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Agmatine Modulation of Noradrenergic Neurotransmission in Isolated Rat Blood Vessels

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Abstract

Agmatine, a vasoactive metabolite of L-arginine, is widely distributed in mammalian tissues including blood vessels. Agmatine binding to imidazoline and α_2-adrenoceptors induces a variety of physiological and pharmacological effects. We investigated the effect of agmatine on contractile responses of the rat pulmonary artery and portal vein induced by electrical stimulation of perivascular nerves and by exogenous adrenergic substances. Experiments were performed on isolated segments of rat main pulmonary artery and its extralobular branches, and portal vein suspended in organ bath containing modified Krebs bicarbonate solution and connected to a force-displacement transducer for isometric tension recording. Electrical field stimulation (EFS) produced tetrodotoxin-sensitive contractile responses of pulmonary artery and portal vein. Besides the well known vasorelaxant actions, we found that agmatine also produced a concentration-dependent inhibition of neurogenic contractions induced by EFS in pulmonary arteries; however, the agmatine treatment did not influence the responses to exogenous noradrenaline. The inhibitory effect on EFS-induced contractions was not abolished by the α_2-adrenoceptor antagonist rauwolscine. In portal vein, in contrast, agmatine increased spontaneous mechanical contractions and enhanced the contractions induced by EFS. The results suggest that agmatine can significantly influence vascular function of pulmonary arteries and portal veins by modulating sympathetically mediated vascular contractions by pre- and postsynaptic mechanisms.

Key Words: agmatine, neurogenic contractions, portal vein, pulmonary artery, rauwolscine

Introduction

L-arginine, an essential amino acid, exerts antihypertensive and antiproliferative effects on the cardiovascular system (5, 26) and reduces inflammatory and oxidative damages in organs and tissues exposed to various pathological and exhaustive conditions (14). In vessels, L-arginine can change the activity of smooth muscle by direct action as an agnist and a modulator of vascular function, or through its metabolites. L-arginine is a substrate for at least five enzymes identified in mammals, including arginase, nitric oxide synthase, arginine decarboxylase, arginine-glycine transaminase and kyotorphine synthase (26). Among these, two pathways are potentially related to regulation of the activity of vascular smooth muscle. One is production of nitric oxide(NO), mediated by nitric oxide synthase (24), and the other is formation of agmatine by the enzyme arginine decarboxylase.

Agmatine is widely distributed in mammalian tissues, including heart and blood vessels (30), where it is synthetized by endothelial cells and stored in endothelial and smooth muscle cells (33). Moreover, a substantial portion of tissue agmatine is probably absorbed from the lumen of the gut, which contains a high amount of agmatine originating from several sources, including ingested food. Agmatine can be further hydrolyzed by agmatinase to putrescin and urea, and metabolized to polyamines (1).

Agmatine has been identified as an endogenous agonist at imidazoline and α_2-adrenoceptors (18, 31, 33), and may be involved in the antihypertensive effect of L-arginine. Agmatine exerts predominantly

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vasorelaxant and blood pressure-reducing properties; intravenous administration of agmatine at higher doses induced a pronunced blood pressure decrease in anesthetized hypertensive rats (9, 29, 32). These effects might be due to its direct action in cardiovascular structures. Agmatine has been reported to stimulate (25), but also to inhibit, NO synthase (8), and to suppress smooth muscle cell proliferation (4, 33). Literature on the effect of agmatine on activity of isolated vascular smooth muscle of different vessels is sparse, with most reports showing that agmatine produces endothelium-dependent relaxation in precontracted isolated arteries (35). Agmatine-mediated relaxation is impaired in vessels isolated from high salt-loaded Dahl hypertensive rats (7).

