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RX 821002 as a Tool for Physiological Investigation of α_2-Adrenoceptors

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ABSTRACT

RX 821002 is the 2-methoxy congener of idazoxan. In binding and tissue studies it behaves as a selective antagonist of α_2-adrenoceptors, with at least 5 times greater affinity for these receptors than any other binding site. It does not select between the different types of α_2-receptor. Although this drug probably has no future as a therapeutic agent, it remains a good probe for physiological activity at α_2-adrenoceptors in animal experiments. A particularly useful feature of this compound is its lack of binding at I_1 and I_2 imidazoline receptors. However, it has relatively high affinity for 5-HT_{1A} receptors (at which it acts as an antagonist) and a tendency to behave as an inverse agonist at α_{2A}-adrenoceptors in some cell culture systems. These potential drawbacks may be overcome by careful design of experiments, and the greater selectivity of RX 821002 renders it much superior to yohimbine or idazoxan as a tool for probing physiological actions at α_2-receptors. It can be compared favorably with other selective antagonists such as atipamezole.

In physiological studies, RX 821002 augments norepinephrine release in the frontal cortex and increases drinking behavior in rat. In rabbit, intrathecal administration of this drug enhances somatic and autonomic motor outflows, showing that tonic adrenergic descending inhibition of withdrawal reflexes and sympathetic pre-ganglionic neurons is strong in this species. The potentiation of reflexes may be considered a pro-nociceptive action. In the same model, RX 821002 antagonizes the inhibitory effects of the μ opioid fentanyl, indicating that exogenous opioids synergize with endogenously released norepinephrine in the spinal cord. Thus, the careful use of RX 821002 has revealed several aspects of the physiological activity of α_2-adrenoceptors in rabbit spinal cord and rat brain. We recommend that RX 821002 and/or compounds with similar selectivity for α_2-adrenoceptors (atipamezole, MK-912, RS-79948) should be used in preference to yohimbine or idazoxan in all future studies of this type.

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INTRODUCTION

The α_2-adrenoceptors, a group of 3 to 4 different gene products (see below), mediate the major central nervous system actions of norepinephrine, including control of mood state, arousal, endocrine function, autonomic and somatic motor outflows, and modulation of sensory inputs, particularly pain (95). The involvement of these receptors in such important processes has made them a key target for drug developers and the clinical uses of α_2-receptor agonists include anesthesia, sedation, and control of hypertension, spasticity and pain (61,63). It is evident that receptors, which mediate such an impressive range of pharmacological effects, should also have major physiological functions, amenable to investigation by use of suitable receptor antagonists.

By virtue of their roles in presynaptic control of central adrenergic and other monoaminergic neurons, it was predicted that α_2-adrenoceptor antagonists might have utility in the treatment of depression, Parkinson's disease and impotence (2). However, clinical trials with α_2-adrenoceptor antagonists have, for various reasons, not been positive (79) and the only current clinical use of such drugs is to reverse the effects of agonists. Nonetheless, the effort put into designing selective antagonists of α_2-adrenoceptors has yielded a number of compounds, including RX 821002, that can, when used with care, indicate physiological activity at α_2-adrenoceptors.

The use of an antagonist in physiological experiments ideally requires a drug that is absolutely specific for the receptor under investigation. As no such agent exists, it is important to use the most selective antagonist(s) available and/or to learn as much as possible about the pharmacology of the molecule in question. Until the 1980s the antagonist of choice for probing the physiology of α_2-adrenoceptors was the alkaloid yohimbine, or its enantiomer rauwolscine (Fig. 1). These drugs give rather poor α_2/α_1 separation (31) and bind with moderate affinity to 5-HT_{1A}-receptors (23), at which they are agonists (27,79). This last factor, added to the recent observation that rauwolscine behaves as a partial agonist at α_{2A}-adrenoceptors in some tissues (78), effectively negates the use of yohimbine and rauwolscine as probes in physiological studies, as it becomes impossible to interpret their actions only in terms of receptor blockade.

The imidazolidine idazoxan (formerly RX 781094, Fig. 1) was developed by Reckitt and Coleman in the late1970s and represented something of a breakthrough in α_2-adrenoceptor antagonist design, giving better α_2/α_1 selectivity than yohimbine (13,31). This agent exhibits some lack of specificity, being a high affinity ligand at imidazoline receptors (see below) and a partial agonist at 5-HT_{1A}-receptors (79). Subsequent development of the idazoxan molecule showed that bulky substitutions at the 2-position of the benzodioxanyl ring gave improved potency and selectivity at the α_2-receptor. Two molecules showed particularly good characteristics: 2-ethoxy-idazoxan (2-(2-ethoxy-2,3-dihydro-benzo[1, 4]dioxin-2-yl)-4,5-dihydro-1H-imidazole, RX 811059) and 2-methoxy-idazoxan (RX 821002, 2-(2-methoxy-2,3-dihydro-benzo[1,4]dioxin-2-yl)-4,5-dihydro-1H-imidazole, Fig. 1) gave improved potency at α_2-adrenoceptors and α_2/α_1 selectivity ratios of > 100 (103,114). Furthermore, RX 821002 retains its pharmacological selectivity after tritiation and makes an excellent radioligand for use in receptor binding studies, autoradiography and PET (3,53,54,56,112). It has become the radioligand of choice for studies on α_2-adrenoceptor pharmacology.

The real significance of the development of substituted idazoxans became apparent in the late 1980s when evidence for the existence of non-adrenergic imidazoline binding

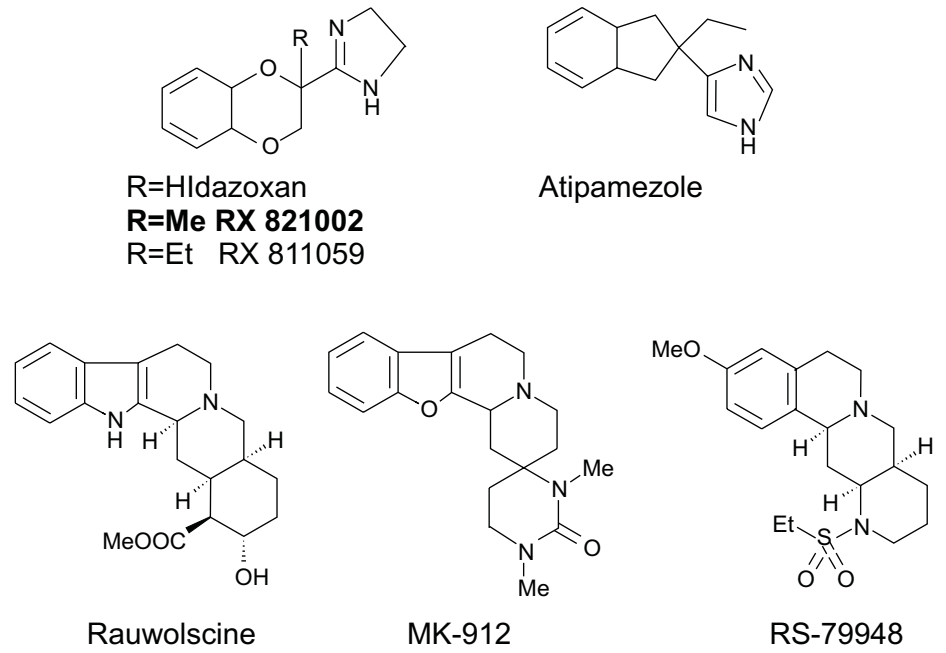

Fig. 1. Chemical structures of the main α_2-adrenoceptor antagonists discussed. Idazoxan and its congeners and atipamezole are imidazoline-based compounds (although strictly speaking the idazoxans are imidazoldines), whereas MK-912 and Rs-79948 have been developed from the yohimbine molecule (rauwolscine is the r-enantiomer of yohimbine).

sites began to emerge (76). These binding sites, now recognized as true receptors in their own right (see below, also 32), were first identified when it was found that, in some tissues, not all idazoxan binding could be displaced by norepinephrine (46,67). It is now known that idazoxan has approximately equal affinity for imidazoline and α_2-adrenoceptors (38). Neither RX 811059 nor RX 821002 has any appreciable affinity for non-adrenergic idazoxan binding sites, rendering these agents the preferred tools for studying α_2-adrenoceptor physiology (53). This review covers the pharmacology of RX 821002 and provides a limited comparison of this agent with other selective α_2-adrenoceptor antagonists. It concludes with a brief overview of some of the physiological functions of norepinephrine that have been revealed or confirmed by the use of this compound, with particular reference to its actions in the spinal cord.

