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Dual effect of agmatine in the bisected rat vas deferens

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Abstract

The functional effects of the amine agmatine, the putative endogenous ligand for α_2-adrenoceptors and imidazoline receptors, in rat vas deferens were investigated by using the epididymal and prostatic portions. Tissues were contracted by electrical stimulation or by exogenous drugs. In electrically stimulated portions, agmatine caused a dual effect on contractions. In the epididymal portion an inhibition on twitch contractions was observed, which was partially antagonised by idazoxan and yohimbine, indicating the involvement of at least a presynaptic α_2-adrenoceptor-mediated mechanism, without the interference of imidazoline receptors. In the prostatic portion, agmatine enhanced the amplitude of twitches. In contractions induced by exogenous drugs, agmatine potentiated, only in the prostatic segment, the effects of noradrenaline (norepinephrine) or ATP; it also enhanced the effect of low concentrations of KCl and blocked the maximum effect of the higher concentrations. Effects induced by agmatine on the exogenous ATP in the prostatic portion were blocked by cromakalim, suggesting a blocking action on the postsynaptic K^+ channels, which explains, in part, the potentiation of the twitch amplitude. It was concluded that agmatine interferes with sympathetic neurotransmission, but the physiological relevance of this needs to be better understood because of the high doses employed to induce its effects.

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Introduction

Agmatine is a guanidine amine with putative neurotransmitter/neuromodulator properties described as an endogenous ligand for the presynaptic α_2-adrenoceptors and the imidazoline receptors (Li et al 1994), although with some uncertainty about its physiological functions (for recent reviews see Reis & Regunathan 2000 and Raasch et al 2001). Besides its reported actions relating to interactions on these sites (Piletz et al 1995; González et al 1996; Molderings et al 2000), other complex and diverse actions for the amine have already been reported (e.g., actions on the nitrergic pathway (Morrisey & Klahr 1997; Demady et al 2001), at the $K^+_{(ATP)}$ channels (Shepherd et al 1996; Molderings & Gothert 1998) and on cholinergic nicotinic neurotransmission (Loring 1990; Santos et al 2001)).

We have previously shown that agmatine enhanced the catecholamine release and the electrically induced twitch contractions of rat vas deferens, and suggested that this effect could be mediated by presynaptic receptors (Jurkiewicz et al 1996). Although the amine produced increasing effects in the whole organ, we found in preliminary studies that, interestingly, it caused opposite effects when the organ was bisected in its epididymal and prostatic portions: an inhibition of the twitches in the epididymal and a potentiation in the prostatic ends (unpublished data). Regarding the rat vas deferens, some physiological differences have already been established between the two segments concerning muscle reactivity (Anton et al 1977; Rhode et al 1986; Nakazawa et al 1987), transduction mechanisms (Burt et al 1996, 1998) and neurotransmission (Sneddon et al 1984; Ventura 1998).

In this study we sought to investigate the effects of agmatine on adrenergic neurotransmission in the bisected rat vas deferens. In the epididymal portion, a reasonable explanation for the inhibitory effect of the twitches by agmatine could be an agonistic action on presynaptic α_2-adrenoceptors and/or imidazoline sites. Therefore, we compared its effects with those obtained with drugs known to act on these sites, such

as clonidine and moxonidine. Both compounds are imidazoline receptor agonists showing high affinity for α_2-adrenoceptors and imidazoline sites (Ernsberger et al 1995). In the prostatic segment, the enhancing effects of agmatine are compatible with a blocking action at potassium channels, facilitating the neurotransmission. Thus, we performed experiments to verify whether these sites are recruited by agmatine to exert its effects. Herein we present the results of such experiments.

Materials and Methods

Biological preparation

Wistar rats (4–5 months old), 350–420 g, were killed with an ether overdose, and vasa deferentia were removed. All animal procedures were conducted according to the Guidelines for the Ethical Care of Experimental Animals and were approved by the Institutional Animal Care and Use Committee. Whole vas was cleared of surrounding tissues and secretions, and then the organ was divided into its corresponding epididymal and prostatic portions. Tissues were kept in aerated nutrient solution, at 32 °C, with the following composition (mM): NaCl 138; KCl 5.7; $CaCl_2$ 1.8; NaH_2PO_4 0.36; $NaHCO_3$ 15; and glucose 5.5, in distilled water (Piccarelli et al 1962). Tissues were mounted in 10-mL organ baths and contractions were recorded through a physiograph (Ugo Basile, Italy) by using isometric transducers (Ugo Basile, type 7006, Italy), with 1 g load. After a 60-min period of equilibration of the preparation, experiments were started.

