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Agmatine, an endogenous modulator of noradrenergic neurotransmission in the rat tail artery

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1 We investigated the vascular effects of agmatine (decarboxylated arginine), an endogenous ligand for α_2-adrenoceptors and non-adrenoceptor imidazoline (I-) receptors, present in endothelium, smooth muscle and plasma, using the rat tail artery as a model.

2 While by itself agmatine (10 nM - 1 mM) was without effect on isolated arterial rings, at the highest concentration used (1 mM) it slightly increased EC_{50} values for contractions elicited respectively by the α_1- and α_2-adrenoceptor agonists methoxamine and clonidine.

3 Agmatine (0.03 - 1 mM) produced a concentration-dependent transient inhibition of the contractions induced by transmural nerve stimulation (TNS; 200 mA, 0.2 ms, 1 Hz, 10 s). This effect was abolished by the α_2-adrenoceptor antagonists, rawolscine and idazoxan.

4 In the presence of rawolscine or idazoxan, agmatine produced a concentration-dependent delayed facilitation of TNS-induced contractions, which was prevented by cocaine.

5 Neither inhibitory nor potentiating actions were produced by agmatine on contractions induced by noradrenaline (NA) administration.

6 Agmatine did not directly affect [^{3}H]-NA uptake in bovine cultured chromaffin cells.

7 Agmatine can regulate vascular function by two opposing actions at sympathetic nerve terminals, with different latencies: a transient inhibition of NA release mediated by prejunctional α_2-adrenoceptors and a cocaine-sensitive delayed facilitation the mechanism of which is undetermined at present.

8 The results reveal the existence of a novel endogenous amine modulating NA release in the perivascular sympathetic terminals.

Keywords: α_2-Adrenoceptors; imidazoline (I-) receptors; noradrenergic transporter; transmural nerve stimulation; sympathetic nerves

Introduction

Agmatine is a polycationic amine synthesized from L-arginine, by the enzyme arginine decarboxylase (ADC). While long recognized as a product of bacteria, plants, and some invertebrates (Tabor & Tabor, 1984), agmatine and ADC have recently been identified in a number of mammalian tissues including brain, stomach, intestine and aorta (Li *et al.*, 1994; Delbarre *et al.*, 1995; Raasch *et al.*, 1995).

Like clonidine and allied drugs, agmatine binds to α_2-adrenoceptors and to the non-adrenoceptor binding sites recognizing some imidazolines and guanidiniums, the imidazoline (I-)receptor, with affinities (K_i) ranging between 1 to 10 μM (Li *et al.*, 1994; Piletz *et al.*, 1995; Pinthong *et al.*, 1995; Reis *et al.*, 1995; Regunathan & Reis, 1996). However, functions of agmatine mediated by α_2- or I-receptors have been uncertain. Agmatine has failed to act as either an agonist or antagonist at pre- or postsynaptic α_2-adrenoceptors in those preparations in which it has been tested *in vitro* including guinea-pig ileum, rat vas deferens, or porcine palmar vein (Pinthong *et al.*, 1995). Its actions at I-receptors are also unclear; agmatine has been reported to generate a small I-receptor-mediated inhibition on noradrenaline (NA) release in rabbit aorta (Molderings & Göthert, 1995), and an increased release of catecholamines from adrenal chromaffin cells (Li *et al.*, 1994).

Agmatine is present in blood vessels, where it is synthesized by endothelial cells and stored in endothelial and smooth muscle cells (Regunathan *et al.*, 1996). Whether agmatine can influence vascular contraction is not known. Most vessels contain α_2-adrenoceptors and I-receptors. Vascular α_2-adrenoceptors are expressed in smooth muscle, endothelium, and perivascular sympathetic nerves. Stimulation of these may, with cellular selectivity, differentially influence vascular tone: stimulation of the muscular receptor directly constricting vessels (Medgett & Langer, 1984; Dyke & Widdop, 1987), stimulation of endothelial receptors by generation of nitric oxide dilating them (Bockman *et al.*, 1993) and stimulation of prejunctional sympathetic receptors also relaxing smooth muscle indirectly by inhibiting NA release (Langer & Hicks, 1984). Vascular tissues also express I-receptors in smooth muscle and endothelium (Regunathan *et al.*, 1996), and presumably sympathetic nerves whose action in regulating NA release may dilate vessels in some beds (Molderings *et al.*, 1991; Molderings & Göthert, 1995).

In this study we have investigated the actions of agmatine on regulating contraction of the rat tail artery. This vessel has the advantages that the function of post-junctional α_2-adrenoceptors in regulating arterial tone has been characterized (Medgett & Langer, 1984; Xiao & Rand, 1989), and its dense sympathetic innervation permits analysis of regulation of vascular contraction by pre-junctional receptors. We demonstrate that agmatine, while without a direct action upon vascular smooth muscle, can inhibit the neurogenically mediated contraction of the rat tail artery by activating prejunctional α_2-adrenoceptors. However, it can also initate after a latent period a pre-junctional and cocaine-sensitive delayed facilitation

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the mechanism of which is uncertain at present. The results point to a potential action of this amine on regulation of vascular tone.