Similarly to NO (13), agmatine was also shown to substantially interact with neural functions. This predestines agmatine to be an important issue in the research of pathomechanisms of various nerve disorders (31). However, these properties may be implicated also in the cardiovascular effects of agmatine – by means of modulation at central but also at peripheral levels of neural control of the heart and vessels. Decreased arterial pressure and sympathetic nerve activity after intravenous application of agmatine has been assumed to be due to blockade of transmission through sympathetic ganglia (40) and inhibition of noradrenaline release from sympathetic nerve endings by activation of presynaptic imidazoline receptors (22, 32). Moreover, agmatine inhibited carotid baroreflex in anesthetized rats (28) and blocked catecholamine release from bovine chromaffin cells (36). However, data on the effect of agmatine on neurotransmission in smooth muscle of different effector organs are scare and contradictory (10, 23, 27, 43, 44), which can be explained by the use of different tissue preparations, and less probably by the use of different agmatine concentrations.

In this study, we investigated the effect of agmatine on contractile responses of the rat pulmonary artery and portal vein induced by electrical stimulation of perivascular nerves and by exogenous adrenergic substances. Since agmatine exhibits pharmacological effects acting also on α_2-adrenergic receptors (18), we investigated possible contributions of α_2-adrenergic receptors in agmatine-induced modulation of adrenergically mediated responses.

Materials and Methods

Experimental Animals

Experiments were performed on 52 adult Wistar rats, weighing 250-350 g, given free access to food and water. The animal protocols used in this study were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health, and approved by the Animal Health and Welfare Division of the State Veterinary and Food Administration of the Slovak Republic.

Preparations of Vascular Samples

On the day of experiments, the rats were decapitated. The main pulmonary artery with its extralobular branches and portal vein were excised and placed in a petri dish filled with a modified Krebs' solution of the following composition (in mM): NaCl 118, KCl 5, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11, $CaNa_2EDTA$ 0.03, ascorbid acid 1.1. For the measurement of contractile activity, pulmonary arteries were cut into rings of 3.5-mm length and a segment of portal vein (12 mm in length) was dissected out and used as a longitudinal preparation. The vessel preparations were fixed on stainless steel hooks and suspended in 20 ml organ baths filled with modified Krebs' solution at 37°C and continuously gassed with 95% O_2 + 5% CO_2. One side of the ring of the pulmonary artery or portal vein segment was mounted vertically by a thread to a force-displacement transducer (Sanborn FT 10, Baltimore MD, USA) to measure changes in isometric tension, which were recorded with a polygraph TZ 4200 (Labora, Prague, Czech Republic). Vascular preparations (pulmonary artery or portal vein) were stretched to 10 mN of resting tension, and the stabilization period was 60 min with washing at 15-min intervals. When necessary, the endothelium was removed by gently rubbing the lumen of the ring with a wooden stick. In de-endothelized pulmonary rings, no relaxant response to 1 μM acetylcholine was observed.

Assessment of Vascular Reactivity

In pulmonary arteries precontracted with 1 μM phenylpephrine, the concentration-response curve for agmatine was determined in a cumulative manner. Control (intact endothelium) and experimental (denuded endothelium) data were obtained from separate vascular preparations. Electrical field stimulation (EFS) of perivascular nerves was provided by an electronic stimulator ST-3 (Medicor, Budapest, Hungary) *via* two platinum electrodes pointed on each side and parallel to the vessel preparation. The following parameters of stimulation were used: square-wave pulses 0.5 ms duration, 1-32 Hz, supramaximal voltage (>40 V), duration of stimulation 20 s. A period of approximately 10 min was allowed between stimulations. EFS-induced responses were pharmacologically tested in preliminary experiments on vessels from 6 animals. In artery and vein, these contrac-

