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Functional properties of agmatine in rat vas deferens

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Abstract

Experiments were performed with rat vas deferens to verify whether agmatine, an endogenous ligand for adrenoceptors and imidazoline receptors, can influence sympathetic neurotransmission, with respect to contractions induced by transmural nerve stimulation, contractions induced by exogenous noradrenaline, and overflow of endogenous noradrenaline. It was shown that agmatine (a) caused a dose-dependent potentiation of electrically induced twitches, up to about 70% in relation to controls, (b) shifted to the right the inhibitory concentration-response curves for clonidine on electrically induced twitches, indicating competitive antagonism at presynaptic α-adrenoceptors, with a pA_2 value of 4.12 ± 0.10, (c) shifted to the right the concentration-response curves for noradrenaline-induced contractions, indicating competitive antagonism at postsynaptic α-adrenoceptors as well, with a pA_2 value of 4.03 ± 0.10, and (d) caused a dose-dependent increase of KCl-induced overflow of noradrenaline, up to about 90% in relation to controls. It is concluded that agmatine has multiple effects on sympathetic neurotransmission in rat vas deferens.

Keywords: Vas deferens; Noradrenaline; Agmatine; Neurotransmission; Imidazoline receptor; Adrenoceptor

1. Introduction

Agmatine (decarboxylated arginine) is an endogenous amine recently found in several mammalian organs including the rat vas deferens (Raasch et al., 1995). Agmatine is a ligand for α_2-adrenoceptors and imidazoline receptors, and was recognised as an endogenous clonidine-displacing substance (Li et al., 1994; Piletz et al., 1995). Thus, it was advanced that agmatine is a putative neurotransmitter or neuromodulator (Li et al., 1994).

It is still uncertain whether agmatine has an effect on noradrenergic nerve terminals. So far the main indication of a functional role for agmatine in the sympathetic system is its ability to release catecholamines from chromaffin cells (Li et al., 1994), in addition to its complex cardiovascular effects, increasing or decreasing blood pressure and noradrenaline overflow (Sun et al., 1995; Szabo et al., 1995; Molderings and Göthert, 1995). The concentration of agmatine in rat vas deferens is about 9.5 pg/mg tissue (Raasch et al., 1995), suggesting that it has a physiological function in this preparation. To analyse the possibility that agmatine influences the sympathetic system in vas deferens, we examined its effect on three steps related to sympathetic neurotransmission in this preparation, such as contractions induced by electrical stimulation, contractions induced by exogenous noradrenaline, and overflow of endogenous noradrenaline from nerve terminals.

2. Material and methods

2.1. Animals

Wistar rats (280–330 g) were killed with ether, and the vasa deferentia were removed (Jurkiewicz and Jurkiewicz, 1976) and kept in nutrient solution (mM: NaCl 138, KCl 5.7, $CaCl_2$ 1.8, NaH_2PO_4 0.36, $NaHCO_3$ 15, and glucose 5.5, in glass-distilled water) to be used in the following experiments.

2.2. Contractile responses to electrical stimulation

Vasa deferentia were mounted in 10 ml organ baths as for isotonic contraction experiments (Jurkiewicz and Jur-

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kiewicz, 1976), but maintained between two parallel platinum electrodes. Electrical stimulation (0.05 Hz, 50 V, 3.0 ms) was done with a Grass S88 stimulator in the absence or presence of agmatine (RBI). Contractions were recorded through a physiograph (Ugo Basile, Italy) using isotonic transducers (type 7006), with a 1 g load and six times amplification.

In some experiments, cumulative addition of the α_2-adrenoceptor agonist clonidine (Sigma) was performed in order to obtain inhibitory dose-response curves for twitch contractions. Simultaneous parallel experiments were done with clonidine in the absence or presence of agmatine (0.1–1.0 mM, 30 min), and log dose-response curves were plotted. To avoid distortions due to the potentiation of twitches by agmatine, the effects of clonidine were normalized, being expressed on the y axis as a percentage of the maximal inhibition, in relation to the respective logarithm of the dose of clonidine, on the x axis (Jurkiewicz and Jurkiewicz, 1976). The concentration of agmatine, [B], and corresponding dose-ratio for clonidine, DR, were used to calculate the pA_2 value, based on the mass law and analytical method, according to the equation:

$$pA_2 = \log(DR - 1) - \log[B]$$

as previously described (Furchgott, 1972; Mackay, 1978; Diaz-Toledo and Jurkiewicz, 1991). The pA_2 value is an estimate of the antagonist-receptor dissociation constant ($pA_2 = -\log K_b$).