Methods

Measurements of isometric tension in rat tail artery rings

Male Sprague-Dawley rats (250–350 g) were anaesthetized with sodium pentobarbitone (50 mg kg^{-1}) and exsanguinated. The tail artery was removed, cleaned of residual blood and placed in oxygenated ice-cold physiological solution with the following composition (mM): NaCl 115, KCl 4.6, $CaCl_2$ 2.5, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.2, EDTA 0.01 and glucose 11. Arterial rings (2 mm length) were suspended on two intraluminal parallel wires, introduced in an organ bath containing the same physiological solution at 37°C, and connected to a Poidem strain gauge for isometric tension recording. All rings were equilibrated at a passive tension of 1 g; experiments started after an equilibration period of 90 min.

Cumulative concentration-response curves were constructed for agmatine (10 nM–1 mM), clonidine (10 nM–3 μM), methoxamine (10 nM–30 μM), and NA (0.1 nM–100 μM). To study the effects of agmatine on clonidine- and methoxamine-induced contractions, two consecutive curves for each agonist were performed in the same artery, allowing an interval of at least 1 h between them. After verifying that responses were identical, agmatine (3 μM–3 mM) was added 30 min before initiation of either the first or the second curve, and results were analysed in a paired way. For NA, a progressive decrease in the contractile response was observed when several concentration-response curves were obtained in the same artery. For that reason, the effects of agmatine and idazoxan on NA-induced contractions were studied in a 'sandwich' experiment. Three NA concentration-response curves were performed in each artery, the first and the third one in the absence of drugs and the second one either in the absence or in the presence of agmatine or idazoxan. After verifying that no difference was found when comparing the first or the third curves in all series, the second curves of control and treated vessels were compared. NA concentration-response curves were always obtained in the presence of 10 μM cocaine, in order to prevent any possible pre-junctional effect of agmatine on NA contraction.

Transmural nerve stimulation (TNS, 200 mA, 0.2 ms, 1 Hz, 10 s) was applied by use of two parallel platinum electrodes, one at each side of the vessel, connected to a CS-20 stimulator (Cibertec, Madrid, Spain). When studying the effects of agmatine on TNS responses, the drug was administered and TNS was applied 2, 12, 17 and 22 min later. The same protocol was repeated in the presence of rauwolscine, idazoxan, or prazosin, added to the organ bath 20 min before agmatine. Cocaine, when used, was present throughout the experiment. Responses to TNS generated in the presence of receptor antagonists or cocaine were considered as 100% when the action of agmatine was evaluated. Absolute values for contractions in the different conditions are presented in the figure legends.

The effect of agmatine on the response to single pulses of 0.1 μM NA was also analyzed at different times in independent experiments, in an attempt to mimic the intensity and duration of TNS-induced contractions. All experiments using TNS or NA were performed in the presence of 1 μM propranolol to prevent any effect mediated by β-adrenoceptors.

Measurements of [^{3}H]-NA uptake in chromaffin cells

To measure directly a possible effect of agmatine on the NA transporter, [^{3}H]-NA uptake was measured in bovine cultured chromaffin cells in the absence and presence of agmatine. Chromaffin cells were isolated from bovine adrenal medullae following standard methods (Livett, 1984) with some modifications (Moro *et al.*, 1990), and plated in 24-multiwell Costar plates at a density of 500,000 cells per well, in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% FCS, 10 μM cytosine arabinoside, 10 μM fluorodeoxiuridine, 50 iu ml^{-1} penicillin and 50 μg ml^{-1} streptomycin. Three days later, cells were pre-incubated with HEPES buffered saline solution (HBS) containing 100 μM pargyline and 300 μM ascorbic acid, for 20 min at 37°C, and then incubated with 65 nM [^{3}H]-NA (10.6 Ci $mmol^{-1}$) in the same medium for 30 min. Desipramine (100 μM), cocaine (10 μM), or agmatine (0.01–1 mM) were added as indicated for the last 10 min of preincubation and maintained throughout the experiment. Uptake was finished by washing the cells three times with HBS at 4°C. Cells were solubilized with 0.2 N sodium hydroxide overnight, the samples were neutralized with 0.2 N HCl and radioactivity was measured in 6 ml of Ready Safe (Beckman) scintillation fluid using a liquid scintillation spectrometer.

Statistical analysis

EC_{50} values are presented as geometric means with confidence limits, and significance was analysed by the Wilcoxon test. The rest of the data are presented as means ± standard errors. Responses in the presence of agmatine at different times were analysed by a one-way ANOVA. Comparisons between two treatments were made by a two-way ANOVA. Differences between individual pairs were further established with the method of Bonferroni. The probability of significance was accepted at $P<0.05$.

Chemicals

The following drugs were used: agmatine sulphate, cytosine arabinoside, clonidine HCl, cocaine, desipramine, fluorodeoxiuridine, idazoxan, methoxamine, N^{ω}-nitro-L-arginine methyl ester, noradrenaline bitartrate, pargyline and propranolol, from Sigma; rawolscine from Research Biochemicals International; [^{3}H]-noradrenaline (10.6 Ci $mmol^{-1}$) from Amersham and collagenase derived from *Clostridium histolyticum* from Boehringer-Mannheim.

Results

Postsynaptic effects of agmatine on rat tail artery

Agmatine did not affect the tension of the rat tail artery rings (Figure 1a). To rule out that a possible contraction effect was masked by a concomitant generation of nitric oxide (NO) by endothelial cells, agmatine was added to the vessels in the presence of the nitric oxide synthase (NOS) inhibitor N^{ω}-nitro-L-arginine methyl esther (L-NAME; 100 μM). Agmatine also failed to contract the vessels after L-NAME treatment (data not shown), confirming the lack of direct effect on agmatine on the vascular smooth muscle.