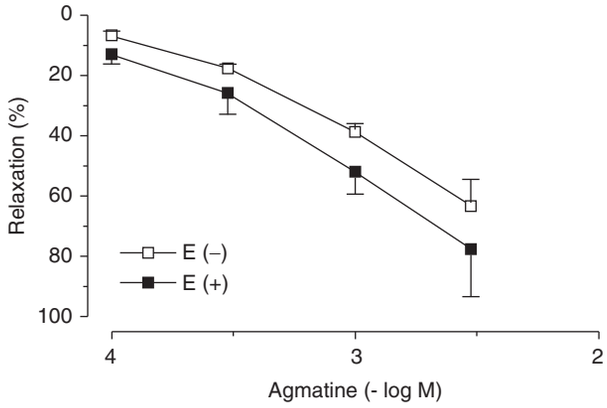

Fig. 1. Concentration-response of agmatine in rat main pulmonary artery in the absence (E–) or presence of intact endothelium (E+). Data are shown as means ± S.E.M.

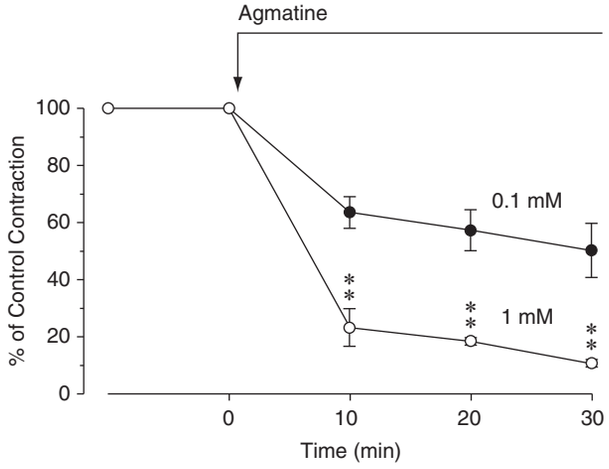

Fig. 2. Effect of agmatine on electrical field (at 4 Hz) stimulation-induced contractions in the rat main pulmonary artery as a function of time. $^{**}P < 0.01$ compared between two different concentrations of agmatine.

tions were abolished by tetrodotoxin indicating the neurogenic nature of the responses. In pulmonary artery, guanethidine or phentolamine abolished the responses at all frequencies of stimulation; therefore, we assumed that the contractile responses were due to release of noradrenaline from depolarized perivascular nerves. After adrenergic blockade in the portal vein, a small residual responses remained, indicating contribution of another neurotransmitter besides noradrenaline in EFS-induced contractions.

Drugs and Data Analysis

All chemicals used were purchased from Sigma-Aldrich (Steinheim, Germany) except noradrenaline hydrogenotartras (Zentiva, Prague, Czech Republic). The results are expressed as means ± S.E.M. The particular responses to pharmacological and electrical stimuli are expressed in relative values as a percentage of control reaction or phenylephrine precontraction, or in absolute values normalized to the cross sectional area of the respective vessel preparation (mN/mm^2). Grouped results were subjected to oneway ANOVA analysis. $P < 0.05$ was considered statistically significant.

Results

Effects of Agmatine on Vascular Active Tension and Spontaneous Mechanical Activity

Agmatine in concentrations of 1 μM-1 mM was without effects in resting rings of isolated pulmonary artery. In 1 μM phenylephrine precontracted arterial preparations, 0.1-3 mM agmatine caused a concentration-dependent relaxation. The relaxation was not significantly different in deendothelized preparations (Fig. 1).

At 1 μM concentration, rauwolscine, a selective α_2-adrenoceptor antagonist, enhanced the magnitude of agmatine-induced relaxation in endothelium-intact pulmonary arteries.

In longitudinal segment of portal vein, agmatine at 100 μM-1 mM concentrations gradually enhanced the magnitude of spontaneous mechanical contractions. In the presence of 1 mM agmatine, the twitch contractions increased 250-290%. The potentiating effect of agmatine on spontaneous twitches was abolished by the calcium antagonist diltiazem (Fig. 4). The enhancement of spontaneous contractions of portal vein was observed also with clonidine, an α_2-adrenergic receptor agonist, in the lower concentrations 0.1-10 μM (data not shown).