2.3. Contractile responses to drugs

Vasa deferentia were mounted in 10 ml organ baths for isotonic contraction experiments (Jurkiewicz and Jurkiewicz, 1976). Dose-response curves for noradrenaline (Sigma) were performed in the absence or presence of agmatine (0.3–1.0 mM, 30 min). The values of pA_2 for agmatine were calculated from the shifts induced on dose-response curves, as described above. It is known that as a consequence of neuronal uptake, but not of extraneuronal uptake (Langeloh and Jurkiewicz, 1982), the shifts induced on dose-response curves for adrenergic agonists are distorted, making it difficult to measure drug-receptor parameters in rat vas deferens (Jurkiewicz and Jurkiewicz, 1976). To avoid this problem, we used cocaine (Merck, 10 μM, 30 min) to block neuronal uptake.

2.4. On-line electrochemical detection of noradrenaline overflow

Four organs were cut into slices (about 1 mm thick) and placed in a 1-ml glass microchamber for superfusion (1.6 $ml \cdot min^{-1}$, 30°C) with regular nutrient solution for 20–30 min, followed by a solution containing KCl (120 mM, by isosmotically replacing NaCl) for about 2.5 min, to release endogenous noradrenaline. The perfusates were pumped through the microchamber to a system for quantitation of noradrenaline by electrooxidation, using a Methrom amperometric electrochemical detector (Garcez do Carmo et al., 1993, 1994). Noradrenaline was oxidised at +0.65 V, leading to a change of the amperometric current that was recorded on chart paper. In general, three stimuli (S_1, S_2, S_3) were made with KCl in each experiment, at 30-min intervals. In some experiments agmatine was added 30 min before S_2 and washed out at the end of this stimulus. In the beginning of experiments, a KCl solution was perfused through an organ-free microchamber as control, to ascertain that this solution was not affecting the detection system. KCl caused usually a fast decline of the baseline which returned to normal values within less than 1 min. In addition, several doses of noradrenaline were perfused through the organ-free microchamber, in order to obtain a standard curve relating noradrenaline concentration to the amperometric current, from which the experimental values were interpolated. Cocaine could not be used in the study of noradrenaline overflow, because of technical problems, since it interfered with the electrochemical detection of noradrenaline.

3. Results

3.1. Effect of agmatine on contractile responses to electrical stimulation

Fig. 1 shows that electrically induced contractions in vas deferens are nerve-dependent, since they were blocked by tetrodotoxin (Fig. 1B). Agmatine induced a dose-dependent potentiation of these contractions, which increased up to 70% in relation to controls (Fig. 1A and Fig. 1C). Since this effect could be due to a pre- or postjunctional action of agmatine, experiments were done to verify whether agmatine can influence the effect of clonidine on prejunctional receptors, the contractile effect of exogenous noradrenaline and the release of endogenous noradrenaline.

3.2. Effect of agmatine on inhibitory dose-response curves for clonidine on twitch contractions

In electrically stimulated vas deferens, clonidine caused a dose-dependent decrease of the amplitude of twitch contractions, assumed to be due to the stimulation of presynaptic inhibitory receptors (Pinthong et al., 1995). Agmatine caused a shift to the right of clonidine dose-response curves (Fig. 2), showing a competitive antagonism, with a pA_2 value of 4.12 ± 0.10 ($n = 6$).

3.3. Effect of agmatine on contractile responses to drugs

In isolated vas deferens, contractions were not obtained with agmatine in concentrations up to 10 mM. However