We therefore examined whether the amine could modulate contractions elicited by the α_2-adrenoceptor agonist, clonidine, by the α_1-adrenoceptor agonist methoxamine, or by the physiological neurotransmitter NA.

Clonidine dose-dependently contracted the tail artery (Figure 1a). When clonidine curves were generated in the presence of 30 μM or 1 mM agmatine, 1 mM agmatine slightly shifted the curve to the right (Figure 1a) with a small but significant increase in the mean EC_{50} value (Table 1). The lower agmatine concentration had no effect.

In agreement with the previous finding that α_2-adrenoceptor agonists potentiate the contraction induced by α_1-adrenoceptor stimulation in the rat tail artery (Xiao & Rand, 1989), clonidine at a sub-threshold concentration increased the contraction elicited by low concentrations of methoxamine (Figure 1b; Table 1). However, agmatine did

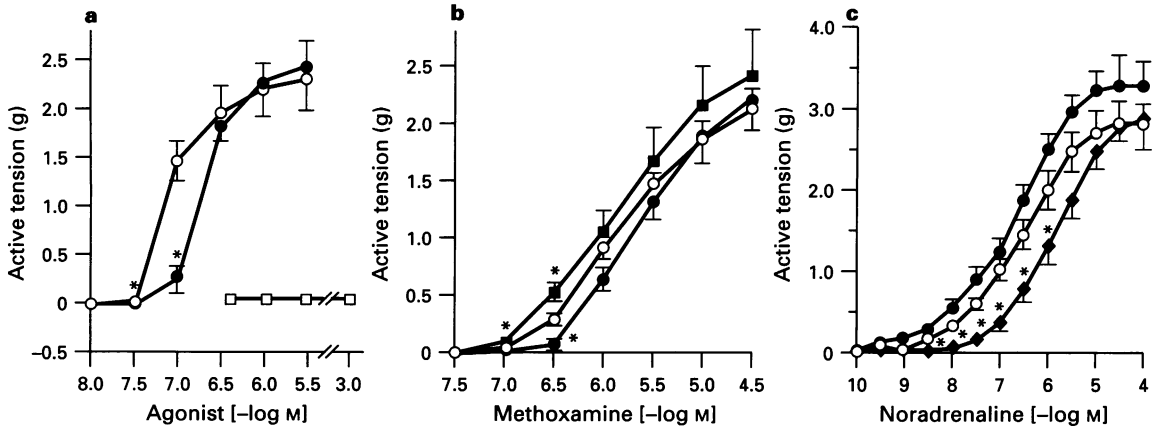

Figure 1 (a) Concentration-response curves for clonidine (○), agmatine (□), and clonidine in the presence of 1 mM agmatine (●) in rat tail artery rings. (b) Concentration-response curves for methoxamine in the absence (○) or in the presence of 10 nM clonidine (■) or 1 mM agmatine (●). (c) Concentration-response curves for noradrenaline in the absence (○) or in the presence of 3 μM idazoxan (◆) or 1 mM agmatine (●). Values represent the mean ± s.e. $n = 5-7$; $^*P < 0.05$.

Table 1 EC_{50} and E_{max} values for adrenoceptor agonists in the rat tail artery, in the absence and presence of different compounds

Agonist	*Compound*	*EC_{50}* (M)	*E_{max}* (g)
Clonidine	–	$0.9\ (0.6-1.3) \times 10^{-7}$	2.44 ± 0.33
Clonidine	Agmatine 1 mM	$2.6\ (1.8-3.7) \times 10^{-7}$*	2.81 ± 0.32
Methoxamine	–	$1.6\ (1.1-2.3) \times 10^{-6}$	2.41 ± 0.31
Methoxamine	Clonidine 10 nM	$1.1\ (0.7-1.7) \times 10^{-6}$*	2.48 ± 0.40
Methoxamine	Agmatine 1 mM	$2.8\ (2.3-3.3) \times 10^{-6}$*	2.41 ± 0.28
Noradrenaline	–	$2.2\ (1.6-2.9) \times 10^{-7}$	2.71 ± 0.28
Noradrenaline	Idazoxan 3 μM	$12.9\ (7.7-28.3) \times 10^{-7}$*	2.89 ± 0.15
Noradrenaline	Agmatine 1 mM	$1.5\ (1.1-2.6) \times 10^{-7}$	3.14 ± 0.21

Data are presented as geometric means and confidence intervals (EC_{50}) or as means ± s.e. (E_{max}). $^*P < 0.05$ (Wilcoxon test).

not mimic this action of clonidine and, in fact, at 1 mM modestly reduced the methoxamine-induced contraction (Figure 1b; Table 1).

The contraction elicited by NA was inhibited by idazoxan, indicating a contribution of α_2-adrenoceptors (Figure 1c, Table 1), as previously described in the rat tail artery (Xiao & Rand, 1989). Agmatine slightly potentiated the contractile response to NA, although the differences were not statistically significant (Figure 1c, Table 1).

Presynaptic actions of agmatine

We examined the effects of agmatine on contractions of rat tail arteries elicited by electrical stimulation of perivascular nerves. TNS-induced contractions were reproducible throughout the duration of the experiment in untreated arteries (Figure 2a, upper tracing) and were abolished by 1 μM phentolamine (data not shown), indicating that they were entirely due to the release of NA from the depolarized nerve endings. As shown in the middle and lower tracings of Figure 2a and in Figure 2c, agmatine produced a concentration-dependent inhibition of the TNS-induced contraction which was recovered within 10 min and followed in some cases by a small and delayed facilitation.