PHARMACOLOGY OF RX 821002

Pharmacokinetics

Very few data have been published on the physicochemical characteristics, or biological distribution and elimination of this compound *in vivo*. Clinical work on the molecule is available through a commercial subscription database (see 79). In our experience it is readily soluble in ionic solutions (150 mM NaCl, Ringer's) and we regularly use it in concentrations of 4 to 8 mg/mL. In rabbit, it has effects that persist in excess of 1 h, but

we have not made definitive measurements of the duration of action. It has central effects when administered systemically in rat (54) and in rabbit (R. W. Clarke, J. Harris, and C. Stanley, unpublished observations), and is, therefore, able to cross blood brain barrier.

Pharmacological Characterization

In vitro studies show RX 821002 to be a potent, surmountable, silent and highly selective antagonist at α_2-adrenoceptors in rat vas deferens. It blocks the action of brimonidine (UK 14, 304) in that tissue, with a pA_2 of 9.4. In rat annococcygeus muscle, an assay for α_1 adrenoceptor activity, it showed much lower affinity and the α_2/α_1 potency ratio calculated from these studies was in excess of 300 (103,114). All subsequent pharmacological studies with the compound have supported these findings (e.g., 68).

Receptor Binding Studies

α-Adrenoceptors

Like all drugs developed before the regular use of multi-receptor screens, RX 821002 has revealed its secrets slowly. No data have yet emerged to render it unusable in physiological studies. It is a competitive antagonist at α_2-adrenoceptors generally with an affinity in the order of 1 to 6 nM, appreciably lower than at other receptors so far investigated (Table 1). It has rapid association kinetics at α_2-adrenoceptors (53,68), but dissociates rather slowly, so that in some assays it behaves in the manner of a non-surmountable antagonist (65).

RX 821002 appeared at time when the molecular characterization of α_2-adrenoceptor types was approaching resolution. It is now accepted that there are three main classes of α_2-adrenoceptor, namely α_{2A}, α_{2B} and α_{2C}, which in humans correspond to the products of the α_2C_{10}, α_2C_2 and α_2C_4 genes, respectively (1,50). There are however marked species differences in the A type receptor. The human, porcine and rabbit α_{2A} adrenoceptors are pharmacologically very similar, but the equivalent receptor in rat, mouse, guinea pig and cattle is distinctive, particularly in respect to its low affinity for yohimbine (1,9,30). In acknowledgement of these differences, this receptor is sometimes known as α_{2D} (107). All types have been found in the CNS, but the dominant α_2-receptor in the brains of all species is type A, accounting for up to 90% of α_2-binding in some brain regions (109,110). For a time it was held that RX 821002 was selective for α_{2A}-receptors, as it is resistant to displacement by prazosin, which has moderate affinity for α_{2B} and α_{2C} receptors (68). However, subsequent studies revealed that RX 821002 has high affinity for all types of α_2-receptor (28,80,108), which presumably explains why [^{3}H]-RX 821002 appears to bind to a single site in displacement studies (e.g., 53). The drug is now used routinely as marker for total α_2-adrenoceptor binding in brain and other tissues (33,54). Nonetheless, RX 821002 shows consistently higher affinity for the α_{2D} (rat/mouse/guinea pig/bovine α_{2A}) than for other α_2-adrenoceptors (107). It should be noted that the two antagonists regularly employed as type-selective (ARC 239 (2-[2-[4(o-methoxyphenyl)-piperazine-1-Yl]-ethyl]-4,4-dimethyl-1,3(2H-4H) isoquinolinedione) and BRL 44408 ((±)-2-((4,5,-dihydro-1H-imidazoyl-2yl)methyl-2,3-dihydro-1-methyl-1H-isoindole) for α_{2A} and α_{2B}, respectively), have significant affinity for 5-HT_{1A} receptors (73).

TABLE 1. Binding constants for RX 821002 at relevant receptors

Receptor	$h\alpha_{2A}$	$h\alpha_{2B}$	$h\alpha_{2C}$	$r\alpha_{2A\ (D)}$	$r\alpha_1$	rI_1	rI_2	rI_3	h5-HT_{1A}	r5-HT_{1A}	gp5-HT_{1B}	hD_2	hD_3
K_D (nM)	0.29 – 5.01	4.42 – 10.2	3.98 – 6.80	0.32 – 0.63	66	> 1000	> 1000	124	24.1 – 25.0	47.9	> 1000	> 1000	> 1000
References	28, 39, 79, 80, 108	28, 39, 79, 80, 108	28, 39, 79, 80, 108	28, 39, 79, 80	114	38	38	12	39, 79	79	39	39	39

h, human receptor; r, rat receptor; gp, guinea pig receptor.

Imidazoline receptors

RX 821002 came to the fore as an agent for differentiating between actions mediated by adrenoceptors and imidazoline receptors. Soon after the introduction of idazoxan, it was reported that a significant proportion of its binding in rabbit brain (46) and adipocytes (67) could not be displaced by epinephrine. This site also showed a high affinity for the guanidium compound, amiloride (75,76). This receptor, originally described as the non-adrenergic idazoxan (or imidazoline) binding site (75,76), was subsequently classified as the I_2 imidazoline receptor. It is an interesting entity, with a predominantly mitochondrial location and a very close relationship with monoamine oxidase (86). Its distribution matches closely, but not exactly, that of the α_2-adrenoceptor (70,71) and its functions remain undefined. Although a number of compounds have been developed that are highly selective for this site (52,84), the endogenous ligand is as yet unidentified and it is not possible to characterize molecules as agonists or antagonists for the receptor. In rat, I_2 selective compounds given *in vivo* enhance release of norepinephrine in the brain (66), are antinociceptive (29,51), antagonize the development of tolerance to morphine (4), and are mildly hyperphagic (6,90). In rabbit such drugs increase arterial blood pressure and spinal reflexes (18).

Another major imidazoline receptor, I_1, was characterized by the epinephrine-resistant binding of *para*-aminoclonidine to bovine medulla (34,35). This site has a completely different pharmacology to I_2 and is apparently membrane bound (32). It has been proposed as a major target for imidazoline-based, centrally acting antihypertensive drugs, particularly moxonidine and rilmenidine (5), but has also been implicated in spinal antinociceptive mechanisms (36,37). However, most drugs that are ligands for these receptors are also α_2-adrenoceptor agonists and it has been difficult to secure definitive evidence for I_1 involvement in blood pressure control (32,43). Recent work with the I_1 selective compound S23515, (±)5-(2-bromophenoxyl)methyl-2-amino-4,5-dihydro-1,3 oxazole, suggests that I_1 receptor ligands are hypotensive and that, in this respect, they act synergistically in combination with α_2-adrenoceptor agonists (7).

It is evident that, notwithstanding our incomplete understanding of imidazoline receptor pharmacology, there is considerable overlap between the putative functions of I_1, I_2 and α_2-adrenoreceptors, so it is important to use drugs that discriminate between them in physiological experiments. RX 821002 can do this, as it shows very low affinity for I_2 or I_1 receptors (38,53,68,77). However, it does bind to a type of non-adrenergic imidazoline receptor in pancreas that is distinct from those defined above and is sometimes called the I_3 receptor (32). RX 821002 acts through this low affinity site to stimulate insulin secretion (11,12). Yet another non-adrenergic binding site for RX 821002 has been located in rat kidney (8), but the significance of this site has yet to be established. Neither of these binding sites has been described in central nervous system, and virtually all RX 821002 binding to rat brain can be displaced by epinephrine (54). It would appear that it is still safe to consider methoxy-idazoxan as selective for adrenoceptors in the CNS.

