors. *Br. J. Pharmacol.,* **99,** 207 – 211.

LORTIE, M.J., NOVOTNY, W.F., PETERSON, O.W., VALLON, V., MALVEY, K., MENDONCA, M., SATRIANO, J., INSEL, P., THOMSON, S.C. & BLANTZ, R.C. (1996). Agmatine, a bioactive metabolite of arginine. Production, degradation, and functional effects in the kidney of the rat. *J. Clin. Invest.,* **97,** 413 – 420.

LYSKO, P.G., COX, J.A., VIGANO, M.A. & HENNEBERRY, R.C. (1989). Excitatory amino acid neurotoxicity at the N-methyl-D-aspartate receptor in cultured neurons: pharmacological characterization. *Brain Res.,* **499,** 258 – 266.

MACMILLAN, L.B., HEIN, L., SMITH, M.S., PIASCIK, M.T. & LIMBIRD, L.E. (1996). Central hypotensive effects of the alpha2a-adrenergic receptor subtype. *Science,* **273,** 801 – 803.

MAIESE, K., PEK, L., BERGER, S.B. & REIS, D.J. (1992). Reduction in focal cerebral ischemia by agents acting at imidazole receptors. *J. Cereb. Blood Flow Metab.,* **12,** 53 – 63.

MALLARD, N.J., HUDSON, A.L. & NUTT, D.J. (1992). Characterization and autoradiographical localization of non- adrenoceptor idazoxan binding sites in the rat brain. *Br. J. Pharmacol.,* **106,** 1019 – 1027.

MAZE, M., SEGAL, I.S. & BLOOR, B.C. (1988). Clonidine and other alpha2 adrenergic agonists: strategies for the rational use of these novel anesthetic agents. *J. Clin. Anesth.,* **1,** 146 – 157.

MEDGETT, I.C. & MCCULLOCH, M.W. (1979). Effects of clonidine, guanfacine and three imidazolidine derivatives related to clonidine on blood pressure, heart rate and gastric acid secretion in the anaesthetized rat. *Arch. Int. Pharmacodyn. Ther.,* **240,** 158 – 168.

MEELEY, M.P., ERNSBERGER, P.R., GRANATA, A.R. & REIS, D.J. (1986). An endogenous clonidine-displacing substance from bovine brain: receptor binding and hypotensive actions in the ventrolateral medulla. *Life Sci.,* **38,** 1119 – 1126.

MEELEY, M.P., ERNSBERGER, P., MCCAULEY, P.M. & REIS, D.J. (1988a). Clonidine-specific antibodies as models for imidazole and alpha 2-adrenergic receptor binding sites: implications for the structure of clonidine-displacing substance. *J. Hypertens.,* **6** (Suppl): S490 – S493.

MEELEY, M.P., HENSLEY, M.L., ERNSBERGER, P., FELSEN, D. & REIS, D.J. (1992). Evidence for a bioactive clonidine-displacing substance in peripheral tissues and serum. *Biochem. Pharmacol.,* **44,** 733 – 740.

MEELEY, M.P., TOWLE, A.C., ERNSBERGER, P. & REIS, D.J. (1988b). A specific antiserum recognizes clonidine-displacing substance: implications for the structure of the brain's own clonidine. *Neurosci. Lett.,* **84,** 84 – 90.

MERCENIER, A., SIMON, J.P., HAAS, D. & STALON, V. (1980). Catabolism of L-arginine by Pseudomonas aeruginosa. *J. Gen. Microbiol.,* **116,** 381 – 389.

MESHUL, C.K., SEIL, F.J. & HERNDON, R.M. (1987). Astrocytes play a role in regulation of synaptic density. *Brain Res.,* **402,** 139 – 145.

MICHEL, M.C., BRODDE, O.E., SCHNEPEL, B., BEHRENDT, J., TSCHADA, R., MOTULSKY, H.J. & INSEL, P.A. (1989). [3H]idazoxan and some other alpha 2-adrenergic drugs also bind with high affinity to a nonadrenergic site. *Mol. Pharmacol.,* **35,** 324 – 330.

MICHEL, M.C. & ERNSBERGER, P. (1992). Keeping an eye on the I site: imidazoline-preferring receptors. *Trends Pharmacol. Sci.,* **13,** 369 – 370.

MICHEL, M.C. & INSEL, P.A. (1989). Are there multiple imidazoline binding sites? *Trends Pharmacol. Sci.,* **10,** 342 – 344.

MICHEL, M.C. & RUMP, L.C. (1996). alpha-Adrenergic regulation of human renal function. *Fundam. Clin. Pharmacol.,* **10,** 493 – 503.

MOLDERINGS, G.J., BÖNISCH, M., GÖTHERT. & BRÜSS, H. (2001). Agmatine and putrescine uptake in the human glioma cell line SK-MG-1. *Naunyn Schmiedebergs Arch. Pharmacol.,* **363,** 671 – 679.

MOLDERINGS, G.J., BURIAN, M., HOMANN, J., NILIUS, M. & GÖTHERT, M. (1999d). Potential relevance of agmatine as a virulence factor of Helicobacter pylori. *Dig. Dis. Sci.,* **44,** 2397 – 2404.