To determine whether the effects of agmatine were generated pre- or postjunctionally, a single dose of NA was added as a pulse to the organ bath to reach a concentration of 0.1 μM. This concentration elicited a contraction comparable in magnitude to that produced by 1 Hz TNS. The effects of 0.1 μM NA were evaluated at different times after addition of agmatine. As seen in Figure 2b and d, agmatine, in contrast to its effects on the TNS reponses, only slightly modified the responses to NA at the highest concentration (Figure 2d). This result suggests that the site of agmatine's action to inhibit the responses to TNS was prejunctional.

To characterize the receptor(s) responsible for agmatine's early inhibition of the contractile response to TNS, vascular rings were exposed to rauwolscine or idazoxan, and the experiments repeated. Both rauwolscine and idazoxan alone comparably reduced the responses to TNS as a result of inhibition of postsynaptic α_2-adrenoceptors (Medgett & Langer, 1984); this reduction was stable for the duration of the experiment in control arteries (see recording in Figure 6a, upper tracing). In the presence of either α_2-adrenoceptor blocker, the early inhibitory action of agmatine on TNS-induced contractions was prevented and, unexpectedly, a delayed significant potentiation of this response was revealed (Figure 3a and b, and Figure 6a lower tracing). In contrast, the α_1-adrenoceptor blocker prazosin, used at a concentration that results in a similar reduction of TNS-induced contractions did not modify the effects of agmatine (Figure 3a and b).

The effect of rauwolscine and idazoxan in preventing the inhibition of TNS-induced contractions elicited by 2 min incubations with agmatine was concentration-dependent (Figure 4). The estimated IC_{50} was approximately 0.1 μM for both antagonists in the presence of 100 μM agmatine. Thus the inhibitory action of agmatine on sympathetic vasoconstriction can be attributed to stimulation by the amine of presynaptic α_2-adrenoceptors.

The delayed potentiation of the TNS responses induced by agmatine in the presence of α_2-adrenoceptor antagonists was concentration-dependent (Figure 5a) with an EC_{50} of approximately 10 μM. Such a potentiation was not observed when contractions were elicited by exogenous NA (Figure 5b) instead of TNS, suggesting a pre-junctional site of action. To investigate the mechanism underlying the presynaptic potentiation of TNS responses induced by agmatine, two possibilities were considered: an increased NA release mediated via undetermined positive regulatory re-