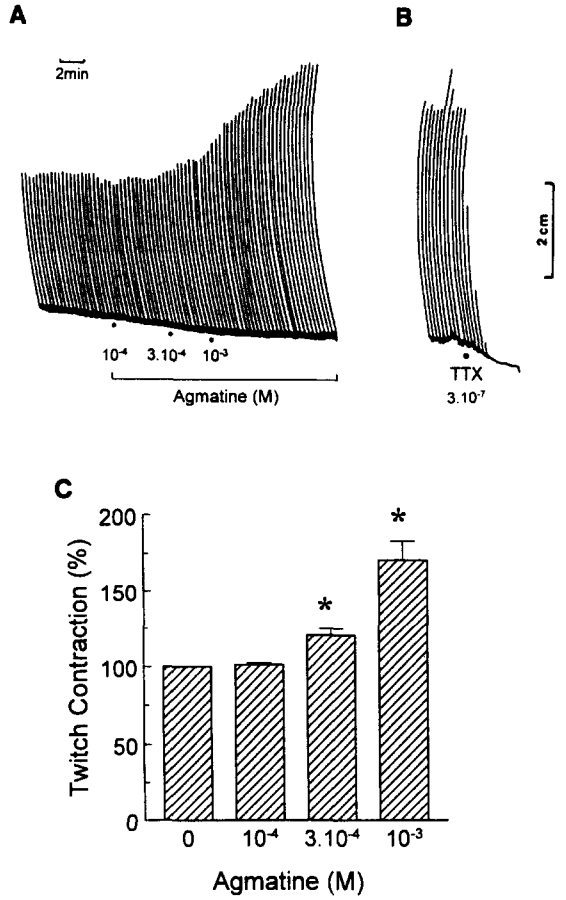

Fig. 1. A: Typical recording of contractions induced by transmural electrical stimulation (0.05 Hz, 50 V, 3.0 ms). Cumulative doses of agmatine (at dots) caused a potentiation of the amplitude of contractions. B: Control experiment showing that Tetrodotoxin caused a complete block of contractions, indicating that the effect is due to nerve stimulation. C: Histogram showing mean effects from experiments similar to that shown in A in the absence or presence of three doses of agmatine. Columns represent mean ± S.E.M. for 15 experiments.

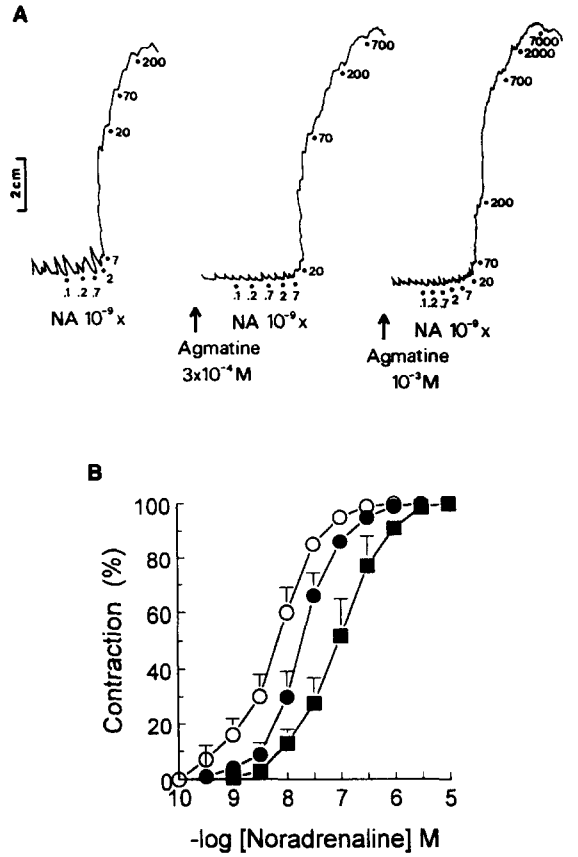

Fig. 3. A: Typical recording; and B: mean cumulative dose-response curves for noradrenaline in the absence (○) or presence of agmatine 0.3 mM (●) and 1.0 mM (■), in cocaine-treated vas deferens (10 μM, 30 min). Points represent mean ± S.E.M. for seven experiments.

agmatine caused a parallel dose-dependent shift to the right of dose-response curves for noradrenaline (Fig. 3A and Fig. 3B), showing competitive antagonism, with a pA_2 value of 4.03 ± 0.10 ($n = 4$). This effect resembled that of a number of other adrenergic antagonists in vas deferens (Jurkiewicz and Jurkiewicz, 1976).

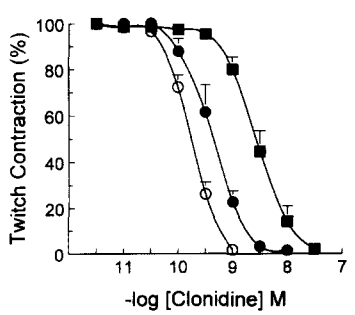

Fig. 2. Mean cumulative dose response curves for clonidine against electrically evoked contractions of the rat vas deferens, in the absence (○) or presence agmatine 0.1 mM (●) and 1.0 mM (■).

3.4. Effect of agmatine on noradrenaline release

Initial perfusion with regular nutrient solution caused an amperometric current of 13.2 ± 1.6 nA, corresponding to a concentration of noradrenaline of 0.17 μM. This was assumed to represent spontaneous overflow from nerve terminals, as it was absent in denervated organs (Garcez do Carmo et al., 1994). This current was taken as the baseline (0% in Fig. 4B).