MOLDERINGS, G.J., BURIAN, M., MENZEL, S., DONECKER, K., HOMANN, J., NILIUS, M. & GÖTHERT, M. (1999c). Imidazoline recognition sites and stomach function. *Ann. N.Y. Acad. Sci.,* **881,** 332 – 343.

MOLDERINGS, G.J., DONECKER, K., BURIAN, M., SIMON, W.A., SCHRODER, D.W. & GÖTHERT, M. (1998b). Characterization of I2 imidazoline and sigma binding sites in the rat and human stomach. *J. Pharmacol. Exp. Ther.,* **285,** 170 – 177.

MOLDERINGS, G.J., DONECKER, K. & GÖTHERT, M. (1995). Characterization of non-adrenergic [3H]clonidine binding sites in rat stomach: high affinity of imidazolines, guanidines and sigma ligands. *Naunyn Schmiedebergs Arch. Pharmacol.,* **351,** 561 – 564.

MOLDERINGS, G.J. & GÖTHERT, M. (1995). Inhibitory presynaptic imidazoline receptors on sympathetic nerves in the rabbit aorta differ from I1- and I2-imidazoline binding sites. *Naunyn Schmiedebergs Arch. Pharmacol.,* **351,** 507 – 516.

MOLDERINGS, G.J., GÖTHERT, M., HOMANN, J. & NILIUS, M. (1998a). Potential relevance of agmatine for the pathogenesis of gastric mucosal lesions. *Naunyn Schmiedebergs Arch. Pharmacol.,* **357** (Suppl): R166.

MOLDERINGS, G.J., HENTRICH, F. & GÖTHERT, M. (1991). Pharmacological characterization of the imidazoline receptor which mediates inhibition of noradrenaline release in the rabbit pulmonary artery. *Naunyn Schmiedebergs Arch. Pharmacol.,* **344,** 630 – 638.

MOLDERINGS, G.J., LIKUNGU, J. & GÖTHERT, M. (1999b). Presynaptic cannabinoid and imidazoline receptors in the human heart and their potential relationship. *Naunyn Schmiedebergs Arch. Pharmacol.,* **360,** 157 – 164.

MOLDERINGS, G.J., LIKUNGU, J., JAKSCHIK, J. & GÖTHERT, M. (1997). Presynaptic imidazoline receptors and non-adrenoceptor [3H]-idazoxan binding sites in human cardiovascular tissues. *Br. J. Pharmacol.,* **122,** 43 – 50.

MOLDERINGS, G.J., MENZEL, S. & GÖTHERT, M. (1999a). Imidazoline derivatives and agmatine induce histamine release from the rat stomach. *Naunyn Schmiedebergs Arch. Pharmacol.,* **360,** 711 – 714.

MOLDERINGS, G.J., MENZEL, S., KATHMANN, M., SCHLICKER, E. & GÖTHERT, M. (2000). Dual interaction of agmatine with the rat alpha(2D)-adrenoceptor: competitive antagonism and allosteric activation. *Br. J. Pharmacol.,* **130,** 1706 – 1712.

MOLDERINGS, G.J., MOURA, D., FINK, K., BÖNISCH, H. & GÖTHERT, M. (1993). Binding of [3H]clonidine to I1-imidazoline sites in bovine adrenal medullary membranes. *Naunyn Schmiedebergs Arch. Pharmacol.,* **348,** 70 – 76.

MOLDERINGS, G.J., SCHMIDT, K., BÖNISCH, H. & GÖTHERT, M. (1996). Inhibition of 5-HT3 receptor function by imidazolines in mouse neuroblastoma cells: potential involvement of sigma 2 binding sites. *Naunyn Schmiedebergs Arch. Pharmacol.,* **354,** 245 – 252.

MORGAN, N.G., CHAN, S.L., BROWN, C.A. & TSOLI, E. (1995). Characterization of the imidazoline binding site involved in regulation of insulin secretion. *Ann. N.Y. Acad. Sci.,* **763,** 361 – 373.

MORGAN, N.G., CHAN, S.L., MOURTADA, M., MONKS, L.K. & RAMSDEN, C.A. (1999). Imidazolines and pancreatic hormone secretion. *Ann. N.Y. Acad. Sci.,* **881,** 217 – 228.

MUNK, S.A., LAI, R.K., BURKE, J.E., ARASASINGHAM, P.N., KHARLAMB, A.B., MANLAPAZ, C.A., PADILLO, E.U., WIJONO, M.K., HASSON, D.W., WHEELER, L.A. & GARST, M.E. (1996). Synthesis and pharmacologic evaluation of 2-endo-amino-3-exo-isopropylbicyclo[2.2.1]heptane: a potent imidazoline 1 receptor specific agent. *J. Med. Chem.,* **39,** 1193 – 1195.

NESTLER, E.J., BERHOW, M.T. & BRODKIN, E.S. (1996). Molecular mechanisms of drug addiction: adaptations in signal transduction pathways. *Mol. Psychiatry,* **1,** 190 – 199.

NICHOLAS, A.P., HOKFELT, T. & PIERIBONE, V.A. (1996). The distribution and significance of CNS adrenoceptors examined with in situ hybridization. *Trends Pharmacol. Sci.,* **17,** 245 – 255.

NICHOLAS, A.P., PIERIBONE, V. & HOKFELT, T. (1993). Distributions of mRNAs for alpha-2 adrenergic receptor subtypes in rat brain: an in situ hybridization study. *J. Comp. Neurol.,* **328,** 575 – 594.