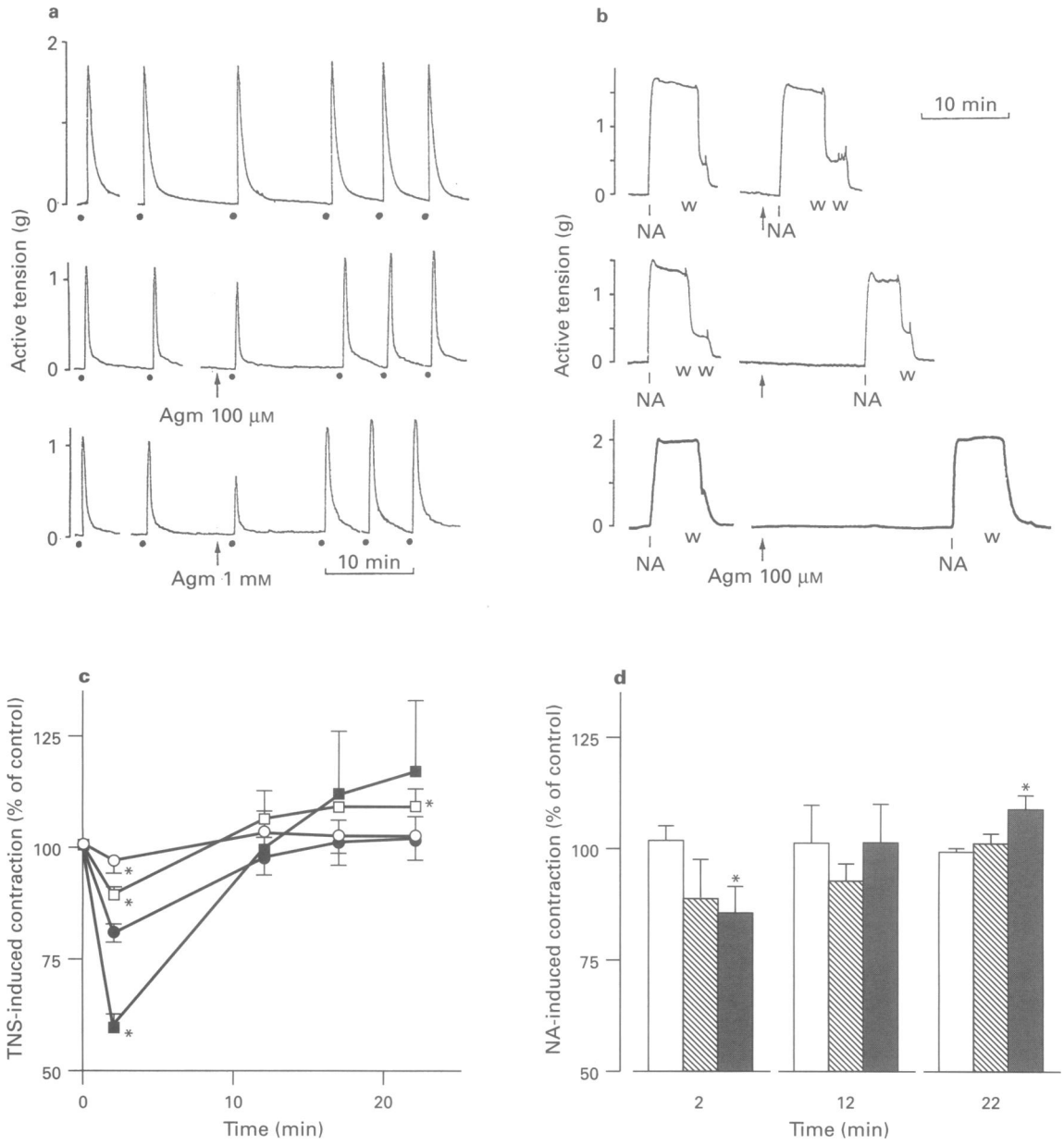

Figure 2 (a) Representative recordings showing the contractile response elicited by transmural nerve stimulation (TNS; black dots) in the rat tail artery, in control conditions (upper tracing) and after addition of 100 μM and 1 mM agmatine (middle and lower tracings). (b) Representative recordings showing the effect of 100 μM agmatine on the contractions produced in the rat tail artery by 0.1 μM noradrenaline (NA), administered at different times after agmatine addition. (c) Effects of increasing concentrations of agmatine on TNS-induced contractions in the rat tail artery, as a function of time. Agmatine: 0.01 mM (○); 0.03 mM (□); 0.1 mM (●); 1 mM (■). Agmatine was added at time 0. (d) Effects of incubation with agmatine for different times on NA-induced contractions in the rat tail artery. Agmatine: 30 μM (open columns); 100 μM (hatched columns); 1 mM (stippled columns). (c and d) Results are presented as percentages of the contraction obtained in the absence of agmatine for each arterial ring. Average initial contractions were 1234 ± 129 mg for TNS-induced responses and 1395 ± 165 mg for NA-induced responses. Values correspond to means ± s.e. $n = 5-9$; $^*P < 0.05$, as compared with values in the absence of agmatine.

ceptors and/or a decrease in NA re-uptake, since both mechanisms will increase NA concentrations in proximity to smooth muscle cells. To examine the latter possibility, experiments were repeated in the presence of cocaine. Responses to TNS in the presence of cocaine and of cocaine plus rauwolscine were stable in control arteries (Figure 6b, upper tracing). Addition of agmatine in the presence of cocaine and rauwolscine did not potentiate TNS-induced contractions (Figure 6b, lower tracing and c). Cocaine did not modify the effect of agmatine alone (Figure 6c).

A possible direct effect of agmatine on the NA-transporter was studied by measuring [^{3}H]-NA uptake in bovine chromaffin cells. Exogenous [^{3}H]-NA is actively taken up by these cells by a high affinity system similar to that seen at sympathetic nerve terminals (Kenigsberg & Trifaro, 1980). [^{3}H]-NA incorporation was prevented by both desipramine and cocaine, but was not modified by agmatine at concentrations up to 1 mM (Figure 7).

Discussion

This study sought to determine whether agmatine, a polycationic amine recently discovered to be present in mammalian tissue and serum (Li *et al.*, 1994; Raasch *et al.*, 1995) will modulate the contraction of the rat tail artery *in vitro*.

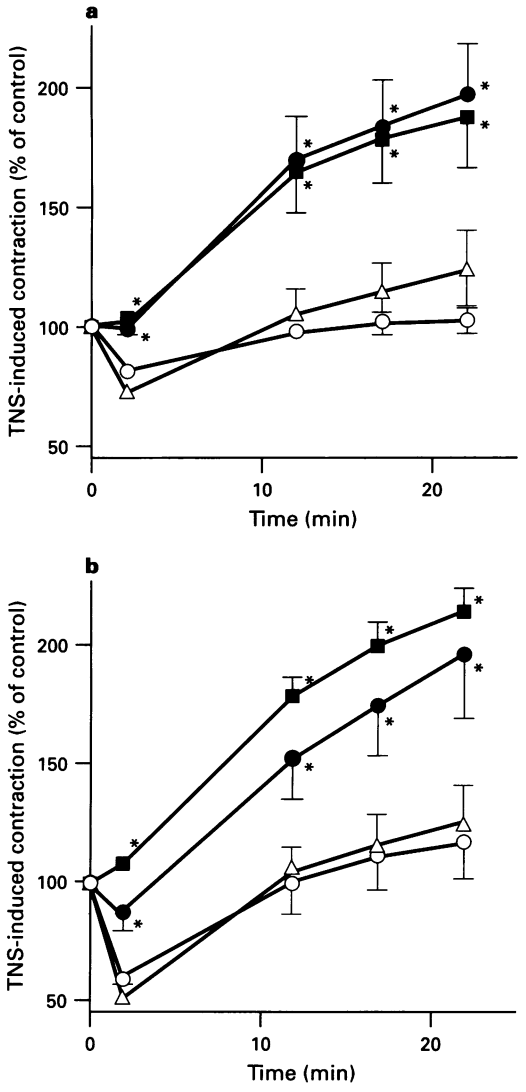

Figure 3 Effect of 0.1 mM (a) or 1 mM (b) agmatine on transmural nerve stimulation (TNS)-induced contraction in the rat tail artery in the absence (○) and presence of 0.1 nM prazosin (△), 1 μM rawolscine (●) or 1 μM idazoxan (■), as a function of time. Data are presented as percentages of the contraction obtained in the absence of agmatine. Control values were (mg): (a) 1188±107 for agmatine alone; 600±87 in the presence of prazosin, 404±121 in the presence of rauwolscine; 374±82 in the presence of idazoxan; (b) 1165±100 for agmatine alone; 481±88 in the presence of prazosin, 644±216 in the presence of rauwolscine; 469±97 in the presence of idazoxan. $n=6-9$; $^*P<0.05$, as comapred with agmatine alone.