Contractile responses to electrical stimulation

Tissues were placed between two parallel platinum electrodes and electrical stimulation was performed with a Grass S88 stimulator at the following parameters: 0.05 Hz, 3 ms duration and 50 V supramaximal voltage.

When electrically driven contractions (twitch response) were stabilised (20 min), concentration–response curves in both epididymal and prostatic portions were constructed for agmatine, clonidine or moxonidine, added as cumulative concentrations either in the absence or presence of idazoxan (3×10^{-9} to 10^{-6} M), an imidazoline receptor and α_2-adrenoceptor antagonist (Langin & Lafontan 1989), or yohimbine (10^{-8} to 10^{-6} M), an α_2-adrenoceptor antagonist (Weitzell et al 1979). When present, the antagonists were added 20 min before starting the curves. Experiments were performed on paired vas deferens from a single rat. One portion of the pair was treated with a single concentration of the antagonist, while the other was treated with vehicle. Responses were measured as the height of the twitch contraction in the presence of each agonist concentration and were expressed as percentage contraction of the height of the basal twitch contraction (in g of tension) induced by electrical stimulation.

The parameters pIC50 (negative logarithm of the dose of drug inducing a 50% inhibitory effect (IC50)) and pA_2 (negative molar concentration of antagonist that produces a two-fold shift to the right of a concentration–response curve of the agonist) were calculated as previously described (Arunlakshana & Schild 1959; Van Rossum 1963).

In experiments with the irreversible antagonist, tissues were exposed to phenoxybenzamine (10^{-6} M) followed by several washouts 15 min later. Cumulative concentration–response curves for agmatine (10^{-5} to 3×10^{-3} M) or clonidine (10^{-11} to 10^{-5} M) in the epididymal portion were constructed 30 min after exposing the preparation to phenoxybenzamine. For receptor protection experiments (Furchgott 1966) a similar protocol was followed; however, before the exposure to phenoxybenzamine, a 15-min incubation with agmatine (10^{-3} M) was performed.

Contractile responses to exogenous ATP, noradrenaline (norepinephrine) and KCl

The contractile effects of single concentrations of adenosine 5′-triphosphate (ATP; 10^{-4} M), noradrenaline (norepinephrine) (10^{-4} M), or KCl (3×10^{-2} M and 3×10^{-1} M) were verified in the absence or presence of agmatine (10^{-4} to 10^{-3} M), tetraethylammonium (10^{-5} to 10^{-4} M) or Bay K 8644 (10^{-9}–10^{-8} M), after a 20-min incubation. In some protocols with ATP, cromakalim (3×10^{-7} M), a $K^+_{(ATP)}$-opener (Hamilton & Weston 1989), was present throughout the experiments. In these studies, the maximum effect (E_{max}) attained by these concentrations was analysed.

Expression of data and statistical analysis

Values are means ± s.e.m. A P value less than 0.05 was termed significant. Pharmacological parameters were analysed by Kruskal–Wallis test followed by Mann–Whitney as a pos-hoc test or by paired Student's t-test where appropriate.

Drugs

Agmatine sulfate was purchased from RBI, and adenosine 5′-triphosphate, Bay K 8644 (1,4-dihydro-2,6-dimethyl-5-nitro-4-[2-(trifluoromethyl)phenyl]pyridine-3-carboxilic acid methyl ester), clonidine hydrochloride, idazoxan hydrochloride, phenoxybenzamine hydrochloride, prazosin hydrochloride, suramin hexasodium, tetraethylammonium chloride, tetrodotoxin and yohimbine hydrochloride from Sigma. Cromakalim (levcromakalim) was from Beecham Pharmaceuticals (UK); moxonidine (free base) was kindly supplied by Eli Lilly (Brazil). Stock solutions of these drugs were prepared in distilled water, except for moxonidine, phenoxybenzamine and tetrodotoxin, which were dissolved in 10^{-2} M acetic acid and then distilled water, and cromakalim and Bay K 8644, which were dissolved in absolute ethanol (50%) and then distilled water. The maximal concentrations of acetic acid and absolute ethanol used in the final solutions for experiments had no effects on vas deferens muscle contraction.