Effects of Agmatine on Neurogenic Contractions in Pulmonary Artery

EFS caused frequency-dependent contractions with maximum tension reached at 32 Hz, representing 29.1 ± 1.3% (n = 6) of 60 mM KCl-induced contraction. The magnitude of these neurogenic contractions was higher by approximately 20% in the main pulmonary artery compared to its branches.

Agmatine produced a concentration-dependent inhibition of neurogenic contractions in the main pulmonary artery induced by EFS at 4 Hz (Fig. 2). After 30 min of its action, 0.1 mM agmatine reduced the EFS-induced contractions to 49.0 ± 9.2% of the control responses (n = 8). Clonidine (0.03-3 μM) produced quantitatively similar inhibition of neurogenic contractions in the pulmonary artery (data not shown).

Rauwolscine at 1 μM inhibited neurogenic contractions but did not prevent the inhibiting effect of

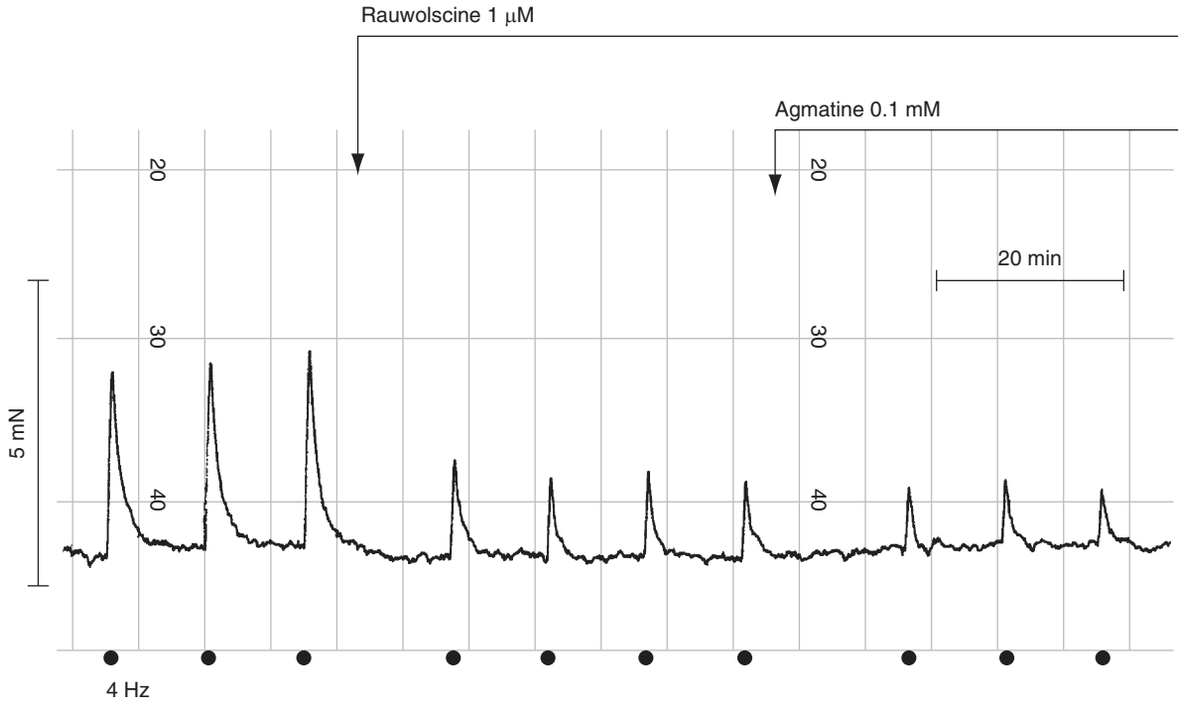

Fig. 3. Representative recording showing the effect of agmatine on contractions induced by EFS at 4 Hz in rat main pulmonary artery after pretreatment with rauwolscine.

agmatine on EFS-induced contractions (Fig. 3). In the presence of 1 μM rauwolscine, 0.1 mM agmatine treatment for 30 min reduced neurogenic contractions in the pulmonary artery to 43.5 ± 4.2% (n = 9), and this inhibition was not significantly different from that in the absence of rauwolscine. Similar results were obtained also with the other selective α_2-adrenoceptor antagonist yohimbine (data not shown).