5-HT_{1A}-Receptors

A major concern over the selectivity of RX 821002 emerges from its interactions with 5-HT_{1A}-receptors. In common with many α_2-ligands, it binds with moderate affinity (20–30 nM) to these sites (42,79,112). There is a greater separation of affinities between rat α_2 and 5-HT_{1A} receptors than for their human counterparts (79, Table 1). These two receptors are found in many of the same CNS locations and there is real potential for confusion between their actions. Fortunately, unlike idazoxan or yohimbine, which are partial

agonists at 5-HT_{1A} receptors, RX 821002 behaves as a pure antagonist at these sites *in vitro* (79) and *in vivo* (81). In the latter case it shows somewhat lower activity at 5-HT_{1A}-receptors than would be predicted from binding data, so that in rabbit, intrathecal doses < 100 μg are α_2-selective (81). Nonetheless, this is a factor that must be considered very carefully and controlled for when interpreting the effects of RX 821002 in physiological studies.

Glutamate receptors

At high micromolar concentrations, RX 821002 displaces dizocilpine (MK-801) from the phencyclidine binding site of the glutamate *N*-methyl-d-aspartate receptor, and is neuroprotective against cell death in cerebellar granule cells caused by glutamate, but not by apoptosis (85). However, RX 821002 had no protective action in a rat model of global brain ischemia in which idazoxan was effective (26). It is unlikely that RX 821002 has much future as a neuroprotective agent, or that its binding to NMDA receptors is a significant feature of its actions in physiological studies. However, it is worth noting that atipamezole (Fig. 1), another highly selective, imidazoline-based α_2-adrenoceptor antagonist, shows much promise as a neuroprotective agent in animal models (59). It is uncertain whether this effect is due to an action at α_2- or imidazoline receptors.

RX 821002 as an Inverse Agonist at α_2-Adrenoceptors

The use of cell culture systems expressing high levels of cloned receptors, with or without specific mutations, has provided a great deal of interesting data for students of receptor pharmacology. Combining expression of human α_{2A}-receptors (particularly with a mutation at Thr^{373}) with rat $G_{\alpha 0}$ protein gives rise to a constitutively active receptor that tonically suppresses production of cyclic AMP (87,113,116). Under these rather artificial circumstances, RX 821002, in common with yohimbine/rauwolscine, usually behaves as an inverse agonist at α_{2A}-adrenoceptors (87,113). More pertinently, a similar result has been obtained with native α_{2A}-adrenoceptors in rat brain slices treated with 100 μM GTP (78). The level of constitutive activity in α_2-adrenoceptors *in vivo* is unknown, but it is clear that the possibility of an inverse agonist action must be considered when interpreting effects obtained with RX 821002 in physiological experiments.

Comparison with Other Selective α_2-Receptor Antagonists

As mentioned above, yohimbine/rauwolscine should not be used to probe the physiological actions of α_2-adrenoceptors because of its partial agonist activity at 5-HT_{1A}-receptors. Idazoxan also cannot be recommended for the same reasons, in addition to its high affinity for I_1 and I_2 receptors (38). However, a number of compounds are available that could be used as alternatives to RX 821002 and that may be superior in some respects, of which the best candidates appear to be atipamezole (45), MK-912 ((2S,12bS)1′,3′-dimethylspiro(1,3,4,5′,6,6′,7,12b-octahydro-2H-benzo[b]furo[2,3-a]quinolizine)-2,4′-pyrimidin-2′-one (89)) and RS-79948-197 ((8aR,12aS,13aS)-5,8, 8a,9,10,11,12,12a,13,13a-decahydro-3-methoxy-12-(ethylsulphonyl)-6H-iso-quino[2,1-g][1,6]naphthyridine (108), Fig. 1). All show slightly higher affinity for α_2-adrenoceptors, compared to RX 821002, but are not particularly selective for any type of α_2-adrenoceptor (108). In PET studies, [^{11}C]-RS-79948-197 gives approximately twice the level of specific binding in rat entorhinal cortex as found with [^{3}H]-RX 821002 (55). Both atipamezole and MK-912 have inverse agonist

activity at α_{2A}-adrenoceptors, although there is less agreement about this than has been reported for RX 821002 (58,78,87,113). RS-79948-197 has not been tested for inverse agonist activity.

The one clear advantage of atipamezole over RX 821002 is in its low affinity for 5-HT_{1A}-receptors, at which it is essentially inactive (79). MK-912 also appears to have good selectivity over "5-HT_1" receptors (89), but has not been tested against 5-HT_{1A} sites specifically. No data are available for RS-79948-197 binding at these receptors, but this compound is known to have low affinity for I_2 imidazoline receptors (55). No published data are available on the affinities of atipamezole or MK-912 at I_1 or I_2 imidazoline receptors. MK-912 is not an imidazoline and, therefore, probably does not bind to these sites. It labels only a single population of α_2-adrenoceptors in guinea pig kidney, which is known to contain both main types of imidazoline receptor (111). Atipamezole, on the other hand, does have an imidazoline structure, and binds with moderate affinity (40 nM) to non-adrenergic binding sites in rat lung and kidney that are distinct from I_1, I_2, or I_3 receptors (98,99). It can also displace dexmedetomidine from non-adrenergic sites in rat spinal cord (97), which are again distinct from the characterized imidazoline receptors. However, it does not fully reverse the antinociceptive action of the selective I_2 ligand BU-224 after intrathecal administration in rat (29). It is difficult to know exactly what this means in terms of "physiological" actions of atipamezole, but these findings show that care must be taken whichever selective antagonist one chooses to probe α_2 adrenoceptor function. In our opinion there is little to choose between any of these drugs in terms of selecting an agent for physiological studies, although RX 821002 and atipamezole have been more thoroughly characterized than the other two, and are available commercially.

PHYSIOLOGICAL STUDIES WITH RX 821002

Pharmacological investigations have utilized selective antagonists extensively and much of the literature on RX 821002 centers on its use in such experiments, or as a selective marker in binding studies. Fewer data are available on the effects of RX 821002 in physiological studies in which the drug has been given without prior administration of an agonist, to probe for endogenous activity at α_2-receptors. RX 821002 reached phase I clinical trials for use as an antidepressant (79), but the data on its activity are available only through a commercial database. A single open access study with RX 811059, the 2-ethoxy congener of idazoxan, showed that this agent induced mild increases in blood pressure and minor alterations in state of attention in human subjects, results similar to those obtained with other selective antagonists (25). It is unlikely that RX 821002 has any future as a human medicine, but it remains a useful tool for animal studies. In this section, the focus is on the effects of RX 821002, when it has been given alone, on release of monoamines in the brain, on behavior, and on spinal cord function.

Release of Monoamines in the CNS and Behavior

α_{2A}-Adrenoceptors (and perhaps also α_{2C}-adrenoceptors) are autoreceptors on the terminals of adrenergic neurons and the cell bodies of locus coeruleus neurons, and are also located on the terminals of other neurons with monoamine transmitters (101,102). Blockade of these receptors should, therefore, increase the release of brain monoamines, which appears to be the case. By systemic administration at low doses to rats RX 821002

increases extracellular norepinephrine and dopamine, but not 5-HT levels, in frontal cortex (39). Similar results for norepinephrine release in frontal cortex were recorded by Hudson et al. (54), who further showed augmented release of norepinephrine in ventral hippocampus, and dopamine in the striatum. RX 821002 also enhances norepinephrine release in frontal cortex after administration of the uptake blocker sibutramine (115). Direct application of the antagonist to locus coeruleus induces a large increase in norepinephrine output in the cingulate cortex *per se* (72), and augments the norepinephrine releasing action of desipramine in the same area (74). These data indicate that α_2-adrenoceptors tonically inhibit release of catecholamines in the frontal cortex, and probably other parts of the brain.