Results

Electrical stimulation produced phasic contractions at both epididymal and prostatic segments that were completely blocked by tetrodotoxin (3×10^{-7} M). Mean twitch contraction was 0.80 ± 0.067 g (n = 31) in epididymal and 2.32 ± 0.13 g (n = 45) in prostatic ends. At the stimulation parameters used (see Methods) the amplitude of the twitch was maintained for at least 2 h. Contractions were completely blocked by suramin (10^{-5} M) in both segments. Prazosin (10^{-7} M) inhibited the twitch by about 20% only in the epididymal end, but not in the prostatic end (data not shown), thus confirming that contractions were mediated essentially by purinergic receptors.

Effect of agmatine over twitch contractions

The effect of agmatine over twitch contractions varied with the portion of the organ. In the epididymal segment, agmatine reduced the amplitude of twitch contractions in a concentration-dependent manner. Maximal inhibition was observed in the concentration range of 10^{-3} to 3×10^{-3} M (Figure 1A). In contrast, in the prostatic segment agmatine potentiated the contractions in a concentration-dependent manner (Figure 1B); at 10^{-3} M the potentiation reached $207.1 \pm 14.2\%$ of control response.

Effects of idazoxan and yohimbine on the effects of agmatine over twitch contractions

The antagonists idazoxan and yohimbine (10^{-7} to 10^{-6} M) partially reverted the inhibition of twitch contractions caused by agmatine in the epididymal end (Table 1).

Neither idazoxan nor yohimbine alone altered the amplitude of contractions in both portions (data not shown).

Effects of idazoxan and yohimbine on inhibitory concentration–response curves for clonidine or moxonidine on twitch contractions

The agonists clonidine and moxonidine completely inhibited the twitch contraction in both epididymal and prostatic portions in a concentration-dependent manner. These inhibitory curves were competitively antagonised by idazoxan and yohimbine. The pIC50 and pA_2 values are shown in Table 2.

Effects of phenoxybenzamine on the inhibitory concentration–response curves for agmatine and clonidine over twitch contractions in epididymal end

Figure 2A shows that previous exposure of the preparation to phenoxybenzamine (10^{-6} M, 15 min) caused a small, although statistically significant ($P < 0.05$), shift to the right of the concentration–response curve for agmatine (pIC50 in the absence of phenoxybenzamine $= 3.84 \pm 0.06$; pIC50 in the presence of phenoxybenzamine $= 3.49 \pm 0.12$), without altering its maximum inhibitory effect. Under the same experimental condition, the 100% inhibition of twitch response by clonidine in the absence of phenoxybenzamine was reduced to $26.3 \pm 4.9\%$ in the presence of the antagonist ($P < 0.01$). The pIC50 was also reduced from 9.01 ± 0.13 to 6.97 ± 0.24 in the presence of phenoxybenzamine ($P < 0.01$; Figure 2B). In receptor protection experiments, when phenoxybenzamine was incubated in the presence of agmatine, the inhibitory effect of clonidine was partially recovered (maximum inhibition: $90.4 \pm 1.8\%$; pIC50 $= 7.88 \pm 0.09$, $P < 0.01$), as shown in Figure 2B.