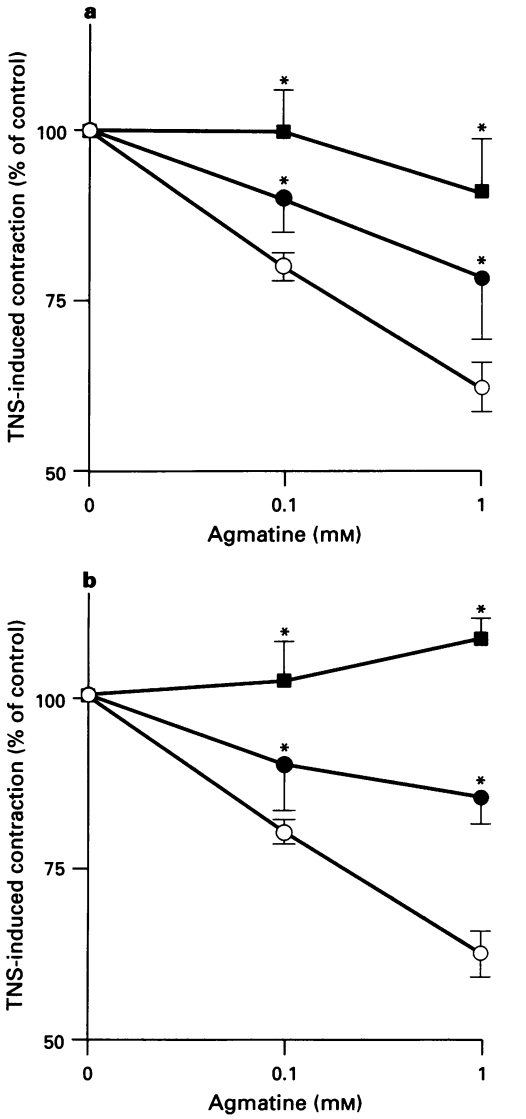

Figure 4 Antagonistic action of 0.1 μM (●) and 1 μM (■) rauwolscine (a) or idazoxan (b) on the inhibitory effect produced by a 2 min incubation with agmatine (○) on transmural nerve stimulation (TNS)-induced contraction in the rat tail artery. Data are presented as in Figure 3. Control values were (mg): 1210±84 for untreated arteries; 1118±179 for arteries treated with 0.1 μM rauwolscine; 510±141 for arteries treated with 1 μM rauwolscine; 923±163 for arteries treated with 0.1 μM idazoxan: 423±64 for arteries treated with 1 μM idazoxan. Values correspond to means±s.e. $n=6-10$; $^*P<0.05$, as compared with agmatine alone.

That agmatine may be relevant to vascular function derives from observations that the amine is stored in vascular endothelium and smooth muscle cells *in vivo* and *in vitro*, that its biosynthetic enzyme, ADC, is present in endothelium (Regunathan *et al.*, 1996), and that vascular smooth muscle and endothelium express receptors to which it binds: α_2-adrenoceptors (Piletz *et al.*, 1995; Pinthong *et al.*, 1995) and imidazoline receptors of the I_2 subclass (Regunathan *et al.*, 1996). Moreover, agmatine may inhibit stimulated proliferation of vascular smooth muscle (Regunathan & Reis, 1996). We demonstrate that agmatine can modulate contraction of blood vessels in two different ways: (1) by acting as an agonist at prejunctional α_2-adrenoceptors, it inhibits neurally evoked vasoconstriction; and (2) by a yet undetermined mechanism, it produces a delayed prejunctional facilitation of sympathetically-mediated vasoconstriction. The results provide evidence that agmatine is an endogenous modulator of neuronal transmission.

Agmatine by itself does not appear to have any direct postjunctional actions on the rat tail artery. Even though agmatine is a ligand at α_2-adrenoceptors (Piletz *et al.*, 1995) it failed to reproduce the responses associated with stimulation of these receptors including direct contraction or potentiation of the vasoconstriction elicited by stimulation of α_1-adrenoceptor agonists (Xiao & Rand, 1989). The finding is in agreement with conclusions of Pinthong *et al.* (1995) in the porcine palmar lateral vein. While the rightward displacement of the clonidine concentration-response curve at high concentrations of agmatine may indicate that the amine acts as a weak α_2-adrenoceptor antagonist, this was not confirmed when concentration-response curves to NA were examined.