To determine whether the effects of agmatine were caused pre- or postsynaptically, a single dose of exogenous 0.1 μM noradrenaline was used to induce a contraction comparable in magnitude to that produced by EFS at 4 Hz. Contractile responses induced by exogenous 0.1 μM noradrenaline (11.2 ± 1.7 mN/mm^2) were not significantly inhibited by 1 mM agmatine (10.4 ± 2.0 mN/mm^2, n = 8).

Effects of Agmatine on Neurogenic Contractions in Portal Vein

Agmatine caused potentiation of EFS-induced contractions at 4 Hz in portal vein (Fig. 4). Rauwolscine (1 μM) showed a tendency to inhibition of neurogenic contractions (not significant) but did not abolish the potentiating effects of agmatine on EFS-induced contractions (Fig. 4). Enhancement of EFS-induced contractions after 60 min of incubation with 1 mM agmatine in the presence of 1 μM rauwolscinereached 246 ± 19.1% (n = 7), and did not significantly differ from data obtained in the absence of rauwolscine (223.5 ± 16.3%, n = 7) (Fig. 5).

Discussion

The present study evaluated the effects of agmatine on vascular tension and EFS-induced contractions in rat low-pressure blood vessels with relatively rich adrenergic innervation: main pulmonary artery and portal vein. The results demonstrated that agmatine inhibited contractile responses induced by EFS in pulmonary artery, and enhanced spontaneous mechanical contractions and contractions induced by EFS in portal vein.

When elucidating the discrepancies between the effects of agmatine on EFS-induced responses in the two used types of vascular preparations, it is necessary to consider the important differences in the functional organization of the smooth muscle and the neuroeffector system in the respective vessels. Somlyo and Somlyo (38) described in low-pressure vessels two major types of vascular smooth muscle with different fundamental characteristics. The first type (main pulmonary artery) contains tonic vascular smooth muscles that are electrically silent and when stimulated, they usually respond to excitatory agents with graded depolarization. The second type (portal-mesenteric vein) contains spike-generating phasic smooth muscles with pronounced cell-to-cell propagation of myogenic