OHARA-IMAIZUMI, M. & KUMAKURA, K. (1992). Effects of imidazole compounds on catecholamine release in adrenal chromaffin cells. *Cell. Mol. Neurobiol.,* **12,** 273 – 283.

OLMOS, G., ALEMANY, R., ESCRIBA, P.V. & GARCIA-SEVILLA, J.A. (1994). The effects of chronic imidazoline drug treatment on glial fibrillary acidic protein concentrations in rat brain. *Br. J. Pharmacol.,* **111,** 997 – 1002.

OLMOS, G., DEGREGORIO-ROCASOLANO, N., PAZ, R.M., GASULL, T., ASSUMPCIO, B.M., TRULLAS, R., VILLARROEL, A., LERMA, J. & GARCIA-SEVILLA, J.A. (1999). Protection by imidazol(ine) drugs and agmatine of glutamate-induced neurotoxicity in cultured cerebellar granule cells through blockade of NMDA receptor. *Br. J. Pharmacol.,* **127,** 1317 – 1326.

OLMOS, G., GABILONDO, A.M., MIRALLES, A., ESCRIBA, P.V. & GARCIA-SEVILLA, J.A. (1993). Chronic treatment with the monoamine oxidase inhibitors clorgyline and pargyline down-regulates non-adrenoceptor [3H]-idazoxan binding sites in the rat brain. *Br. J. Pharmacol.,* **108,** 597 – 603.

OLMOS, G., RIBERA, J. & GARCIA-SEVILLA, J.A. (1996). Imidazoli(di)ne compounds interact with the phencyclidine site of NMDA receptors in the rat brain. *Eur. J. Pharmacol.,* **310,** 273 – 276.

OTAKE, K., RUGGIERO, D.A., REGUNATHAN, S., WANG, H., MILNER, T.A. & REIS, D.J. (1998). Regional localization of agmatine in the rat brain: an immunocytochemical study. *Brain Res.,* **787,** 1 – 14.

PANAGIOTIDIS, C.A., BLACKBURN, S., LOW, K.B. & CANELLAKIS, E.S. (1987). Biosynthesis of polyamines in ornithine decarboxylase, arginine decarboxylase, and agmatine ureohydrolase deletion mutants of Escherichia coli strain K-12. *Proc. Natl. Acad. Sci. U.S.A.,* **84,** 4423 – 4427.

PARKER, C.A., HUDSON, A.L., NUTT, D.J., DILLON, M.P., EGLEN, R.M., CHAN, S.L., MORGAN, N.G. & CROSBY, J. (1999a). Extraction of active clonidine-displacing substance from bovine lung and comparison with clonidine-displacing substance extracted from other tissues. *Eur. J. Pharmacol.,* **378,** 213 – 221.

PARKER, C.A., HUDSON, A.L., NUTT, D.J., DILLON, M.P., EGLEN, R.M. & CROSBY, J. (1999b). Comparison of crude methanolic CDS extracts from various tissues. *Ann. N.Y. Acad. Sci.,* **881,** 92 – 96.

PEGG, A.E. & MCCANN, P.P. (1982). Polyamine metabolism and function. *Am. J. Physiol.,* **243,** C212 – C221.

PENNER, S.B. & SMYTH, D.D. (1996). Natriuresis following central and peripheral administration of agmatine in the rat. *Pharmacology,* **53,** 160 – 169.

PERSSON, L., HOLM, I. & HEBY, O. (1986). Translational regulation of ornithine decarboxylase by polyamines. *FEBS Lett.,* **205,** 175 – 178.

PETERSON, W.L. (1996). Gastrin and acid in relation to Helicobacter pylori. *Aliment. Pharmacol. Ther.,* **10** (Suppl 1): 97 – 102.

PILETZ, J.E., CHIKKALA, D.N. & ERNSBERGER, P. (1995). Comparison of the properties of agmatine and endogenous clonidine-displacing substance at imidazoline and alpha-2 adrenergic receptors. *J. Pharmacol. Exp. Ther.,* **272,** 581 – 587.

PILETZ, J.E. & HALARIS, A. (1995). Involvement of I1-imidazoline receptors in mood disorders. *Ann. N.Y. Acad. Sci.,* **763,** 510 – 519.

PILETZ, J.E., HALARIS, A., NELSON, J., QU, Y. & BARI, M. (1996c). Platelet I1-imidazoline binding sites are elevated in depression but not generalized anxiety disorder. *J. Psychiatr. Res.,* **30,** 147 – 168.

PILETZ, J.E., HALARIS, A.E., CHIKKALA, D. & QU, Y. (1996b). Platelet I1-imidazoline binding sites are decreased by two dissimilar antidepressant agents in depressed patients. *J. Psychiatr. Res.,* **30,** 169 – 184.

PILETZ, J.E., IVANOV, T.R., SHARP, J.D., ERNSBERGER, P., CHANG, C.H., PICKARD, R.T., GOLD, G., ROTH, B., ZHU, H., JONES, J.C., BALDWIN, J. & REIS, D.J. (2000). Imidazoline receptor antisera-selected (IRAS) cDNA: cloning and characterization. *DNA Cell. Biol.,* **19,** 319 – 329.