Agmatine has been proposed as an alternative substrate for NOS in rat aortic rings (Ishikawa *et al.*, 1995); in addition, it could activate NOS by acting on endothelial α_2-adrenoceptors (Bockman *et al.*, 1993). However, although

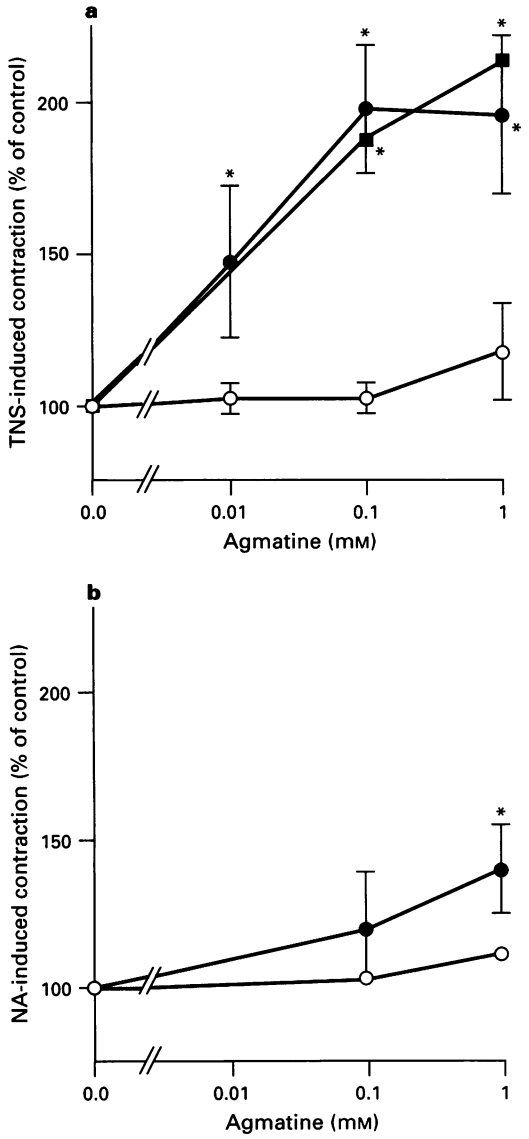

Figure 5 Effect of 22 min incubation with agmatine on the contractions elicited by transmural nerve stimulation (TNS) (a) or noradrenaline (NA) (b) in the rat tail artery, in the absence (○) and presence of 1 μM rauwolscine (●) or idazoxan (■). Data are presented as in Figure 3. Control values were (mg): (a) 1174±119 for agmatine alone; 510±141 for arteries treated with 1 μM rauwolscine; 423±64 for arteries treated with 1 μM idazoxan; (b) 1489±154 for agmatine alone; 920±152 for arteries treated with 1 μM idazoxan; (b) 1489±154 for agmatine alone; 920±152 for ateries treated with 1 μM rauwolscine. $n=6-9$; $^{*}P<0.05$, as compared with agmatine alone.

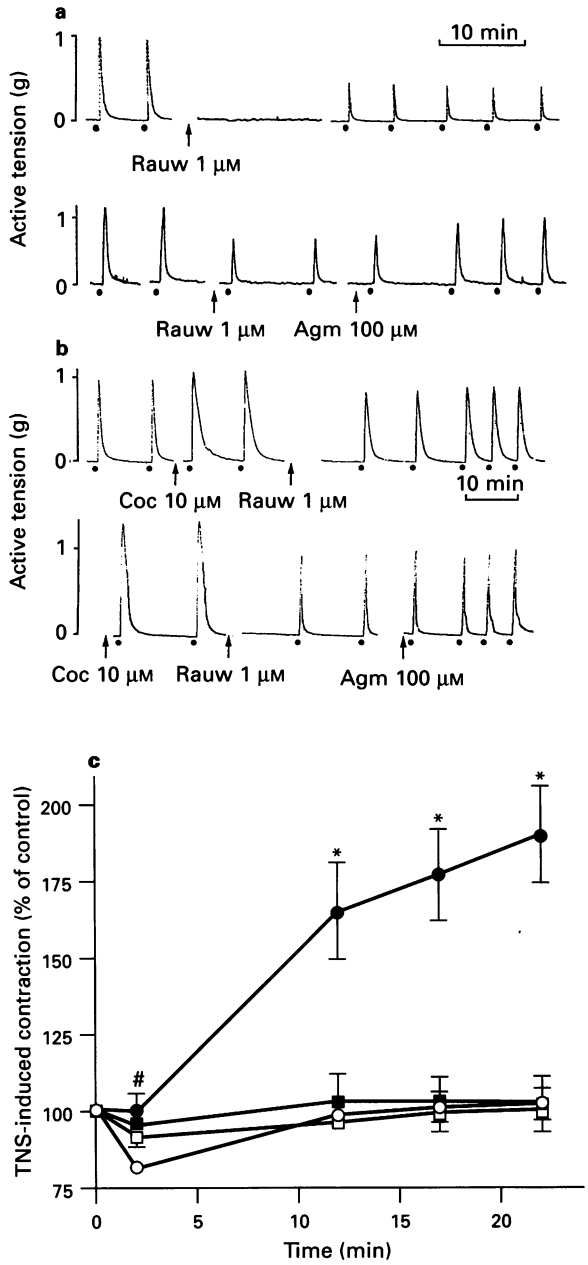

Figure 6 (a) Upper tracing. Representative recording showing the effects of 1 μM rauwolscine on the contractions elicited by transmural nerve stimulation (TNS; black dots) in a rat tail artery. The lower tracing shows the effect of 100 μM agmatine on TNS-induced contractions in an artery treated as above. (b) Upper tracing. Representative recording showing the effects of 1 μM rauwolscine on the contractions elicited by TNS in a rat tail artery pretreated with cocaine (coc). The lower tracing shows the effect of 100 μM agmatine on TNS-induced contractions in an artery tretaed as above. (c) Effect of 100 μM agmatine on the contractions elicited by transmural nerve stimulation (TNS) in the rat tail artery, in the absence (○) and in the presence of cocaine (□), rauwolscine (●), or cocaine plus rauwolscine (■). Data are presented as in Figure 3. Control values were (mg): 1188±107 for agmatine alone; 1822±335 in the presence of cocaine; 404±121 in the presence of rauwolscine; 1004±181 in the presence of cocaine plus rauwolscine. $n=8-10$. $^{*}P<0.05$ as compared with values in other conditions; $^{\#}P<0.05$ as compared with values obtained with agmatine alone.