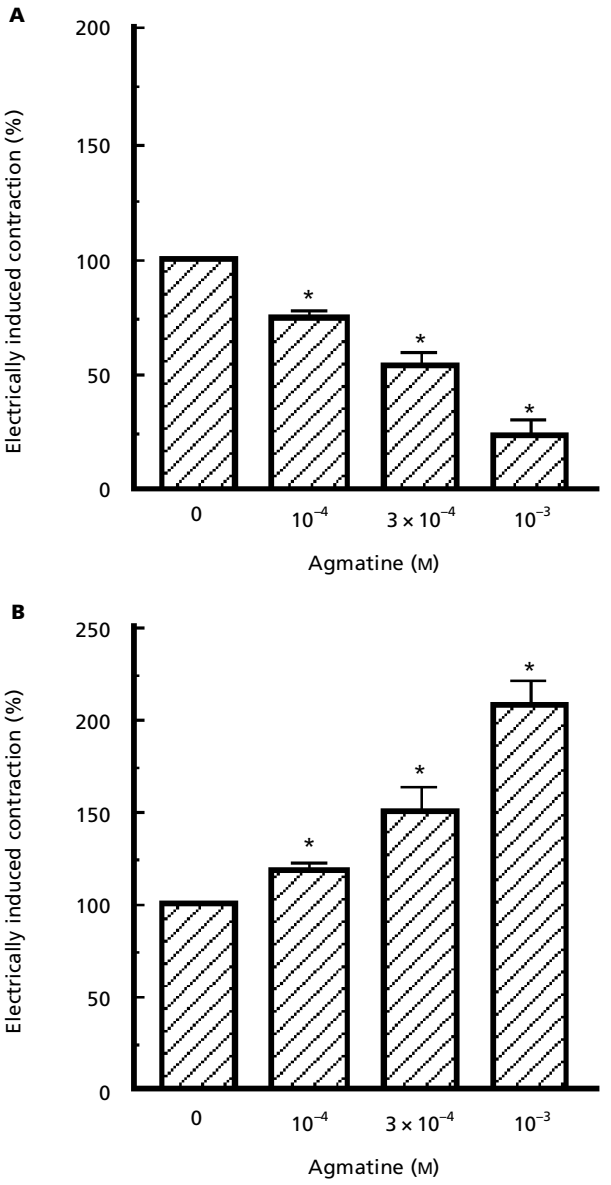

Figure 1 The effect of agmatine on electrically induced contraction in the epididymal (A) and prostatic (B) portion of the rat vas deferens. Responses in the presence of agmatine are expressed as a percentage of response in the absence of agmatine. Vertical bars represent mean ± s.e.m from at least 30 experiments. *$P < 0.01$ compared with the corresponding control (absence of agmatine).

Table 1 Effect of agmatine on the electrically induced contraction in the epididymal portion of the rat vas deferens in the absence or presence of idazoxan or yohimbine.

	Contraction (%) in presence of agmatine			
	0	10^{-4} M	3×10^{-4} M	10^{-3} M
Control	100	81.9 ± 2.3	64.9 ± 3.2	51.1 ± 3.2
Yohimbine 10^{-7} M	100	95.7 ± 1.9*	73.7 ± 5.8	46.4 ± 5.8
Yohimbine 10^{-6} M	100	97.8 ± 1.8*	83.6 ± 3.8*	78.4 ± 4.9*
Idazoxan 10^{-7} M	100	97.6 ± 3.3*	77.5 ± 4.0*	71.5 ± 5.4*
Idazoxan 10^{-6} M	100	94.7 ± 1.0*	92.0 ± 1.2*	80.3 ± 3.7*

*$P < 0.01$ compared with respective control, n = 7 (yohimbine) or 8 (idazoxan).

Table 2 Parameters measured from cumulative inhibitory concentration–response curves for clonidine and moxonidine on electrically induced contraction in the absence or presence of idazoxan or yohimbine.

	pIC50	pA_2	
		Yohimbine	Idazoxan
Epididymal portion			
Clonidine (n = 8)	9.10 ± 0.10	8.25 ± 0.12	9.51 ± 0.13
Moxonidine (n = 5)	7.50 ± 0.11	8.32 ± 0.23	8.40 ± 0.06
Prostatic portion			
Clonidine (n = 6)	8.80 ± 0.05	8.30 ± 0.05	8.98 ± 0.15
Moxonidine (n = 6)	7.50 ± 0.07	8.20 ± 0.13	8.90 ± 0.14

Effect of agmatine on the responses to exogenous ATP, noradrenaline and KCl

Addition of agmatine in the absence of either drugs or electrical stimulation caused an irregular and low-intensity automatism, in both portions of the rat vas deferens.

In the prostatic portion, 10^{-4} M ATP produced a contraction of 0.69 ± 0.04 g, which was enhanced by agmatine in a concentration-dependent manner, reaching 402.4 ± 45.7% at 10^{-3} M (Figure 3). The K^+-channel blocker tetraethylammonium and the Ca^{2+}-channel opener BAY K 8644 also potentiated the effect of exogenous ATP (tetraethylammonium: 364.03 ± 73.3% at 10^{-4} M; BAY K 8644: 231.4 ± 37.4% at 10^{-8} M).

The presence of the $K^+_{(ATP)}$-channel opener cromakalim (3×10^{-7} M) reverted the effects of agmatine and tetraethylammonium, but not those of BAY K 8644 (Figure 3). Cromakalim (3×10^{-7} M) caused a low, although statistically significant, relaxant effect (14.0 ± 0.08%, $P < 0.05$) on contractions by ATP. However, this effect can not be regarded as being responsible for the reversion of the potentiation by agmatine or tetraethylammonium, since the effects of these two drugs on the ATP-induced contractions were normalised, being calculated in percentage values considering as 100% the respective control stimulus by ATP (in the absence or presence of cromakalim).