PILETZ, J.E., ZHU, H. & CHIKKALA, D.N. (1996a). Comparison of ligand binding affinities at human I1-imidazoline binding sites and the high affinity state of alpha-2 adrenoceptor subtypes. *J. Pharmacol. Exp. Ther.,* **279,** 694 – 702.

PINTHONG, D., HUSSAIN, J.F., KENDALL, D.A. & WILSON, V.G. (1995b). Comparison of the interaction of agmatine and crude methanolic extracts of bovine lung and brain with alpha 2-adrenoceptor binding sites. *Br. J. Pharmacol.,* **115,** 689 – 695.

PINTHONG, D., WRIGHT, I.K., HANMER, C., MILLNS, P., MASON, R., KENDALL, D.A. & WILSON, V.G. (1995a). Agmatine recognizes alpha 2-adrenoceptor binding sites but neither activates nor inhibits alpha 2-adrenoceptors. *Naunyn Schmiedebergs Arch. Pharmacol.,* **351,** 10 – 16.

PLANT, T.D. & HENQUIN, J.C. (1990). Phentolamine and yohimbine inhibit ATP-sensitive K + channels in mouse pancreatic beta-cells. *Br. J. Pharmacol.,* **101,** 115 – 120.

PURCELL, W.M., DOYLE, K.M., BAGGA, L. & DERKS, M. (1994). Histamine release from mast cells by polyamines: an NMDA receptor- mediated event? *Biochem. Soc. Trans.,* **22,** 398S.

QUAN, D.B., WANDRES, D.L. & SCHROEDER, D.J. (1993). Clonidine in pain management. *Ann. Pharmacother.,* **27,** 313 – 315.

QUIK, M. (1985). Inhibition of nicotinic receptor mediated ion fluxes in rat sympathetic ganglia by BGT II-S1 a potent phospholipase. *Brain Res.,* **325,** 79 – 88.

RAASCH, W., CHUN, K.R.J., DENDORFER, A. & DOMINIAK, P. (2000). Positive inotropic effects of imidazoline derivatives are not mediated via imidazoline binding sites but alpha1-adrenergic receptors. *Jpn. J. Pharmacol.,* **84,** 1 – 6.

RAASCH, W., LIST, B., HÄUSER, W., SCHÄFER, U. & DOMINIAK, P. (1999). Presynaptic release of noradrenaline is mediated not only through α_2-adrenoceptors but also through imidazoline binding sites. *Dtsch. Med. Wschr.,* **124,** S114.

RAASCH, W., MUHLE, H. & DOMINIAK, P. (1996). Monoamine oxidase (MAO) is inhibited by imidazoline binding site I_2 specific ligands in vitro. *Pharm. Pharmacol. Lett.,* **6,** 42 – 45.

RAASCH, W., MUHLE, H. & DOMINIAK, P. (1999). Modulation of MAO activity by imidazoline and guanidine derivatives. *Ann. N.Y. Acad. Sci.,* **881,** 313 – 331.

RAASCH, W., REGUNATHAN, S., LI, G. & REIS, D.J. (1995a). Agmatine, the bacterial amine, is widely distributed in mammalian tissues. *Life Sci.,* **56,** 2319 – 2330.

RAASCH, W., REGUNATHAN, S., LI, G. & REIS, D.J. (1995b). Agmatine is widely and unequally distributed in rat organs. *Ann. N.Y. Acad. Sci.,* **763,** 330 – 334.

RADDATZ, R. & LANIER, S.M. (1997). Relationship between imidazoline/guanidinium receptive sites and monoamine oxidase A and B. *Neurochem. Int.,* **30,** 109 – 117.

RADDATZ, R., PARINI, A. & LANIER, S.M. (1995). Imidazoline/guanidinium binding domains on monoamine oxidases. Relationship to subtypes of imidazoline-binding proteins and tissue-specific interaction of imidazoline ligands with monoamine oxidase B. *J. Biol. Chem.,* **270,** 27961 – 27968.

RADDATZ, R., PARINI, A. & LANIER, S.M. (1997). Localization of the imidazoline binding domain on monoamine oxidase B. *Mol. Pharmacol.,* **52,** 549 – 553.

RADDATZ, R., SAVIC, S.L., BAKTHAVACHALAM, V., LESNICK, J., JASPER, J.R., MCGRATH, C.R., PARINI, A. & LANIER, S.M. (2000). Imidazoline-binding domains on monoamine oxidase B and subpopulations of enzyme. *J. Pharmacol. Exp. Ther.,* **292,** 1135 – 1145.

RADDATZ, R., SAVIC, S.L. & LANIER, S.M. (1999). Imidazoline binding domains on MAO-B. Localization and accessibility. *Ann. N.Y. Acad. Sci.,* **881,** 26 – 31.

RAMAKRISHNA, S. & ADIGA, P.R. (1975). Arginine decarboxylase from Lathyrus sativus seedlings. Purification and properites. *Eur. J. Biochem.,* **59,** 377 – 386.

REGUNATHAN, S., EVINGER, M.J., MEELEY, M.P. & REIS, D.J. (1991b). Effects of clonidine and other imidazole-receptor binding agents on second messenger systems and calcium influx in bovine adrenal chromaffin cells. *Biochem. Pharmacol.,* **42,** 2011 – 2018.