agmatine produces a small relaxation in precontracted rat tail arteries (unpublished results), blockade of NOS by L-NAME did not reveal any contractile effect of agmatine in this vessel.

In contrast, agmatine significantly and substantially modified the vascular responses to stimulation of perivascular nerves in two different ways. The first was to inhibit rapidly and the second to facilitate, after a delay, the TNS-induced contraction. The biphasic action of agmatine suggested the presence of two different mechanisms of action appearing with different latencies. Although regulation of sympathetic nerve-smooth muscle signal transmission may differ between one organ and another, the coexistence of two opposed actions each with a different latency and time course may explain the failure of other investigators to detect any effect of agmatine on TNS-induced contractions in guinea-pig ileum or rat vas deferens (Pinthong *et al.*, 1995). Both the inhibitory and facilitatory mechanisms appeared to be prejunctional since agmatine did not substantially affect the vascular responses to directly applied NA at the concentrations at which it influenced TNS-evoked contractions.

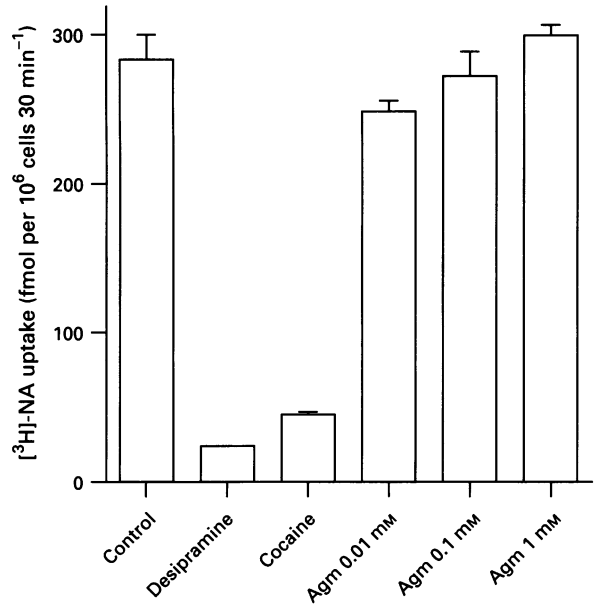

Figure 7 Uptake of [^{3}H]-noradrenaline ([^{3}H]-NA) by bovine chromaffin cells. Cells were incubated for 30 min at 37°C with 65 nM [^{3}H]-NA in the absence and presence of 100 μM desipramine, 10 μM cocaine, or 0.01–1 mM agmatine (Agm). Data represent the mean ± s.e. of the results obtained in four independent cultures.

Since both imidazolines and α_2-adrenoceptor agonists inhibit NA release in vascular preparations (Langer & Hicks, 1984; Molderings & Göthert, 1995), the inhibitory effect of agmatine was analysed in the presence of either rauwolscine, a pure α_2-adrenoceptor antagonist, or idazoxan, an α_2-adrenoceptor antagonist which also binds to imidazoline receptors, preferably of the I_2 subclass. The comparability of the blockade produced by both antagonists indicated that the inhibition by agmatine of TNS-induced contractions was the result of stimulation of α_2-adrenoceptors.

The delayed and persistent facilitation of TNS responses produced by agmatine in the presence of α_2-adrenoceptor antagonists was in turn prevented by cocaine, an inhibitor of the NA uptake system in sympathetic nerve terminals. This may explain why only an inhibition was detected when the effects of agmatine on [^{3}H]-NA efflux by the rabbit aorta were studied (Molderings & Göthert, 1995), since these experiments were routinely performed in the presence of cocaine. The ability of cocaine to prevent the prejunctional potentiating action of agmatine suggested an involvement of the NA transporter in its mechanism of action. However, we could not demonstrate a direct action of agmatine on this transport system, using cultured chromaffin cells, which have a high affinity NA uptake system similar to that of sympathetic nerve terminals (Kenigsberg & Trifaro, 1980). Therefore, the cocaine-sensitive mechanism behind the potentiating effect of agmatine remains undetermined.

The present study therefore provides further evidence that agmatine may have a function in the biology of blood vessels. This endogenous amine, stored and synthesized in neurones (Wang *et al.*, 1994), blood vessels (Regunathan *et al.*, 1996) and present in serum may influence vascular contraction via presynaptic mechanisms, as demonstrated here in the rat tail artery. Agmatine has a cocaine-sensitive potentiating effect on sympathetically mediated contractions. This effect is opposed by an inhibition of NA release resulting from stimulation of α_2-adrenoceptors. The existence of a naturally occurring substance with such effects may reveal a novel physiological mechanism of sympathetic regulation.

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