No doubt these changes in amine release underlie the behavioral actions of RX 821002 and other selective antagonists. In human subjects, blockade of α_2-receptors is generally mildly anxiogenic (62,64). Animal studies with yohimbine have discovered a rather subtle spectrum of behavioral alterations, not all of which are shared by more selective antagonists. The actions that have received most attention are anxiogenesis (usually measured by exploration of new environments) and enhanced male sexual performance (94). To our knowledge, RX 821002 has not been evaluated for the former action, although there is evidence for and against the involvement of α_2-adrenoceptors in anxiogenesis in studies using more selective antagonists (44,92). Somewhat surprisingly, low dose RX 821002 slightly decreases erectile function and pelvic thrusting in the dog (118). It is clear that all behavioral data obtained with yohimbine alone must be considered suspect unless supported by one or more of the more selective antagonists. For instance, yohimbine has been shown to increase drinking behavior induced by angiotensin II (10). The enhanced water intake is a marked feature of the behavioral effects of RX 821002 when it is given alone (57,90), thereby suggesting that control of water intake is a true physiological role of central α_2-adrenoceptors.

Spinal Cord Function

In the spinal cord, α_2-adrenoceptors are associated with inhibition of pain, reflex function (with concurrent activation of the locomotor central pattern generator), and autonomic outflow. RX 821002, applied to the spinal cord of rats, has no effect on baseline withdrawal reflex thresholds in control or inflamed states (41,51). This finding is in keeping with the generally weak effects of α_2-adrenoceptor antagonists on reflex function in this species and presumably indicates a low level of tonic activity in spinally-projecting adrenergic neurons (although see 96). There is some evidence that adrenergic descending activity increases inflammation in rat (100).

In contrast, α_2-adrenoceptor antagonists, including RX 821002, powerfully enhance withdrawal reflexes in the rabbit, an action that could be considered pro-nociceptive. These studies have focussed primarily on reflexes evoked in medial gastrocnemius (MG) motoneurons by stimulation of afferents from the heel. In decerebrated rabbits, MG responses to electrical stimulation of the sural nerve (which carries input from the heel) are enhanced to approximately 4-fold by RX 821002, RX 811059 and idazoxan, are increased only 2-fold by yohimbine, and not facilitated at all by prazosin (47,48). Similar results are obtained in anesthetized animals with little preparative surgery (83), or when MG activity is evoked by mechanical stimulation at the heel (17), showing that adrenergic tone is not an epiphenomenon of surgical preparation or electrical stimulation. In recent studies we have shown that RX 821002 increases reflexes evoked by all classes of cutaneous afferent axons (14). When rabbits are spinalized in the presence of idazoxan or RX 821002, re-

flexes evoked by large myelinated sural nerve afferents decrease in size (17,20,47,48,83), indicating that part of its effect is mediated through release of descending facilitatory systems (see below). No effect of RX 821002 is seen in spinalized preparations (81), showing that its action is dependent on the integrity of descending (presumably adrenergic) pathways. Interestingly, idazoxan does induce a small increase in reflexes in spinalized animals (15), presumably through interaction with I_2 receptors (17) or 5-HT_{1A} receptors (19). In unpublished experiments we have found that reflexes evoked by electrical stimulation of the toes in the flexor muscles (tibialis anterior and semitendinous) are potentiated by RX 821002. This finding suggests that adrenergic tone is a general phenomenon in rabbit lumbosacral spinal cord.

The heel-MG pathway is also enhanced by the selective 5-HT_{1A} receptor antagonist WAY-100635 (*N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclohexanecarboxamide) (16), which immediately raises the question of selectivity of action of RX 821002. However, the maximal effect of the latter drug is reached with an intrathecal dose of 65 μg (48), below the level at which it begins to block 5-HT_{1A} receptors *in vivo* (81). Collectively, these data provide unequivocal evidence that spinal reflex pathways in the rabbit are subject to tonic descending inhibition mediated by norepinephrine acting at α_2-receptors. As the effects on reflexes of RX 821002 are abolished by spinalization, it is very unlikely that they are due to an inverse agonist action. Spinalization severs all adrenergic pathways to the cord, but is not likely to alter any constitutive activity at spinal α_2-receptors. Thus, tonic inhibition of spinal reflexes in rabbit appears to be a true physiological role of spinal α_2-adrenoceptors.

The idazoxan-induced increase in reflexes is inhibited by prazosin and by the 5-HT_2 receptor antagonist ICI 170809 (16,47). We believe that at least some of the reflex facilitating action of α_2-receptor antagonists in rabbit arises from enhanced release of norepinephrine and 5-HT from the terminals of descending fibers in the ventral horn (93). This would be in keeping with the role of these receptors as presynaptic controllers of monoamine release in the CNS (see above).

α_2-Adrenoceptors located in the dorsal horn of the spinal cord have long been associated with analgesia (117). As in the rest of the CNS, the α_{2A} receptor is the predominant type in the cord (69,104,109) and appears to be responsible for analgesic actions of α_2-receptor agonists *per se* and for their well known synergistic interactions with opioid μ receptor agonists (105). Evidence that tonic adrenergic control of rabbit cord is also directed at dorsal horn neurons arises from the fact that RX 821002 inhibits the spinal inhibitory effects of the μ-opioid fentanyl given by either i.v. or intrathecal, but not intracerebroventricular routes (20). Similar effects have been seen with idazoxan in rat (40,49) and mouse (106). Our interpretation of these results is that there is a synergistic interaction between endogenously released norepinephrine and exogenously administered opioids at dorsal horn neurons. This is a new way of thinking about the interactions between analgesic opioids and endogenous monoamines. The spinal inhibitory actions of fentanyl, applied to the fourth ventricle, are not sensitive to RX 821002, but are reduced by WAY-100635 (21). These observations provide further evidence that the α_2- and 5-HT_{1A}-blocking properties of RX 821002 are readily distinguishable *in vivo*. The involvement of monoamines in the inhibitory actions of opioids has long been suspected but infrequently demonstrated in a convincing way, and provides a rationale for combination analgesic therapies in which opioids can be given with monoamine uptake inhibitors.

In summary, we believe that tonic adrenergic control of spinal withdrawal reflexes is the result of direct inhibition of dorsal horn neurons combined with presynaptic inhibition

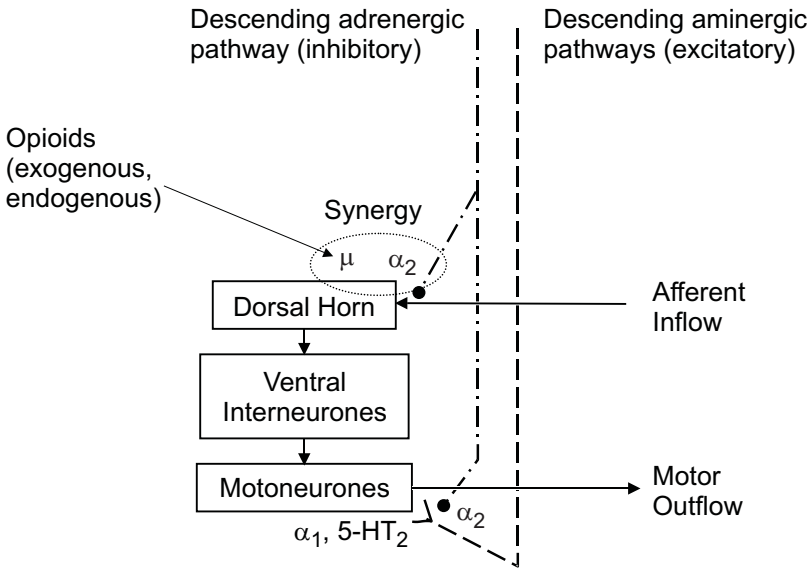

Fig. 2. Concept diagram illustrating possible sites at which endogenous norepinephrine may suppress transmission through spinal withdrawal reflex pathways. The pathway is composed of primary afferent fibers (afferent inflow) and at least two groups of interneurons, that drive the final output from α-motoneurons. Our view is that α_2 adrenoceptor antagonists such as RX 821002 block endogenous adrenergic activity at α_2 receptors in the dorsal horn (which may be pre- or post-synaptic to primary afferents) and at presynaptic receptors on the terminals of excitatory descending systems in the ventral horn (see text). Similar mechanisms may operate in sympathetic preganglionic neurons.

of excitatory monoamines in the ventral horn (Fig. 2). This type of complex control is characteristic of the involvement of monoamines in spinal cord function.