REGUNATHAN, S., FEINSTEIN, D.L., RAASCH, W. & REIS, D.J. (1995a). Agmatine (decarboxylated arginine) is synthesized and stored in astrocytes. *Neuroreport,* **6,** 1897 – 1900.

REGUNATHAN, S., FEINSTEIN, D.L. & REIS, D.J. (1999). Anti-proliferative and anti-inflammatory actions of imidazoline agents. Are imidazoline receptors involved? *Ann. N.Y. Acad. Sci.,* **881,** 410 – 419.

REGUNATHAN, S., MEELEY, M.P. & REIS, D.J. (1991a). Clonidine-displacing substance from bovine brain binds to imidazoline receptors and releases catecholamines in adrenal chromaffin cells. *Mol. Pharmacol.,* **40,** 884 – 888.

REGUNATHAN, S., MEELEY, M.P. & REIS, D.J. (1993). Expression of non-adrenergic imidazoline sites in chromaffin cells and mitochondrial membranes of bovine adrenal medulla. *Biochem. Pharmacol.,* **45,** 1667 – 1675.

REGUNATHAN, S. & REIS, D.J. (1997). Stimulation of imidazoline receptors inhibits proliferation of human coronary artery vascular smooth muscle cells. *Hypertension,* **30,** 295 – 300.

REGUNATHAN, S. & REIS, D.J. (2000). Characterization of arginine decarboxylase in rat brain and liver: distinction from ornithine decarboxylase. *J. Neurochem.,* **74,** 2201 – 2208.

REGUNATHAN, S., SASTRE, M. & REIS, D.J. (1996b). Agmatine is released from synaptosomes and adrenal chromaffin cells by depolarization. *Soc. Neurosci.,* (Abstr. 22): 1564.

REGUNATHAN, S., YOUNGSON, C., RAASCH, W., WANG, H. & REIS, D.J. (1996a). Imidazoline receptors and agmatine in blood vessels: a novel system inhibiting vascular smooth muscle proliferation. *J. Pharmacol. Exp. Ther.,* **276,** 1272 – 1282.

REGUNATHAN, S., YOUNGSON, C., WANG, H. & REIS, D.J. (1995b). Imidazoline receptors in vascular smooth muscle and endothelial cells. *Ann. N.Y. Acad. Sci.,* **763,** 580 – 590.

REID, J.L., PANFILOV, V., MACPHEE, G. & ELLIOTT, H.L. (1995). Clinical pharmacology of drugs acting on imidazoline and adrenergic receptors. Studies with clonidine, moxonidine, rilmenidine, and atenolol. *Ann. N.Y. Acad. Sci.,* **763,** 673 – 678.

REIS, D.J. & REGUNATHAN, S. (1998). Agmatine: a novel neurotransmitter? *Adv. Pharmacol.,* **42,** 645 – 649.

REIS, D.J., LI, G., REGUNATHAN, S., BARROW, C.J. & COOPER, R. (1994). Identifying clonidine-displacing substance. *Science,* **266,** 462 – 464.

REIS, D.J., REGUNATHAN, S. & MEELEY, M.P. (1992). Imidazole receptors and clonidine-displacing substance in relationship to control of blood pressure, neuroprotection, and adrenomedullary secretion. *Am. J. Hypertens.,* **5,** 51S – 57S.

REIS, D.J., RUGGIERO, D.A. & MORRISON, S.F. (1989). The C1 area of the rostral ventrolateral medulla oblongata. A critical brainstem region for control of resting and reflex integration of arterial pressure. *Am. J. Hypertens.,* **2,** 363S – 374S.

REIS, D.J., YANG, X.C. & MILNER, T.A. (1998). Agmatine containing axon terminals in rat hippocampus form synapses on pyramidal cells. *Neurosci. Lett.,* **250,** 185 – 188.

REMAURY, A., RADDATZ, R., ORDENER, C., SAVIC, S., SHIH, J.C., CHEN, K., SEIF, I., DE MAEYER, E., LANIER, S.M. & PARINI, A. (2000). Analysis of the pharmacological and molecular heterogeneity of I(2)- imidazoline-binding proteins using monoamine oxidase-deficient mouse models. *Mol. Pharmacol.,* **58,** 1085 – 1090.

RUFFOLO, R.R., TUROWSKI, B.S. & PATIL, P.N. (1977). Lack of cross-desensitization between structurally dissimilar alpha-adrenoceptor agonists. *J. Pharm. Pharmacol.,* **29,** 378 – 380.

RUGGIERO, D.A., REGUNATHAN, S., WANG, H., MILNER, T.A. & REIS, D.J. (1995). Distribution of imidazoline receptor binding protein in the central nervous system. *Ann. N.Y. Acad. Sci.,* **763,** 208 – 221.

RUSTENBECK, I., HERRMANN, C., RATZKA, P. & HASSELBLATT, A. (1997). Imidazoline/guanidinium binding sites and their relation to inhibition of K(ATP) channels in pancreatic B-cells. *Naunyn Schmiedebergs Arch. Pharmacol.,* **356,** 410 – 417.

SANCHEZ-BLAZQUEZ, P., BORONAT, M.A., OLMOS, G., GARCIA-SEVILLA, J.A. & GARZON, J. (2000). Activation of I(2)-imidazoline receptors enhances supraspinal morphine analgesia in mice: a model to detect agonist and antagonist activities at these receptors. *Br. J. Pharmacol.,* **130,** 146 – 152.