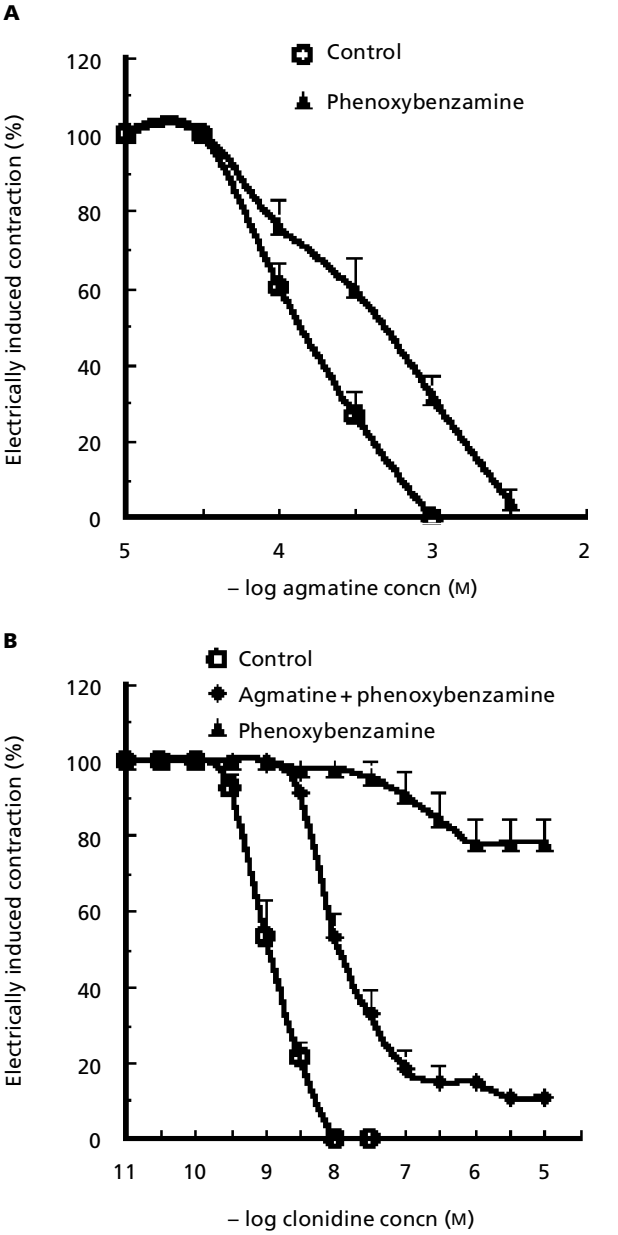

Figure 2 Mean cumulative concentration–response curves for agmatine (A) or clonidine (B) on electrically induced contraction in the epididymal portion of rat vas deferens after irreversible blockade induced by 10^{-6} M phenoxybenzamine (incubated for 15 min), without or with receptor protection by agmatine. Points represent mean ± s.e.m. from at least 6 experiments, and are expressed as a percentage of response in the absence of drugs.

In experiments with KCl, agmatine or tetraethylammonium enhanced the contractile effect of low K^+ concentration (3×10^{-2} M, 0.83 ± 0.14 g) in the prostatic segment (Figure 4A). Agmatine (10^{-3} M) potentiated the contraction to 232.06 ± 29.8%. Tetraethylammonium (10^{-3} M) increased contractions to 242.2 ± 30.5%.

When tested against maximum K^+ concentration (3×10^{-1} M, 4.2 ± 0.13 g), either agmatine or

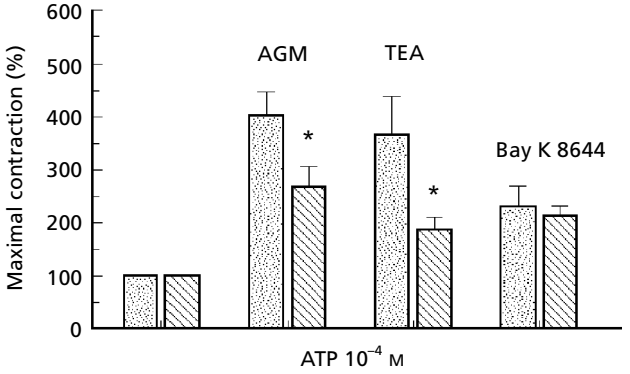

Figure 3 Maximal effect attained by 10^{-4} M ATP in the prostatic portion of rat vas deferens after incubation for 20 min with 10^{-3} M agmatine (AGM), 10^{-4} M tetraethylammonium (TEA) or 10^{-8} M BAY K 8644, in the absence (shadow bars) or presence (striped bars) of 3×10^{-7} M cromakalim (CRK). ATP response (with or without cromakalim) obtained in the absence of agmatine, TEA or BAY K 8644 was taken as 100%. Vertical bars represent mean ± s.e.m. from at least 6 experiments. *$P < 0.02$ compared with the corresponding control, without cromakalim.