Adrenergic inhibition of spinal function can be augmented by noxious stimuli or by activation of certain brain stem nuclei. These types of studies have suffered particularly from the use of poor antagonists, as most experimenters have used yohimbine, idazoxan or even phentolamine to try to block the effects of brain stimulation. Stimulation of any of the noradrenergic nuclei with spinal cord projections (A5, A6, and A7, see 60,91) gives inhibition of spinal neurons, but none of the many studies performed has used selective antagonists to attempt to reverse the effects obtained. We have shown that stimulation within the periaqueductal gray matter inhibits spinal withdrawal reflexes in rabbit and that this effect is partially antagonized by intrathecal RX 821002 (82), although the dose required (200 μg) was rather high. A similar result has been obtained with idazoxan in the rat (88). We have also shown that inhibition of the heel-MG reflex by noxious stimulation of the toes is reversed by co-administration of RX 821002 with naloxone in decerebrated rabbit (22). In our opinion the brain stem sources of adrenergic control of the spinal cord, and their activation by peripheral noxious stimuli, need to be investigated more thoroughly with modern selective antagonists.

Understanding these systems offers important possibilities for developing therapeutic use of endogenous pain control mechanisms, and motor control.

Autonomic outflow from the spinal cord is also under the control of adrenergic descending fibers (24). In rabbit intrathecal RX 821002, RX 811059 and yohimbine, but not

idazoxan, increase arterial blood pressure and, at higher doses, heart rate (48). This effect appears to be spinally mediated as it is not seen in spinalized animals (i.e., the pressor effect is not due to leakage of drug into the circulation, 81), and presumably arises from disinhibition of sympathetic preganglionic neurons. In man, intravenous atipamezole and RX 811059 cause increases in arterial blood pressure when given alone, but this effect is likely to have a peripheral component (25,62).

CONCLUSIONS

The use of pharmacological tools to probe physiology has risks. The more a drug comes under scrutiny, the greater the likelihood that some damaging new fact will emerge to confound the interpretation of experiments using that agent. For this reason, most conclusions from experiments of this nature must be considered provisional, but this is not a problem unique to physiological pharmacology. A respected colleague of ours once offered the suggestion that one should never be the first person to use a new drug, on the grounds that today's selective antagonist will be tomorrow's uninterpretable turkey. We think this is a counsel of despair. Anyone carrying out this type of work knows that no agent is perfect and that there is a risk that new findings will require modification of their conclusions. The risk can be minimized by using more than one drug (preferably with differing structural bases) and by very careful research into the agents chosen. While there is no disgrace in having to rethink one's views in the light of new discoveries, there is no excuse for researchers continuing to use agents that have been shown to be non-selective. Any data that have been obtained with yohimbine or even idazoxan must be considered suspect and should be re-examined with RX 821002 or one of the other selective drugs described above.

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REFERENCES

1. Aantaa R, Marjamaki A, Scheinin M. Molecular pharmacology of α_2-adrenoceptor subtypes. *Ann Med* 1995;27:439–449.
2. Berlan M, Montastruc J-L, Lafontan M. Pharmacological prospects for α_2-adrenoceptor antagonist therapy. *Trends Pharmacol Sci* 1992;13:277–282.
3. Boer GJ, Van der Hee R, Feenstra MGP. α_2-Adrenoceptor assayed in rat brain by the new ligand [^{3}H]RX821002. *Biogenic Amines* 1993;9:259–269.
4. Boronat MA, Olmos G, Garcia Sevilla JA. Attenuation of tolerance to opioid-induced antinociception and protection against morphine-induced decrease of neurofilament proteins by idazoxan and other I_2-imidazoline ligands. *Br J Pharmacol* 1998;125:175–185.
5. Bousquet P. I_1 receptors, cardiovascular function, and metabolism. *Am J Hypertens* 2001;14:317S–321S.
6. Brown CM, MacKinnon AC, Redfern WS, et al. RS-45041-190: A selective, high-affinity ligand for I_2 imidazoline receptors. *Br J Pharmacol* 1995;116:1737–1744.
7. Bruban V, Feldman J, Greney H, et al. Respective contributions of alpha-adrenergic and non-adrenergic mechanisms in the hypotensive effect of imidazoline-like drugs. *Br J Pharmacol* 2001;133:261–266.