SAPER, C.B., AKIL, H. & WATSON, S.J. (1986). Lateral hypothalamic innervation of the cerebral cortex: immunoreactive staining for a peptide resembling but immunochemically distinct from pituitary/arcuate alpha-melanocyte stimulating hormone. *Brain Res. Bull.,* **16,** 107 – 120.

SASTRE, M., ESCRIBA, P.V., REIS, D.J. & GARCIA-SEVILLA, J.A. (1995). Decreased number and immunoreactivity of I2-imidazoline receptors in the frontal cortex of suicide victims. *Ann. N.Y. Acad. Sci.,* **763,** 520 – 522.

SASTRE, M., GALEA, E., FEINSTEIN, D., REIS, D.J. & REGUNATHAN, S. (1998). Metabolism of agmatine in macrophages: modulation by lipopolysaccharide and inhibitory cytokines. *Biochem. J.,* **330,** 1405 – 1409.

SASTRE, M., REGUNATHAN, S., GALEA, E. & REIS, D.J. (1996b). Agmatinase activity in rat brain: a metabolic pathway for the degradation of agmatine. *J. Neurochem.,* **67,** 1761 – 1765.

SASTRE, M., REGUNATHAN, S. & REIS, D.J. (1997). Uptake of agmatine into rat brain synaptosomes: possible role of cation channels. *J. Neurochem.,* **69,** 2421 – 2426.

SASTRE, M., VENTAYOL, P. & GARCIA-SEVILLA, J.A. (1996a). Decreased density of I2-imidazoline receptors in the postmortem brain of heroin addicts. *Neuroreport,* **7,** 509 – 512.

SATISHCHANDRAN, C. & BOYLE, S.M. (1986). Purification and properties of agmatine ureohydrolyase, a putrescine biosynthetic enzyme in Escherichia coli. *J. Bacteriol.,* **165,** 843 – 848.

SATRIANO, J., MATSUFUJI, S., MURAKAMI, Y., LORTIE, M.J., SCHWARTZ, D., KELLY, C.J., HAYASHI, S. & BLANTZ, R.C. (1998). Agmatine suppresses proliferation by frameshift induction of antizyme and attenuation of cellular polyamine levels. *J. Biol. Chem.,* **273,** 15313 – 15316.

SCHÄFER, U., RAASCH, W., CHUN, J.K.R., DENDORFER, A., QADRI, F. & DOMINIAK, P. (1999b). The cardiovascular effects of agmatine are not mediated via peripheral binding sites but via blockade of the ganglionic transmission. *Naunyn Schmiedebergs Arch. Pharmacol.,* **359** (Suppl): R25.

SCHÄFER, RAASCH, W., DENDORFER, A., QADRI, F. & DOMINIAK, P. (1998). Effects of agmatine on the cardiovascular system. *Kidney Blood Press Res.,* **21,** A131.

SCHÄFER, U., RAASCH, W., QADRI, F., CHUN, J. & DOMINIAK, P. (1999a). Effects of agmatine on the cardiovascular system of spontaneously hypertensive rats. *Ann. N.Y. Acad. Sci.,* **881,** 97 – 101.

SCHULZ, A. & HASSELBLATT, A. (1989a). Dual action of clonidine on insulin release: suppression, but stimulation when alpha 2-adrenoceptors are blocked. *Naunyn Schmiedebergs Arch. Pharmacol.,* **340,** 712 – 714.

SCHULZ, A. & HASSELBLATT, A. (1989b). An insulin-releasing property of imidazoline derivatives is not limited to compounds that block alpha-adrenoceptors. *Naunyn Schmiedebergs Arch. Pharmacol.,* **340,** 321 – 327.

SCHWARTZ, D., PETERSON, O.W., MENDONCA, M., SATRIANO, J., LORTIE, M. & BLANTZ, R.C. (1997). Agmatine affects glomerular filtration via a nitric oxide synthase- dependent mechanism. *Am. J. Physiol.,* **272,** F597 – F601.

SEILER, N. & DEZEURE, F. (1990). Polyamine transport in mammalian cells. *Int. J. Biochem.,* **22,** 211 – 218.

SENER, A., LEBRUN, P., BLACHIER, F. & MALAISSE, W.J. (1989). Stimulus-secretion coupling of arginine-induced insulin release. Insulinotropic action of agmatine. *Biochem. Pharmacol.,* **38,** 327 – 330.

SINGH, G., HUSSAIN, J.F., MACKINNON, A., BROWN, C.M., KENDALL, D.A. & WILSON, V.G. (1995). Evidence for the presence of a non-catecholamine, clonidine-displacing substance in crude, methanolic extracts of bovine brain and lung. *Naunyn Schmiedebergs Arch. Pharmacol.,* **351,** 17 – 26.

SJOHOLM, A. (1993). Role of polyamines in the regulation of proliferation and hormone production by insulin-secreting cells. *Am. J. Physiol.,* **264,** C501 – C518.

SKOGLUND, G., LUNDQUIST, I. & AHREN, B. (1988). Selective alpha 2-adrenoceptor activation by clonidine: effects on 45Ca2+ efflux and insulin secretion from isolated rat islets. *Acta Physiol Scand.,* **132,** 289 – 296.