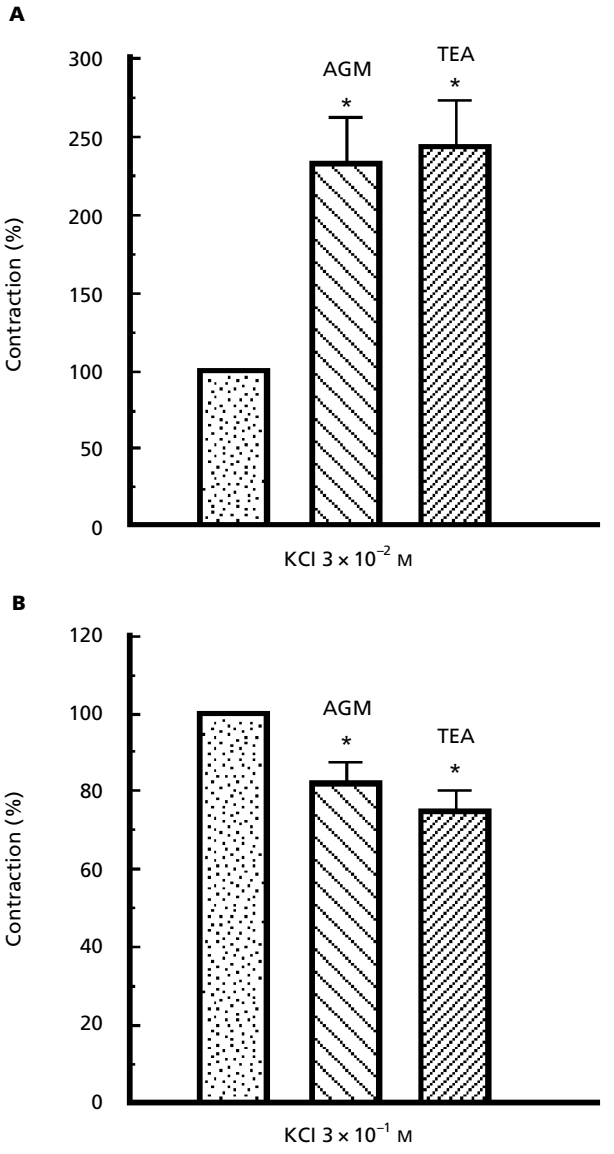

Figure 4 Effect obtained by 3×10^{-2} M KCl (A) and by 3×10^{-1} M KCl (B) in the prostatic portion of rat vas deferens after incubation for 20 min with 10^{-3} M agmatine (AGM) or 10^{-3} M tetraethylammonium (TEA). Vertical bars represent mean ± s.e.m. from at least 5 experiments and are expressed as a percentage of response obtained in the absence of agmatine or TEA (control). *$P < 0.01$ compared with the corresponding control.

tetraethylammonium (Figure 4B) reduced K^+ contractile effect. In the presence of agmatine, the contraction was reduced to 82.1 ± 5.3% at 10^{-3} M. In the presence of tetraethylammonium, the contraction was reduced to 74.4 ± 5.7% at 10^{-3} M. In control experiments, sequential application of KCl produced reproducible contractile responses throughout the duration of experiments.

Agmatine and tetraethylammonium also potentiated the contractions induced by maximal concentrations of noradrenaline (10^{-4} M): at 3×10^{-4} M agmatine enhanced the contraction to 144.4 ± 21.7% ($P < 0.05$), and 3×10^{-4} M tetraethylammonium, to 166.9 ± 8.7% ($P < 0.05$).

In the epididymal segment, neither agmatine nor tetraethylammonium altered the responses to ATP, noradrenaline or KCl (data not shown).

Discussion

The opposite effects of agmatine obtained in the different segments of the rat vas deferens indicate that they might result from different mechanisms of action and, hence, they will be discussed separately.

Inhibitory effect in the epididymal portion

Since agmatine was described as a ligand for α_2-adrenoceptors and imidazoline receptors, a reasonable supposition to explain its inhibitory effect in this segment could be an agonistic action on these receptor sites. An α_2-adrenoceptor-mediated mechanism was demonstrated, but we did not obtain any indications for the involvement of imidazoline receptors.