8. Callado LF, Gabilondo AM, Meana JJ. [^{3}H]RX821002 (2-methoxyidazoxan) binds to α_2-adrenoceptor subtypes and a non-adrenoceptor imidazoline binding site in rat kidney. *Eur J Pharmacol* 1996;316:359–368.
9. Calzada BC, De Artinano AA. Alpha-adrenoceptor subtypes. *Pharmacol Res* 2001;44:195–208.
10. Camargo LA, Saad WA. Role of the α_1- and α_2-adrenoceptors of the paraventricular nucleus on the water and salt intake, renal excretion, and arterial pressure induced by angiotensin II injection into the medial septal area. *Brain Res Bull* 2001;54:595–602.
11. Chan SLF, Brown CA, Scarpello KE, Morgan NG. The imidazoline site involved in control of insulin-secretion: characteristics that distinguish it from I_1 and I_2 sites. *Br J Pharmacol* 1994;112:1065–1070.
12. Chan SLF, Scarpello KE, Morgan NG. Identification and characterization of non-adrenergic binding sites in insulin-secreting cells with the imidazoline RX821002. *Adv Exp Med Biol* 1997;426:159–163.
13. Chapleo CB. The discovery of idazoxan. *Chem Brit* 1986;22:313–314.
14. Clarke RW, Eves S, Harris J, Peachey JE, Stuart E. Interactions between cutaneous afferent inputs to a withdrawal reflex in the decerebrated rabbit and their control by descending and segmental systems. *Neurosci.* 2002; in press.
15. Clarke RW, Ford TW, Taylor JS. Adrenergic and opioidergic modulation of a spinal reflex in the rabbit. *J Physiol* 1988;404:407–417.
16. Clarke RW, Harris J, Houghton AK. Spinal 5-HT-receptors and tonic modulation of transmission through a withdrawal reflex pathway in the decerebrated rabbit. *Br J Pharmacol* 1996;119:1167–1176.
17. Clarke RW, Harris J, Houghton AK. Endogenous adrenergic control of reflexes evoked by mechanical stimulation of the heel in the decerebrated rabbit. *Neurosci Lett* 2001;308:189–192.
18. Clarke RW, Harris J, Ogilvie J. Imidazoline I_2-receptors and spinal reflexes in the decerebrated rabbit. *Neuropharmacology* 2000;39:1904–1912.
19. Clarke RW, Ogilvie J, Houghton AK. Enhancement and depression of spinal reflexes by 8-hydroxy-2-(di-n-propylamino)tetralin in the decerebrated and spinalized rabbit: involvement of 5-HT_{1A}- and non-5-HT_{1A}-receptors. *Br J Pharmacol* 1997;122:631–638.
20. Clarke RW, Parry-Baggott C, Houghton AK, Ogilvie J. The involvement of bulbo-spinal pathways in fentanyl-induced inhibition of spinal withdrawal reflexes in the decerebrated rabbit. *Pain* 1998;78:197–207.
21. Clarke RW, Ward RE. The role of 5-HT_{1A}-receptors in fentanyl-induced bulbospinal inhibition of a spinal withdrawal reflex in the rabbit. *Pain* 2000;85:239–245.
22. Clarke RW, Wych BE, Harris J. Adaptive changes in withdrawal reflexes after noxious stimulation at the heel and the toes in the decerebrated rabbit. *Neurosci Lett* 2001;304:120–122.
23. Convents A, De Keyser J, De Backer J-P, Vauquelin G. [^{3}H]Rauwolscine labels α_2-adrenoceptors and 5-HT_{1A} receptors in human cerebral cortex. *Eur J Pharmacol* 1989;159:307–310.
24. Coote JH, Lewis DI. Bulbospinal catecholamine neurones and sympathetic pattern generation. *J Physiol Pharmacol* 1995;46:259–271.
25. Coupland NJ, Bailey JE, Wilson SJ, Potter WZ, Nutt DJ. A pharmacodynamic study of the α_2-adrenergic receptor antagonist ethoxyidazoxan in healthy volunteers. *Clin Pharmacol Ther* 1994;56:420–429.
26. Craven JA, Conway EL. Effects of α_2-adrenoceptor antagonists and imidazoline$_2$-receptor ligands on neuronal damage in global ischaemia in the rat. *Clin Exp Pharmacol Physiol* 1997;24:204–207.
27. De Vos H, Czerwiec E, De Backer J-P, De Potter W, Vauquelin G. [^{3}H]Rauwolscine behaves as an agonist for the 5-HT_{1A} receptors in human frontal cortex membranes. *Eur J Pharmacol* 1991;207:1–8.
28. Devedjian JC, Esclapez F, DenisPouxviel C, Paris H. Further characterization of human α_2-adrenoceptor subtypes: [^{3}H]RX821002 binding and definition of additional selective drugs. *Eur J Pharmacol* 1994;252: 43–49.
29. Diaz A, Mayet S, Dickenson AH. BU-224 produces spinal antinociception as an agonist at imidazoline I_2 receptors. *Eur J Pharmacol* 1997;333:9–15.
30. Docherty JR. Subtypes of functional α_1- and α_2-adrenoceptors. *Eur J Pharmacol* 1998;361:1–15.
31. Doxey JC, Lane AC, Roach AG, Verdee NK. Comparison of the α-adrenoceptor antagonist profile of idazoxan (RX781094), yohimbine, rauwolscine and corynanthine. *Naunyn-Schmiedeberg's Arch Pharmacol* 1984;325:136–144.
32. Eglen RM, Hudson AL, Kendall DA, et al. "Seeing through a glass darkly": casting light on imidazoline "I" sites. *Trends Pharmacol Sci* 1998;19:381–390.
33. Erdbrugger W, Raulf M, Otto T, Michel MC. Does [^{3}H]2-methoxy-idazoxan (RX 821002) detect more α_2-adrenoceptor agonist high-affinity sites than [^{3}H]rauwolscine? A comparison of nine tissues and cell lines. *J Pharmacol Exp Ther* 1995;273:1287–1294.
34. Ernsberger P, Graves ME, Graff LM, et al. I_1-imidazoline receptors — Definition, characterization, distribution, and transmembrane signalling. *Ann N Y Acad Sci* 1995;763:22–42.
35. Ernsberger P, Meeley MP, Mann JJ, Reis DJ. Clonidine binds to imidazole binding sites as well as α_2-adrenoceptors in the ventrolateral medulla. *Eur J Pharmacol* 1987;134:1–13.
36. Fairbanks CA, Posthumus IJ, Kitto KF, Stone LS, Wilcox GL. Moxonidine, a selective imidazoline/α_2 adrenergic receptor agonist, synergizes with morphine and deltorphin II to inhibit substance P-induced behavior in mice. *Pain* 2000;84:13–20.

37. Fairbanks CA, Wilcox GL. Moxonidine, a selective α_2-adrenergic and imidazoline receptor agonist, produces spinal antinociception in mice. *J Pharmacol Exp Ther* 1999;290:403–412.
38. Flamez A, De Backer JP, Czerwiec E, Ladure P, Vauquelin G. Pharmacological characterization of I_1 and I_2 imidazoline receptors in human striatum. *Neurochem Int* 1997;30:25–29.
39. Gobert A, Rivet JM, Audinot V, Newman-Tancredi A, Cistarelli L, Millan MJ. Simultaneous quantification of serotonin, dopamine and norepinephrine levels in single frontal cortex dialysates of freely-moving rats reveals a complex pattern of reciprocal auto- and heteroreceptor-mediated control of release. *Neurosci* 1998;84:413–429.
40. Goodchild CS, Guo Z, Davies A, Gent JP. Antinociceptive actions of intrathecal xylazine: Interactions with spinal cord opioid pathways. *Br J Anaesth* 1996;76:544–551.
41. Gray AM, Pache DM, Sewell RDE. Do α_2-adrenoceptors play an integral role in the antinociceptive mechanism of action of antidepressant compounds? *Eur J Pharmacol* 1999;378:161–168.
42. Grijalba B, Callado LF, Meana JJ, Garcia-Sevilla JA, Pazos A. α_2-Adrenoceptor subtypes in the human brain: A pharmacological delineation of [^{3}H]RX-821002 binding to membranes and tissue sections. *Eur J Pharmacol* 1996;310:83–93.
43. Guyenet PG. Is the hypotensive effect of clonidine and related drugs due to imidazoline binding sites? *Am J Physiol* 1997;42:R1580–R1584.
44. Haapalinna A, MacDonald E, Viitamaa T, Salonen JS, Sirvio J, Virtanen R. Comparison of the effects of acute and subchronic administration of atipamezole on reaction to novelty and active avoidance learning in rats. *Naunyn-Schmiedeberg's Arch Pharmacol* 1999;359:194–203.
45. Haapalinna A, Viitamaa T, MacDonald E, et al. Evaluation of the effects of a specific α_2-adrenoceptor antagonist, atipamezole, on α_1 and α_2-adrenoceptor subtype binding, brain neurochemistry and behavior in comparison with yohimbine. *Naunyn-Schmiedeberg's Arch Pharmacol* 1997;356:570–582.
46. Hamilton CA, Reid JL, Yakubu MA. [^{3}H]Yohimbine and [^{3}H]Idazoxan bind to different sites on rabbit forebrain and kidney membranes. *Eur J Pharmacol* 1988;146:345–348.
47. Harris J, Clarke RW. An analysis of adrenergic influences on the sural gastrocnemius reflex of the decerebrated rabbit. *Exp Brain Res* 1992;92:310–317.
48. Harris J, Clarke RW. Motor and cardiovascular effects of selective α_2-adrenoceptor antagonists in the decerebrated rabbit. *Eur J Pharmacol* 1993;237:323–328.
49. Herrero JF, Solano RE. The antinociceptive effect of the μ-opioid fentanyl is reduced in the presence of the α_2-adrenergic antagonist idazoxan in inflammation. *Brain Res* 1999;840:106–114.
50. Hieble JP, Bondinell WE, Ruffolo RR. α- and β-Adrenoceptors: From the gene to the clinic. 1. Molecular biology and adrenoceptor subclassification. *J Med Chem* 1995;38:3415–3444.
51. Houghton AK, Westlund KN. An I_2 imidazoline ligand, RS 45041, potentiates hyperalgesia in acute arthritis. *Neuroreport* 1996;7:1497–1501.
52. Hudson AL, Chapleo CB, Lewis JW, et al. Identification of ligands selective for central I_2-imidazoline binding sites. *Neurochem Int* 1997;30:47–53.
53. Hudson AL, Mallard NJ, Tyacke R, Nutt DJ. [^{3}H]-RX821002: a highly selective ligand for the identification of α_2-adrenoceptors in the rat brain. *Molecular Neuropharmacology* 1992;1:219–229.
54. Hudson AL, Robinson ESJ, Lalies MD, Tyacke RJ, Jackson HC, Nutt DJ. In vitro and *in vivo* approaches to the characterization of the α_2-adrenoceptor. *J Auton Pharmacol* 1999;19:311–320.
55. Hume SP, Ashworth S, Lammertsma AA, et al. Evaluation in rat of RS-79948-97 as a potential PET ligand for central α_2-adrenoceptors. *Eur J Pharmacol* 1996;317:67–73.
56. Hume SP, Lammertsma AA, Opackajuffry J, et al. Quantification of *in vivo* binding of [^{3}H]RX 821002 in rat brain-evaluation as a radioligand for central α_2-adrenoceptors. *Nucl Med Biol-Int J Rad App B* 1992;19:841–849.
57. Jackson HC, Griffin IJ, Nutt DJ. The effects of idazoxan and other α_2-adrenoceptor antagonists on food and water-intake in the rat. *Br J Pharmacol* 1991;104:258–262.
58. Jansson CC, Kukkonen JP, Nasman J, et al. Protean agonism at α_{2A}-adrenoceptors. *Mol Pharmacol* 1998;53:963–968.
59. Jolkkonen J, Puurunen K, Rantakomi S, Harkonen A, Haapalinna A, Sivenius J. Behavioral effects of the α-adrenoceptor antagonist, atipamezole, after focal cerebral ischemia in rats. *Eur J Pharmacol* 2000;400:211–219.
60. Jones SL. Descending noradrenergic influences on pain. *Prog Brain Res* 1991;88:381–394.
61. Kamibayashi T, Maze M. Clinical uses of α_2-adrenergic agonists. *Anesthesiol* 2000;93:1345–1349.
62. Karhuvaara S, Kallio A, Scheinin M, Anttila M, Salonen JS, Scheinin H. Pharmacological effects and pharmacokinetics of atipamezole, a novel α_2-adrenoceptor antagonist — A randomized, double-blind crossover study in healthy male-volunteers. *Br J Clin Pharmacol* 1990;30:97–106.
63. Khan ZP, Ferguson CN, Jones RM. α_2- and imidazoline receptor agonists — Their pharmacology and therapeutic role. Anaesthesia 1999;54:146–165.
64. Krystal JH, McDougle CJ, Woods SW, Price LH, Heninger GR, Charney DS. Dose-response relationship for oral idazoxan effects in healthy human subjects — comparison with oral yohimbine. *Psychopharmacology (Berl)* 1992;108:313–319.