SMYTH, D.D. & PENNER, S.B. (1995). Renal I1-imidazoline receptor-selective compounds mediate natriuresis in the rat. *J. Cardiovasc. Pharmacol.,* **26** (Suppl 2): S63 – S67.

SMYTH, D.D. & PENNER, S.B. (1999). Peripheral and central imidazoline receptor-mediated natriuresis in the rat. *Ann. N.Y. Acad. Sci.,* **881,** 344 – 357.

SOBHANI, I., BADO, A., CHERIFI, Y., MOIZO, L., LAIGNEAU, J.P., POSPAI, D., MIGNON, M. & LEWIN. (1996). Helicobacter pylori stimulates gastric acid secretion via platelet activating factor. *J. Physiol Pharmacol.,* **47,** 177 – 185.

SOMLYO, A.P. & SOMLYO, A.V. (1994). Signal transduction and regulation in smooth muscle. *Nature,* **372,** 231 – 236.

STEFFEN, G. & DOMINIAK, P. (1996). Significance of imidazolines for catecholamine release and for voltage-gated calcium channels in the bovine chromaffin cell. *Naunyn Schmiedebergs Arch. Pharmacol.,* **353** (Suppl): R4.

STICKLE, D., BOHRER, A., BERGER, R., MORRISSEY, J., KLAHR, S. & TURK, J. (1996). Quantitation of the putative neurotransmitter agmatine as the hexafluoroacetylacetonate derivative by stable isotope dilution gas chromatography and negative-ion chemical ionization mass spectrometry. *Anal. Biochem.,* **238,** 129 – 136.

STIENE-MARTIN, A., GURWELL, J.A. & HAUSER, K.F. (1991). Morphine alters astrocyte growth in primary cultures of mouse glial cells: evidence for a direct effect of opiates on neural maturation. *Brain Res. Dev. Brain Res.,* **60,** 1 – 7.

STIENE-MARTIN, A. & HAUSER, K.F. (1993). Morphine suppresses DNA synthesis in cultured murine astrocytes from cortex, hippocampus and striatum. *Neurosci. Lett.,* **157,** 1 – 3.

SUN, M.K., REGUNATHAN, S. & REIS, D.J. (1995). Cardiovascular responses to agmatine, a clonidine-displacing substance, in anesthetized rat. *Clin. Exp. Hypertens.,* **17,** 115 – 128.

SYNETOS, D., MANOLOPOULOS, V.G., ATLAS, D. & PIPILI-SYNETOS, E. (1991). Human plasma-derived material with clonidine displacing substance (CDS)- like properties contracts the isolated rat aorta. *J. Auton. Pharmacol.,* **11,** 343 – 351.

SZABO, B., URBAN, R., LIMBERGER, N. & STARKE, K. (1995). Cardiovascular effects of agmatine, a ''clonidine-displacing substance'', in conscious rabbits. *Naunyn Schmiedebergs Arch. Pharmacol.,* **351,** 268 – 273.

TABOR, C.W. & TABOR, H. (1984). Polyamines. *Annu. Rev. Biochem.,* **53,** 749 – 790.

TESSON, F., LIMON-BOULEZ, I., URBAN, P., PUYPE, M., VANDEKERCKHOVE, J., COUPRY, I., POMPON, D. & PARINI, A. (1995). Localization of I2-imidazoline binding sites on monoamine oxidases. *J. Biol. Chem.,* **270,** 9856 – 9861.

TESSON, F., LIMON, I. & PARINI, A. (1992). Tissue-specific localization of mitochondrial imidazoline-guanidinium receptive sites. *Eur. J. Pharmacol.,* **219,** 335 – 338.

TESSON, F. & PARINI, A. (1991). Identification of an imidazoline-guanidinium receptive site in mitochondria from rabbit cerebral cortex. *Eur. J. Pharmacol.,* **208,** 81 – 83.

TESSON, F., PRIP-BUUS, C., LEMOINE, A., PEGORIER, J.P. & PARINI, A. (1991). Subcellular distribution of imidazoline-guanidinium-receptive sites in human and rabbit liver. Major localization to the mitochondrial outer membrane. *J. Biol. Chem.,* **266,** 155 – 160.

TOMB, J.F., WHITE, O., KERLAVAGE, A.R., CLAYTON, R.A., SUTTON, G.G., FLEISCHMANN, R.D., KETCHUM, K.A., KLENK, H.P., GILL, S., DOUGHERTY, B.A., NELSON, K., QUACKENBUSH, J., ZHOU, L., KIRKNESS, E.F., PETERSON, S., LOFTUS, B., RICHARDSON, D., DODSON, R., KHALAK, H.G., GLODEK, A., MCKENNEY, K., FITZEGERALD, L.M., LEE, N., ADAMS, M.D. & VENTER, J.C. (1997). The complete genome sequence of the gastric pathogen Helicobacter pylori. *Nature,* **388,** 539–547.

TRUJILLO, K.A. (1995). Effects of noncompetitive N-methyl-D-aspartate receptor antagonists on opiate tolerance and physical dependence. *Neuropsychopharmacology,* **13,** 301–307.