In our experiments, the α_2-adrenoceptor and imidazoline agonists clonidine and moxonidine inhibited the twitch contractions in both epididymal and prostatic segments with pIC50 values, respectively, of about 9.0 and 7.5 (Table 2). These values are better explained by actions on the adrenoceptors and cannot be fitted in the hypothesis of actions on imidazoline receptors, since the IC50 obtained for moxonidine was about 30-times higher than for clonidine. In this way, it has already been established that, considering imidazoline receptors, this relation is inverse, being that the affinity of moxonidine for imidazoline sites is higher than that of clonidine (Schlicker et al 1990; Ernsberger et al 1995; Eglen et al 1998). Furthermore, the

antagonism by idazoxan and yohimbine of the inhibitory effect by clonidine and moxonidine, with pA_2 values in the nanomolar range, is also compatible with interactions on α_2-adrenoceptors. It is already well established that yohimbine shows very low affinity (mM) for the imidazoline receptors (Doxey & Roach 1980; Ernsberger et al 1995). Therefore, we can conclude on the basis of our experiments that imidazoline receptors are not present in the rat vas deferens, as already reported (Avellar & Markus 1996).

Since we discarded the presence of imidazoline sites in our biological preparation, the inhibitory effect of agmatine in the epididymal end of the vas deferens could be attributed to interactions on the α_2-adrenoceptors. Indeed, the α_2-adrenoceptor antagonists idazoxan and yohimbine antagonised the inhibitory curve for agmatine (Table 1), thus suggesting an action of the amine on these receptors. Our results, with protocols of protection experiments (Figure 2), also support the idea of actions mediated by presynaptic α_2-adrenoceptors. The ability of the classical irreversible α-adrenergic antagonist phenoxybenzamine (Browne et al 1994) in blocking the concentration–response curve of clonidine was strongly reversed when this antagonist was incubated in the presence of agmatine, thus indicating that agmatine protected the receptor where clonidine interacted against the action of phenoxybenzamine. Therefore, agmatine does recognise and interact with presynaptic α_2-adrenoceptors. Taking all these results together, we feel that they strongly indicate an α_2-adrenoceptor-mediated action by agmatine. Nevertheless, we should consider that the antagonism by idazoxan and yohimbine was obtained only at a concentration range higher than that demanded to inhibit the effects of clonidine and moxonidine. This might lead us to point out that the interaction of the amine with the α_2-adrenergic receptor is not similar to the other agonists utilised. It is well known that the potency of an antagonist must be considered upon results obtained in ideal conditions, such as the occurrence of only one type of action (Furchgott 1972). Thus, a supposition to explain the low potency of the antagonists could be that agmatine acts by more than one mechanism. The hypothesis of another mechanism unrelated to α_2-adrenoceptors is also supported by the experiments with phenoxybenzamine (Figure 2). Whereas phenoxybenzamine exerted almost a full blockade on inhibitory concentration–response curves for clonidine, the same dose of the antagonist caused just a little (although significant) rightward shift of the curve for agmatine, without altering its maximum effect. This suggests that most of the effect of the amine in the epididymal portion may be attributed to a phenoxybenzamine insensitive pathway.

Thus, our results showed that the inhibitory effect of agmatine in the epididymal segment seems to occur via a double mechanism: one due to interactions on presynaptic α_2-adrenoceptors, and the other by a mechanism unrelated to this receptor site.

Excitatory effect in the prostatic segment

A reasonable explanation for the potentiation obtained in the prostatic end of the vas deferens (Figure 1B) could be an antagonistic action at the prejunctional α_2-adrenoceptors. This suggestion is in line with the idea that agmatine is an intriguing molecule. Molderings et al (2000) observed in the rat vena cava that the amine is a positive modulator at an allosteric site of the α_2-adrenoceptors and an antagonist at the ligand recognition site of the same receptor. However, in our case, an antagonist action by the amine on these presynaptic receptors is unlikely since in our experiments the antagonists idazoxan and yohimbine when given alone over the twitches did not reproduce the potentiation by agmatine. This was of no surprise since at low frequencies the prejunctional α_2-adrenoceptors are not activated by endogenous noradrenaline, as already pointed out by Starke et al (1989).

Since agmatine enhanced the response to exogenous ATP (Figure 3) or noradrenaline in the prostatic portion (but not in the epididymal), the possibility exists that the potentiating effect at the electrically-stimulated prostatic portion could be due to a facilitatory postsynaptic action on the released neurotransmitters.