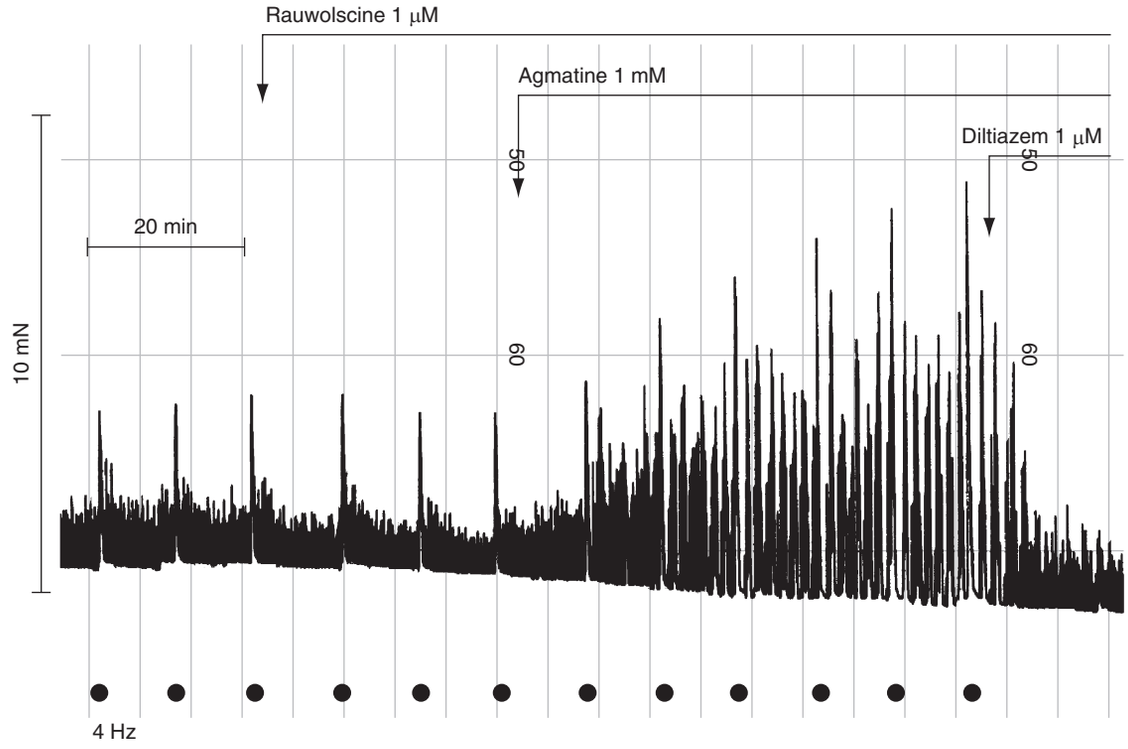

Fig. 4. Representative recording showing the effect of agmatine on spontaneous and EFS-induced contractions in the longitudinal segment of portal vein after pretreatment with rauwolscine.

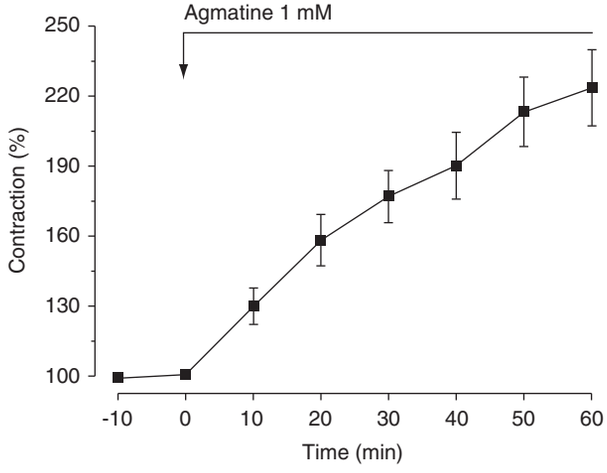

Fig. 5. Effect of agmatine on contractions induced by EFS at 4 Hz in the longitudinal segment of rat portal vein as a function of time. Data are presented as percentages of the control contractions obtained in the absence of agmatine.

activity (3). It is conceivable that the portal vein with its spontaneously generated contractions and discrete neurogenic influence enforced by the myogenic conduction of activity on the one hand, and the pulmonary artery with tonic smooth muscle and more diffuse spread and action of the transmitter on the other, represent the extremes in a spectrum of vascular neuroeffector organization (3). Therefore, we assume that these vessels respond differently to various neurohumoral substances acting as direct agonists or modulating the responses to other important vasoactive stimuli.

In our experiments, agmatine by itself did not evoke any direct change of the resting tone in the pulmonary artery. However, after phenylephrine precontraction, agmatine induced concentration-dependent relaxant responses. Similar relaxations were also observed on precontracted rat tail artery and aorta rings (6, 10, 11). The magnitude of agmatine-induced relaxation was unchanged in deendothelized pulmonary arteries. This finding excludes the significant participation of the endothelium-derived NO in agmatine-induced relaxation of the rat pulmonary artery. Addition of rauwolscine, an α_2-adrenoceptor blocker, into the incubation bath did not abolish this relaxation. These data failed to confirm the participation of α_2-adrenergic receptors in this reaction. In summary, such observations point to agmatine being another important L-arginine-derived metabolite, which can produce vascular dilatation independently of NO. This is in accordance with the results of Gadkari *et al.* (7), who demonstrated that inhibition of arginine decarboxylase by difluoromethylarginine reduced L-arginine-induced vasorelaxation, suggesting that this inhibition was mediated, at least in part, by agmatine formation.