TSOLI, E., CHAN, S.L. & MORGAN, N.G. (1995). The imidazoline I1 receptor agonist, moxonidine, inhibits insulin secretion from isolated rat islets of Langerhans. *Eur. J. Pharmacol.,* **284,** 199–203.

ULIBARRI, I., SOTO, J., RUIZ, J., BALLESTEROS, J., JAUREGUI, J.V. & MEANA, J.J. (1999). I2-imidazoline receptors in platelets of patients with Parkinson's disease and Alzheimer's type dementia. *Ann. N.Y. Acad. Sci.,* **881,** 199–202.

VAN BIESEN, T., HAWES, B.E., LUTTRELL, D.K., KRUEGER, K.M., TOUHARA, K., PORFIRI, E., SAKAUE, M., LUTTRELL, L.M. & LEFKOWITZ, R.J. (1995). Receptor-tyrosine-kinase- and G beta gamma-mediated MAP kinase activation by a common signalling pathway. *Nature,* **376,** 781–784.

VARGIU, C., CABELLA, C., BELLIARDO, S., CRAVANZOLA, C., GRILLO, M.A. & COLOMBATTO, S. (1999). Agmatine modulates polyamine content in hepatocytes by inducing spermidine/spermine acetyltransferase. *Eur. J. Biochem.,* **259,** 933–938.

WANG, H., REGUNATHAN, S., MCGOWAN, D., BRAMWELL, S. & REIS, D.J. (1997). An antiserum to idazoxan recognizes an immunoreactive substance in human serum and cerebral spinal fluid which is not agmatine. *Neurochem. Int.,* **30,** 85–94.

WANG, H., REGUNATHAN, S., YOUNGSON, C., BRAMWELL, S. & REIS, D.J. (1995). An antibody to agmatine localizes the amine in bovine adrenal chromaffin cells. *Neurosci. Lett.,* **183,** 17–21.

WEITZEL, G., RENNER, R. & GUGLIELMI, H. (1971). Metabolic effects of arginine derivatives. I. Insulin like activity of arginyl compounds in vitro. *Hoppe Seylers. Z. Physiol Chem.,* **352,** 1617–1630.

WEITZEL, G., RENNER, R. & GUGLIELMI, H. (1972b). Metabolic effects of arginine derivatives. 2. Antilipolytic action of arginyl compounds. *Hoppe Seylers. Z. Physiol Chem.,* **353,** 535–539.

WEITZEL, G., STOCK, W. & GUGLIELMI, H. (1972a). Metabolic effects of arginine derivatives. 3. Insulin-like activity of L-arginine-N,N-dialkylamides in vitro. *Hoppe Seylers. Z. Physiol Chem.,* **353,** 1661–1670.

WENNMALM, A. & JUNSTAD, M. (1976). Prostaglandin-mediated inhibition of noradrenaline release: its resistance to variations in rate of prostaglandine synthesis. *Acta Physiol Scand.,* **97,** 60–65.

YAMAMOTO, S., NAKAO, H., YAMASAKI, K., TAKASHINA, K., SUEMOTO, Y. & SHINODA, S. (1988). Activities and properties of putrescine-biosynthetic enzymes in Vibrio parahaemolyticus. *Microbiol. Immunol.,* **32,** 675–687.

YANG, X.C. & REIS, D.J. (1999). Agmatine selectively blocks the N-methyl-D-aspartate subclass of glutamate receptor channels in rat hippocampal neurons. *J. Pharmacol Exp. Ther.,* **288,** 544–549.

ZEMBOWICZ, A., CHLOPICKI, S., RADZISZEWSKI, W., VANE, J.R. & GRYGLEWSKI, R.J. (1992a). NG-hydroxy-L-arginine and hydroxyguanidine potentiate the biological activity of endothelium-derived relaxing factor released from the rabbit aorta. *Biochem. Biophys. Res. Commun.,* **189,** 711–716.

ZEMBOWICZ, A., HECKER, M., MACARTHUR, H., SESSA, W.C. & VANE, J.R. (1991). Nitric oxide and another potent vasodilator are formed from NG-hydroxy- L-arginine by cultured endothelial cells. *Proc. Natl. Acad. Sci. U.S.A.,* **88,** 11172–11176.

ZEMBOWICZ, A., SWIERKOSZ, T.A., SOUTHAN, G.J., HECKER, M., GRYGLEWSKI, R.J. & VANE, J.R. (1992c). Mechanisms of the endothelium-dependent relaxation induced by NG-hydroxy-L-arginine. *J. Cardiovasc. Pharmacol.,* **20** (Suppl 12): S57–S59.

ZEMBOWICZ, A., SWIERKOSZ, T.A., SOUTHAN, G.J., HECKER, M. & VANE, J.R. (1992b). Potentiation of the vasorelaxant activity of nitric oxide by hydroxyguanidine: implications for the nature of endothelium-derived relaxing factor. *Br. J. Pharmacol.,* **107,** 1001–1007.

ZHU, Q.M., LESNICK, J.D., JASPER, J.R., MACLENNAN, S.J., DILLON, M.P., EGLEN, R.M. & BLUE, D.R. (1999). Cardiovascular effects of rilmenidine, moxonidine and clonidine in conscious wild-type and D79N alpha2A-adrenoceptor transgenic mice. *Br. J. Pharmacol.,* **126,** 1522–1530.

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