Imidazolines and a series of chemically related compounds, like the guanidines, have been shown to antagonise the smooth muscle relaxant actions of $K_{(ATP)}$-channel openers in vascular and non-vascular smooth muscle (Challinor-Rogers & McPherson 1994; Shepherd et al 1996; Molderings & Gothert 1998). A blocking action on K^+ channels impairs the outward flow of potassium, and this can facilitate depolarisation and potentiate the contractile effects of different agonists. Therefore, the enhancing effect of agmatine on contractions by ATP and noradrenaline could be regarded as an antagonist action on the K^+ channel. Actually, the actions of agmatine were quite similar to those of tetraethylammonium, a known K^+-channel blocker (Docherty & Brady 1995), since they both augmented maximum effect of noradrenaline and ATP in the prostatic end without altering responses of these agonists in the epididymal end. Although the similarity between the effects of agmatine and tetraethylammonium are suggestive rather than conclusive, the inhibition by cromakalim on the potentiating effect by agmatine and tetraethylammonium on contractions by ATP strongly supports a K^+-mediated action. Indeed, this effect of cromakalim was not reproduced when the enhancement of the ATP response was induced by Bay K 8644, a Ca^{2+}-channel activator that does not interact with K^+channels (García et al 1984).

Other indications of interaction of agmatine with K^+ channels were obtained from the experiments with KCl. Both agmatine and tetraethylammonium, only in the prostatic segment, increased the response to low concentrations of KCl, whereas the response to higher K^+ concentrations was reduced. The ability of inducing differential effects, depending on the concentration of added potassium (low or high concentrations), is a typical behaviour of drugs that affect the K^+ channels on vascular and non-vascular smooth muscles. Enhancing the effect of low doses without altering or inhibiting the effect of high doses of KCl characterise a blocker of K^+ channels (Hamilton & Weston 1989; Edwards & Weston 1990). Thus, our results showed that agmatine might modulate the activity of the

K^+ channel in the prostatic portion of the rat vas deferens, at least at postsynaptic sites, which could explain the potentiation of the twitches.

In the rat vas deferens, previous studies with agmatine demonstrated conflicting results. Pinthong et al (1995), studying the prostatic portion, observed that the amine did not alter the amplitude of electrically-induced contractions, although it interacted with α_2-adrenoceptors. In our previous studies (Jurkiewicz et al 1996), we demonstrated that in the whole organ the amine potentiated the twitches, and in this study we verified this potentiation in the prostatic segment, but not in the epididymal end.

A possible explanation for the discrepancies between our results and those obtained from the other authors may be the ability of agmatine to cause diverse effects in the same biological preparation. It is plausible that, depending on the experimental conditions employed, a determined effect may prevail. Since α_2-adrenoceptors are present in the prostatic portion (Table 2; Ventura & Pennefather 1994), the possibility remains that agmatine could also act on these sites in this portion, causing an inhibitory effect, besides blocking the K^+-channels. So, the final effect in the prostatic portion could be due to a balance between these actions, with the potentiating effect prevailing. On the other hand, in the epididymal portion, where the interference with the K^+ channels seems not to affect the contractions (which was confirmed by the incapacity of tetraethylammonium in significantly affecting the epididymal portion), the inhibitory effect of agmatine would prevail. In the case of Pinthong et al (1995), the absence of effects with agmatine could be attributed to a nullification of the opposite effects under their experimental conditions.

The ability of the amine to cause opposite effects in the same tissue by different mechanisms has already been described. González et al (1996), using the rat tail artery, demonstrated that agmatine inhibited transmural nerve stimulation-induced contraction by an α_2-adrenergic-mediated mechanism, since it was antagonised by idazoxan and rauwolscine (10^{-7} and 10^{-6} M). This inhibition was followed by a delayed facilitation, which was greater in the presence of the α_2-adrenergic antagonists.

In conclusion, we demonstrated here that agmatine inhibited the amplitude of twitch contractions in the epididymal end by a mechanism partially mediated by presynaptic α_2-adrenoceptors. In the prostatic portion, the potentiation of twitches was likely related to, at least, a postsynaptic blocking action on K^+ channels. Whether or not agmatine also acts on presynaptic K^+ channels remains to be investigated.

A possible physiological significance for the actions of agmatine, besides the coexistence of these opposite effects in each segment of the rat vas deferens, needs to be better understood because of the high doses employed to induce its effects, and also remains to be investigated.

Taking all our results together, we can conclude that agmatine still remains an intriguing and versatile molecule, with actions that cannot be totally attributed to those first described by Li et al (1994).

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