On the other hand, in our previous measurements performed on aortic rings, presence of 1 mM agmatine in the incubating medium attenuated acetylcholine-induced NO-dependent relaxant response; however, this was significant only at the highest concentration at 10 μM of the dose-response curve to acetylcholine (8). This suggests that under particular conditions, agmatine can interfere with the NO-generating pathway and acts as a competitive inhibitor of NO synthase (8).

In our experiments, agmatine also inhibited the contractile responses elicited by EFS of perivascular nerves in the rat main pulmonary artery and its two branches. The contractions were fully renewed by washing agmatine out of the incubating bath. The inhibitory effects might be partially ascribed to the blockade of L-type voltage-dependent Ca^{2+} channels by agmatine, which was demonstrated by Li *et al.* (19) in ventricular myocytes, and by Weng *et al.* (42) in rat hippocampal neurons. In addition, similarly to NO (2), agmatine seems to exert direct effects on K^+ channels and might lead to hyperpolarization of the vascular smooth muscle (35). These effects could contribute also to the observed agmatine-induced relaxation in precontracted arteries.

To determine whether the inhibitory action of agmatine on neurogenic contractions of the pulmonary artery was generated pre- or postsynaptically, a single dose of 0.1 μM noradrenaline was added to the bath. Agmatine, in contrast to its effect on inhibition of EFS-induced contraction, did not significantly modify the responses to exogenous noradrenaline, suggesting that the site of agmatine action in inhibiting the responses to EFS was mainly presynaptic. To further examine the mechanism of this effect, we studied the involvement of presynaptic α_2-adrenoceptors. It is well known that agmatine can interact with α_2-adrenergic and imidazoline receptors in various organs (18, 34). Vascular α_2-adrenoceptors are expressed in endothelium, smooth muscle and perivascular sympathetic nerves. The density of the α_2-adrenoceptors varies among different blood vessels even in the same animal. Activation of these receptors was found to influence vascular tone differently: stimulation of endothelial α_2-adrenoceptors (generation of NO) and of presynaptic receptors (inhibition of noradrenaline release) relaxed smooth muscle (16, 20), while stimulation of postsynaptic α_2-adrenoceptors on vascular smooth muscle directly contracted vessels (21). Gonzáles *et al.* (10) found that agmatine produced transient inhibition followed by delayed facilitation of EFS-induced contraction in the rat tail artery. The inhibitory effects were abolished by α_2-adrenergic receptor antagonists. To characterize the receptor(s) responsible for the inhibition of contractile responses to EFS by agmatine in our experiments, the rings of pulmonary artery were initially exposed to rauwolscine or yohimbine, the selective α_2-adrenoceptor antagonists. In accordance with the results of other authors (21), both rauwolscine and yohimbine alone comparably reduced the responses to EFS as a result of inhibition of postsynaptic α_2-adrenoceptors; this reduction was stable for the duration of the experiment in the control arteries. In the presence of either of the α_2-adrenergic receptor blockers studied, the inhibitory action of agmatine on EFS-induced contractions was not prevented, and gradual inhibition of EFS-induced contractions continued (Fig. 3). The fact that rauwolscine failed to reverse the reduction of neurogenic contractions by agmatine indicated that inhibitory postsynaptic effect of the used α_2-adrenoceptor antagonists was probably greater than that of presynaptic, and that combined application with agmatine might produce a cumulative inhibitory effect.