65. Kukkonen JP, Ge HF, Jansson CC, et al. Different apparent modes of inhibition of α_{2A}-adrenoceptor by α_2-adrenoceptor antagonists. *Eur J Pharmacol* 1997;335:99–105.
66. Lalies MD, Nutt DJ. The effect of a selective I_2-site ligand, 2-α_2-benzofuranyl)-2-imidazoline, on *in vivo* noradrenaline release in rat brain. *Br J Pharmacol* 1995;114:413P.
67. Langin D, Lafontan M. [^{3}H]Idazoxan binding at non-α_2-adrenoceptors in rabbit adipocyte membranes. *Eur J Pharmacol* 1989;159:199–203.
68. Langin D, Lafontan M, Stillings MR, Paris H. [^{3}H]RX821002: a new tool for the identification of α_{2A}-adrenoceptors. *Eur J Pharmacol* 1989;167:95–104.
69. Lawhead RG, Blaxall HS, Bylund DB. α_{2A} is the predominant α_2 adrenergic receptor subtype in human spinal cord. *Anesthesiol* 1992;77:983–991.
70. Lione LA, Nutt DJ, Hudson AL. Characterization and autoradiographical localization of imidazoline$_2$ (I_2) sites labelled by [^{3}H]2-(-2-benzofuranyl)-2-imidazoline in rat-brain. *Br J Pharmacol* 1995;116:P 338.
71. Mallard NJ, Hudson AL, Nutt DJ. Characterization and autoradiographical localization of nonadrenoceptor idazoxan binding sites in the rat brain. *Br J Pharmacol* 1992;106:1019–1027.
72. Mateo Y, Meana JJ. Determination of the somatodendritic α_2-adrenoceptor subtype located in rat locus coeruleus that modulates cortical noradrenaline release *in vivo*. *Eur J Pharmacol* 1999;379:53–57.
73. Meana JJ, Callado LF, Pazos A, Grijalba B, Garcia-Sevilla JA. The subtype-selective α_2-adrenoceptor antagonists BRL 44408 and ARC 239 also recognize 5-HT$_{1A}$ receptors in the rat brain. *Eur J Pharmacol* 1996;312:385–388.
74. Meana JJ, Mateo Y, Pineda J. Somatodendritic α_{2A}-adrenoceptors in the locus coeruleus are involved in the modulation of cortical noradrenaline release by the antidepressant desipramine. *Naunyn-Schmiedeberg's Arch Pharmacol* 1998;358:35–43.
75. Michel MC, Ernsberger P. Keeping an eye on the I site: imidazoline preferring receptors. *Trends Pharmacol Sci* 1992;13:369–370.
76. Michel MC, Insel PA. Are there multiple imidazoline binding sites? *Trends Pharmacol Sci* 1989;10: 342–344.
77. Miralles A, Olmos G, Sastre M, Barturen F, Martin I, Garcia-Sevilla JA. Discrimination and pharmacological characterization of I_2-imidazoline sites with [^{3}H]idazoxan and α_2 adrenoceptors with [^{3}H]RX821002 α_2-methoxy idazoxan) in the human and rat brains. *J Pharmacol Exp Ther* 1993;264:1187–1197.
78. Murrin LC, Gerety ME, Happe HK, Bylund DB. Inverse agonism at α_2-adrenoceptors in native tissue. *Eur J Pharmacol* 2000;398:185–191.
79. Newman-Tancredi A, Nicolas JP, Audinot V, et al. Actions of α_2 adrenoceptor ligands at α_{2A} and 5-HT$_{1A}$ receptors: the antagonist, atipamezole, and the agonist, dexmedetomidine, are highly selective for α_{2A} adrenoceptors. *Naunyn-Schmiedeberg's Arch Pharmacol* 1998;358:197–206.
80. O'Rourke MF, Blaxall HS, Iversen LJ, Bylund DB. Characterization of [^{3}H]RX821002 binding to α_2 adrenergic receptor subtypes. *J Pharmacol Exp Ther* 1994;268:1362–1367.
81. Ogilvie J, Clarke RW. Effect of RX 821002 at 5-HT$_{1A}$-receptors in rabbit spinal cord *in vivo*. *Br J Pharmacol 1998;123:1138–1142.*
82. Ogilvie J, Houghton AK, Bhandari RNB, Clarke RW. Suppression of a withdrawal reflex by chemical stimulation in the periaqueductal gray matter of the decerebrated rabbit: involvement of α_2-adrenoceptors. *J Physiol* 1996;491:112P.
83. Ogilvie J, Simpson DAA, Clarke RW. Tonic adrenergic and serotonergic inhibition of a withdrawal reflex in rabbits subjected to different levels of surgical preparation. *Neurosci* 1999;89:1247–1258.
84. Olmos G, Alemany R, Boronat MA, GarciaSevilla JA. Pharmacologic and molecular discrimination of I_2-imidazoline receptor subtypes. *Ann N Y Acad Sci* 1999;881:144–1460.
85. Olmos G, DeGregorio-Rocasolano N, Regalado MP, et al. Protection by imidazol(ine) drugs and agmatine of glutamate-induced neurotoxicity in cultured cerebellar granule cells through blockade of NMDA receptor. *Br J Pharmacol* 1999;127:1317–1326.
86. Parini A, Gargalidis Moudanos C, Pizzinat N, Lanier SM. The elusive family of imidazoline binding sites. *Trends Pharmacol Sci* 1996;17:13–16.
87. Pauwels PJ, Tardif S, Wurch T, Colpaert FC. Facilitation of constitutive α_2-adrenoceptor activity by both single amino acid mutation (Thr^{373}Lys) and $G_{\alpha 0}$ protein coexpression: Evidence for inverse agonism. *J Pharmacol Exp Ther* 2000;292:654–663.
88. Peng YB, Lin Q, Willis WD. Involvement of α_2 adrenoceptors in the periaqueductal gray-induced inhibition of dorsal horn cell activity in rats. *J Pharmacol Exp Ther* 1996;278:125–135.
89. Pettibone DJ, Clineschmidt BV, Lotti VJ, et al. Pharmacological profile of a new potent and specific α_2-adrenoceptor antagonist, L-657, 743. *Naunyn-Schmiedeberg's Arch Pharmacol* 1987;336:169–175.
90. Polidori C, Gentili F, Pigini M, Quaglia W, Panocka I, Massi M. Hyperphagic effect of novel compounds with high affinity for imidazoline I_2 binding sites. *Eur J Pharmacol* 2000;392:41–49.
91. Proudfit HK, Clark FM. The projections of locus coeruleus neurons to the spinal cord. *Prog Brain Res* 1991;88:123–141.