Perfusion of KCl (120 mM) caused an increase of the current from 13.2 ± 1.6 nA to 21.2 ± 2.5 nA, corresponding to an increase of noradrenaline from 0.17 μM to 0.35 μM. During subsequent stimuli, noradrenaline overflow declined progressively, as previously described (Jurkiewicz et al., 1991), and shown in Fig. 4. Agmatine (up to 1 mM), did not cause significant changes in the baseline (Fig. 4A). However, when KCl was perfused in the presence of agmatine, a clear dose-dependent increase, up to about 90%, was observed in noradrenaline overflow (Fig. 4A and

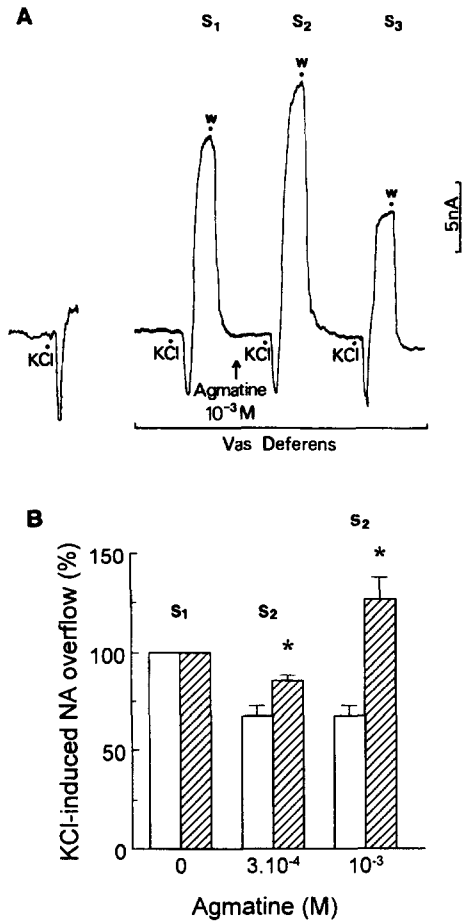

Fig. 4. A: Typical recording of KCl-induced (120 mM, 2.5 min) overflow of noradrenaline from perfused slices of vasa deferentia. After a control perfusion of KCl through the organ-free chamber, the organ slices were added to the chamber and three stimuli (S_1 to S_3) were given with KCl at 30 min intervals. Agmatine (1.0 mM) was added 30 min before (arrow) and washed out immediately after S_2, at w. B: Histogram showing means ± S.E.M. for stimuli S_1 and S_2 on experiments similar to that shown in A, in the absence (open bars) or presence (hatched bars) of 0.3 or 1.0 mM agmatine in S_2. Speed of recorder (usually 0.5 mm/min) was increased to 4 mm/min after initiation of KCl perfusion. Columns represent at least six experiments. $^*P < 0.05$ in relation to S_2 control.

Fig. 4B), as it would be expected with a block of presynaptic inhibitory receptors.

4. Discussion

The enhancement of contractions (Fig. 1) and of noradrenaline overflow (Fig. 4) elicited by agmatine as well as its rightward shift of the noradrenaline (Fig. 3) and clonidine (Fig. 2) concentration-response curves indicate that agmatine can influence sympathetic neurotransmission in rat vas deferens.

The simplest explanation for our results is that agmatine is an antagonist of postsynaptic excitatory as well as of presynaptic inhibitory α-adrenoceptors. The block of postsynaptic α-adrenoceptors in smooth muscle is expected to decrease the amplitude of contractions (as shown in Fig. 3). In contrast, the block of presynaptic α-adrenoceptors is expected to antagonise the effect of the presynaptic agonist clonidine (Fig. 2); furthermore, it is expected to increase the output of endogenous mediator (Fig. 4) and consequently to increase the amplitude of contractions (Fig. 1). According to this hypothesis, the resulting effect of agmatine on nerve-induced contraction depends on the balance between the inhibition of postsynaptic and presynaptic α-adrenoceptors. Under the present experimental conditions, the presynaptic effect seemed to counteract and predominate over the blockade of postsynaptic adrenoceptors in smooth muscle, as judged from the potentiation seen in Fig. 1.

That electrically induced twitch responses in vas deferens are potentiated by α-adrenergic antagonists is well known (Drew, 1977; Jurkiewicz et al., 1991). It is also known that these antagonists are able to shift dose-response curves for exogenous noradrenaline in the vas deferens (Jurkiewicz and Jurkiewicz, 1976; Caricati Neto et al., 1992; Jurkiewicz et al., 1992). Therefore, the effect of agmatine was similar to that of a number of competitive antagonists such as phentolamine, piperoxan, yohimbine, and tolazoline (Drew, 1977; Jurkiewicz and Jurkiewicz, 1976).