As with agmatine, the same inhibition of EFS-induced contractions was observed in the presence of an α_2-adrenoceptor agonist clonidine. The inhibitory effects of agmatine and clonidine on the EFS-induced contractions of the rat pulmonary artery were similar. The comparability of the inhibition produced by agmatine and clonidine also indicated that the inhibition of EFS-induced contractions by agmatine was the result of stimulation of presynaptic α_2-adrenoceptors. This is in agreement with findings of Su and Kubo (39) in the rat mesenteric bed showing that clonidine decreased ^{3}H-noradrenaline efflux evoked by perivascular nerve stimulation.

In the experiments on isolated portal veins, we investigated the effect of agmatine on its spontaneous mechanical activity and on contractions induced by EFS. Phasic spontaneous contractions are known to be highly dependent on Ca^{2+} concentrations; they are significantly depressed in conditions of low extracellular Ca^{2+} (41). The present results show that agmatine at concentration 1 mM enhanced spontaneous activity approximately by 150-190%. Enhancement of spontaneous twitches was reduced by diltiazem and other calcium antagonists, or in a solution with decreased level of Ca^{2+} (data not shown), which suggests that augmentation of these contractions is mediated mainly by the increase in Ca^{2+} influx across cell membrane. Such a mechanism might be partially involved also in agmatine-induced potentiation of EFS-induced contractile responses in the portal vein observed in our experiments. Similarly, in other smooth muscle, the rat vas deferens, agmatine caused a dose-dependent enhancement of electrically induced twitches up to about 70% in relation to the controls (15). These findings are contradictory to the observed inhibitory effects of agmatine on neurogenic contractions in the pulmonary artery reported here. However, as mentioned above, the responses of various vessel

preparations to the same vasoactive substances may vary substantially, with respect to their function and the respective structural organisation along with the density and distribution of various receptors and ion channels. Santos *et al.* (37) previously showed that in vas deferens, agmatine produced a dual effect, depending on the segment of the organ studied, namely a potentiation of neurotransmission in the prostatic end and an inhibition in the epididymal portion. In the prostatic segment, the potentiating effect was suggested to be mediated by a blocking action on K^+ channels. In the epididymal segment, the inhibition of electrically induced twitch contractions was attributed to a mechanism partially mediated by presynaptic α_2-adrenoceptors (37).

The adrenoceptors in the rat portal vein that iniciates contractions seem to be of both α_1 and α_2 types (12, 17). In our observations, the agmatine-induced increase in neurogenic contractions was not changed by the application of rauwolscine; therefore, the activation of α_2-adrenergic receptors does not seem to participate in this process although we cannot exclude possible involvement. In the experiments on rat vena cava, Molderings *et al.* (23) demonstrated that agmatine did not directly modulate the electrically evoked release of noradrenaline from sympathetic nerve endings. However, these authors also showed that in a relatively lower concentration of 10 μM, agmatine could enhance the presynaptic α_2-adrenoceptor-mediated inhibition induced by full α_2-adrenoceptor agonists like noradrenaline and moxonidine. On the other hand, 1 mM agmatine might act as a competitive inhibitor on α_2-adrenergic receptors and suppressed the inhibitory effects of α_2-adrenoceptor agonists on noradrenaline release from sympathetic nerve terminals (23). Therefore, the agmatine influence on sympathetic neurotransmission seems complex. Further studies, especially on direct measurements of noradrenaline release from perivascular nerves along with the use of particular agonists and antagonists of presynaptic receptors, would elucidate in detail mechanisms of agmatine neuromodulation in vessels.

In conclusion, the present study demonstrated that agmatine might enhance as well as inhibit vascular contractile responses to sympathoneural stimulation depending on the vessel type. Therefore, agmatine may have an important function in the biology of blood vessels not only as a direct agonist but also as a modulator of vascular contractions by pre- and postsynaptic mechanisms.

Acknowledgments

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