92. Redfern WS, Williams A. A re-evaluation of the role of α_2-adrenoceptors in the anxiogenic effects of yohimbine, using the selective antagonist delequamine in the rat. *Br J Pharmacol* 1995;116:2081–2089.
93. Rekling JC, Funk GD, Bayliss DA, Dong XW, Feldman JL. Synaptic control of motoneuronal excitability. *Physiol Rev* 2000;80:767–852.
94. Rodriguez-Manzo G. Yohimbine interacts with the dopaminergic system to reverse sexual satiation: further evidence for a role of sexual motivation in sexual exhaustion. *Eur J Pharmacol* 1999;372:1–8.
95. Ruffolo RR, Bondinell WE, Hieble JP. α-Adrenoceptors and β-adrenoceptors — from the gene to the clinic. 2. Structure-activity-relationships and therapeutic applications. *J Med Chem* 1995;38:3681–3716.
96. Sagen J, Proudfit HK. Effect of intrathecally-administered noradrenergic antagonists on nociception in the rat. *Brain Res* 1984;310:295–301.
97. Savola MKT, Savola JM. [^{3}H]Dexmedetomidine, an α_2-adrenoceptor agonist, detects a novel imidazole binding site in adult rat spinal cord. *Eur J Pharmacol* 1996;304:315–323.
98. Sjoholm B, Lahdesmaki J, Pyykko K, Hillila M, Scheinin M. Non-adrenergic binding of [^{3}H]atipamezole in rat kidney — regional distribution and comparison to α_2-adrenoceptors. *Br J Pharmacol* 1999;128: 1215–1222.
99. Sjoholm B, Savola JM, Scheinin M. Nonadrenergic binding of [^{3}H]atipamezole in rat lung. A novel imidazole binding site? *Ann N Y Acad Sci* 1995;763:66–77.
100. Stanfa LC, Dickenson AH. Enhanced α_2 adrenergic controls and spinal morphine potency in inflammation. *Neuroreport* 1994;5:469–472.
101. Starke K. Presynaptic autoreceptors in the third decade: focus on α_2-adrenoceptors. *J Neurochem* 2001;78: 685–693.
102. Starke K, Trendelenburg U, Limberger N. Presynaptic α_2-adrenoceptors: Subtype determination. *Pharmacol Commun* 1995;6:99–108.
103. Stillings MR, Chapleo CB, Butler RCM, et al. α-Adrenoceptor reagents. 3. Synthesis of some 2-substituted 1,4-benzodioxans as selective presynaptic α_2-adrenoceptor antagonists. *J Med Chem* 1985;28:1054–1062.
104. Stone LS, Broberger C, Vulchanova L, et al. Differential distribution of α_{2A} and α_{2C} adrenergic receptor immunoreactivity in the rat spinal cord. *J Neurosci* 1998;18:5928–5937.
105. Stone LS, MacMillan LB, Kitto KF, Limbird LE, Wilcox GL. The α_{2A} adrenergic receptor subtype mediates spinal analgesia evoked by α_2 agonists and is necessary for spinal adrenergic — opioid synergy. *J Neurosci* 1997;17:7157–7165.
106. Su RB, Li J, Gao K, Pei G, Qin BY. Influence of idazoxan on analgesia, tolerance, and physical dependence of morphine in mice and rats *in vivo*. *Acta Pharmacol Sin* 2000;21:1011–1015.
107. Trendelenburg AU, Wahl CA, Starke K. Antagonists that differentiate between α_{2A}- and α_{2D}-adrenoceptors. *Naunyn-Schmiedeberg's Arch Pharmacol* 1996;353:245–249.
108. Uhlen S, Dambrova M, Nasman J, et al. [^{3}H]RS79948-197 binding to human, rat, guinea pig and pig α_{2A}-, α_{2B}-, and α_{2C}-adrenoceptors. Comparison with MK912, RX821002, rauwolscine and yohimbine. *Eur J Pharmacol* 1998;343:93–101.
109. Uhlen S, Lindblom J, Johnson A, Wikberg JES. Autoradiographic studies of central α_{2A}- and α_{2C}-adrenoceptors in the rat using [^{3}H]MK912 and subtype-selective drugs. *Brain Res* 1997;770:261–266.
110. Uhlen S, Lindblom J, Tiger G, Wikberg JES. Quantification of α_{2A} and α_{2C} adrenoceptors in the rat striatum and in different regions of the spinal cord. *Acta Physiol Scand* 1997;160:407–412.
111. Uhlen S, Muceniece R, Rangel N, Tiger G, Wikberg JES. Comparison of the binding activities of some drugs on α_{2A}, α_{2B} and α_{2C}-adrenoceptors and non-adrenergic imidazoline sites in the guinea pig. *Pharmacol Toxicol* 1995;76:353–364.
112. Vauquelin G, De Vos H, De Backer JP, Ebinger G. Identification of α_2 adrenergic receptors in human frontal cortex membranes by binding of [^{3}H]RX 821002, the 2-methoxy analog of [^{3}H]idazoxan. *Neurochem Int* 1990;17:537–546.
113. Wade SM, Lan KL, Moore DJ, Neubig RR. Inverse agonist activity at the α_2-adrenergic receptor. *Mol Pharmacol* 2001;59:532–542.
114. Welbourn AP, Chapleo CB, Lane AC, et al. α-Adrenoceptor reagents. 4. Resolution of some potent selective prejunctional α_2-adrenoceptor antagonists. *J Med Chem* 1986;29:2000–2003.
115. Wortley KE, Heal DJ, Stanford SC. Modulation of sibutramine-induced increases in extracellular noradrenaline concentration in rat frontal cortex and hypothalamus by α_2-adrenoceptors. *Br J Pharmacol* 1999; 128:659–666.
116. Wurch T, Colpaert FC, Pauwels PJ. G-protein activation by putative antagonists at mutant Thr(373)Lys α_{2A} adrenergic receptors. *Br J Pharmacol* 1999;126:939–948.
117. Yaksh TL. Spinal systems and pain processing: development of novel analgesic drugs with mechanistically defined models. *Trends Pharmacol Sci* 1999;20:329–337.
118. Yonezawa A, Ando R, Watanabe C, et al. α_{2A}-Adrenoceptor antagonists. Effects on ejaculation, penile erection and pelvic thrusting behavior in dogs. *Pharmacol Biochem Behav* 2001;70:141–147.