Concerning α-adrenoceptor subtypes, although it is tempting to suggest that the receptor interacting with agmatine in rat vas deferens is an α_2-adrenoceptor, the analysis of this point is out of the scope of the present discussion, mainly because additional experiments would be necessary. We believe that the functional characterisation of receptor subtypes (Bylund et al., 1994) should be carried out under ideal conditions, as described by Furchgott (1972), to avoid erroneous interpretations. For instance, it is known that curves for exogenous noradrenaline are due predominantly to an interaction with α_1-adrenoceptors (Pupo et al., 1995), but the so-called α_2-adrenoceptor antagonists, such as yohimbine, can shift these curves, if given in appropriate doses (Jurkiewicz and Jurkiewicz, 1976). In addition, noradrenaline can interact with β-adrenoceptors, causing an inhibition of electrically induced twitches (Diaz-Toledo and Jurkiewicz, 1990, 1991). Furthermore, at least one α_2-adrenoceptor agonist, clonidine, releases endogenous substances from the vas deferens, and a number of cotransmitters can be released by electrical stimulation (Jurkiewicz and Jurkiewicz, 1991). Moreover, adrenergic neurotransmission in this organ seems to be influenced by nitric oxide pathways (Vladimirova et al., 1994).

Assuming that imidazoline receptor subtypes are present in the vas deferens, the question arises as to whether the effects of agmatine can be ascribed to an interaction with these receptors. Although binding studies have not been done in vas deferens, it is known that in chromaffin cells the ED_{50} for agmatine to release catecholamines is 5 μM (Li et al., 1994), and the estimated affinity in binding

experiments with brain or chromaffin cells is about 1–15 μM (Li et al., 1994; Pinthong et al., 1995). Subsequent radioligand binding assays on human and bovine tissues (Piletz et al., 1995) showed that agmatine binds at two different sites: at I_1 receptors ($K_{i\ high}$ affinity constant = 33–127 nM and $K_{i\ low}$ = about 280 μM) and at α_2-adrenoceptors (K_i = 26–164 μM). Because in our experiments the effects of agmatine were obtained with doses higher than 100 μM, the possibility that the effects are due to an interaction with the high-affinity imidazoline site can be ruled out. Therefore our results are only compatible with an interaction of agmatine with the low-affinity imidazoline site, if present in vas deferens. However this interpretation is suggestive rather than conclusive, considering the limitations of a comparison between functional and binding experiments in different tissues.

One could argue that some of our results might not be in accord with previous publications. For instance, Pinthong et al. (1995) were unable to detect changes in electrically stimulated vas deferens, by using agmatine (300 μM). This result might not be inconsistent with our data, since we could only obtain a clear shift when the dose of agmatine was three times higher than that used by these authors (Fig. 2). Regarding noradrenaline overflow, Szabo et al. (1995) have shown that agmatine increases the plasma concentration of noradrenaline in rabbits, in vivo, but fails to modify [^{3}H]noradrenaline overflow induced by electrical stimulation of brain cortex slices. However, they used up to 100 μM agmatine, a dose that was also ineffective in our experiments in vas deferens. Using lower doses of agmatine (1–10 μM) in rabbit aortas, Molderings and Göthert (1995) reported a reduction of electrically induced [^{3}H]noradrenaline overflow, in contrast with the potentiation shown in vas deferens (Fig. 4). This might be due to the fact that the aortas, but not the vasa deferentia, were pre-treated with α_2-adrenoceptor antagonists before agmatine. In addition, considering that there is a large regional variation in agmatine tissue concentration, from about 0.45 pg/mg to 71 pg/mg (Raasch et al., 1995), the possibility exists that the effect of agmatine on adrenergic neurotransmission can vary according to the organ and species studied.

Our data raised the possibility that the effect of agmatine is physiologically relevant, and put forward the question of whether this amine can be suggested to be a modulator or cotransmitter in the vas deferens. We believe that this suggestion is still open for discussion, mainly because the doses of agmatine necessary to induce an effect are relatively high compared to the concentration of endogenous agmatine (Raasch et al., 1995), and the mechanism involved in the release or activation of endogenous agmatine is still unknown.

In conclusion, evidence has been provided that agmatine influences multiple steps in sympathetic neurotransmission in the rat vas deferens. Its effect is consistent with a block of excitatory postjunctional and inhibitory prejunctional α-adrenoceptors. However, interactions with other sites, such as imidazoline receptors, remain to be investigated.

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