



---

**Short communication**

## Use of transgenic (knockout) mice reveals a site distinct from the $\alpha_{2A}$ -adrenoceptors for agmatine in the vas deferens

Wilson C. Santos<sup>1,3</sup>, Lucia Garcez-do-Carmo<sup>2</sup>, Eliane C. da Silva<sup>2</sup>, Ricardo de Pascual<sup>3</sup>, Neide H. Jurkiewicz<sup>2</sup>, Aron Jurkiewicz<sup>2</sup>, Luis Gandía<sup>3</sup>

<sup>1</sup>Departamento de Farmácia e Administração Farmacêutica, Faculdade de Farmácia, Universidade Federal Fluminense, Rua Mário Viana, 523-Santa Rosa, CEP 24241-000, Niterói, RJ, Brasil

<sup>2</sup>Departamento de Farmacologia, Universidade Federal de São Paulo, Rua Botucatu, 862-Vila Clementino, CEP 04021-023, São Paulo, SP, Brasil

<sup>3</sup>Instituto Teófilo Hernando, Departamento de Farmacología y Terapéutica, Facultad de Medicina, C/Arzobispo Morcillo, 4, 28029 Madrid, Spain

**Correspondence:** Wilson C. Santos, e-mail: wsantos@vm.uff.br

---

**Abstract:**

The inhibitory effect of agmatine on electrically induced contractions was studied in vas deferens of Adra 2a transgenic mice lacking  $\alpha_{2A}$ -adrenoceptors. Agmatine and clonidine caused a concentration-dependent inhibition of twitches. However, while agmatine showed a similar pIC<sub>50</sub> value in control and transgenic mice, the pIC<sub>50</sub> value for clonidine was about 30-fold lower in knockout mice. In both strains, yohimbine shifted the curve for clonidine, but not for agmatine, even when a 100-fold higher concentration of yohimbine was employed. Our results indicate that inhibition by agmatine in mouse vas deferens is not simply due to interactions with  $\alpha_2$ -adrenoceptors in our experimental conditions.

**Key words:**

vas deferens, transgenic (knockout) mice, agmatine;  $\alpha_{2A}$ -adrenoceptors, neurotransmission

---

**Abbreviations:** Adra 2a mice – knockout mice lacking  $\alpha_{2A}$  adrenoceptors (C57BL/6-*Adra2atmlLel* mice)

---

### Introduction

Agmatine is an endogenous amine with  $\alpha$ -adrenergic and imidazoline ligand properties [8, 20], whose effects in diverse biological preparations have high-

lighted its putative role as a neurotransmitter or neuromodulator [11, 19, 21]. In the rat vas deferens, we previously showed that agmatine possesses pre- and post-synaptic effects [9] and that it inhibits the electrically induced twitch contraction in the epididymal portion of this organ [22]. This effect was ascribed, to some extension, as an action of the amine on prejunctional  $\alpha_2$ -adrenoceptors, although it was only partially reverted by antagonists of prejunctional  $\alpha_2$ -adrenoceptors [22]. Furthermore, we recently showed that a nitrergic pathway activated by agmatine might play

---

a role in its inhibitory effect, although no definition was obtained as to whether it resulted from a direct or an indirect mechanism [6]. Thus, the possibility that the amine interacts not only with  $\alpha_2$ -adrenoceptors but also with other sites is still open to investigation.

The  $\alpha_2$ -adrenoceptors have been implicated in various physiological processes [10]. Multiple  $\alpha_2$ -adrenergic receptors subtypes ( $\alpha_{2A}$ ,  $\alpha_{2B}$  and  $\alpha_{2C}$ ) have been identified by both pharmacological and molecular approaches [4, 13, 16]. Knockout mice lacking the  $\alpha_{2A}$ -adrenoceptor, which mediates the response of several alpha-adrenergic agonists in central and peripheral nerves [12], have been genetically engineered. Under our point of view, this knockout model could be a suitable model for further studying the site of action of agmatine on sympathetic neurotransmission. Thus, we addressed functional experiments to investigate whether the prejunctional  $\alpha_2$ -adrenoceptors might account for the inhibition by the amine in mouse vas deferens. The vas deferens was submitted to electrical field stimulation (EFS), and the inhibition induced by agmatine or by the  $\alpha_2$ -adrenergic agonist clonidine [5] was compared.

---

## Materials and Methods

### Animals

Knockout mice, lacking  $\alpha_{2A}$  adrenoceptors (C57BL/6-*Adra2atm1Lel* mice, which will be referred to here as Adra 2a mice) were purchased from The Jackson Laboratory, USA [13], bred and maintained in our animal facilities under Specific-Pathogen Free conditions. These animals and their respective normal controls (C57BL/6) were used in the following experiments. 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.

### Biological preparation

Mice weighing about 25 g and 3 months old were killed by ether overdose, and the vasa deferentia were rapidly removed and dissected out from fat and connective tissues. The vas deferens was suspended in a 10-ml organ bath with an oxygenated nutrient solu-

tion kept at 30°C in glass-distilled water with the following composition (mM): NaCl 138, KCl 5.7, CaCl<sub>2</sub> 1.8, NaHCO<sub>3</sub> 15, NaH<sub>2</sub>PO<sub>4</sub> 0.36, and glucose 5.5 [2]. Tissues were allowed to equilibrate for a 60-min period before starting the experiments. During this time tension was adjusted to a final value of 0.5 g. Contractions were recorded on a two channel physiograph (Ugo Basile, Italy) by using isometric transducers (Ugo Basile, type 7006).

### Contractile responses to electrical stimulation

For electrical field stimulation protocols the tissues were placed between two parallel platinum electrodes and electrical stimulation was performed with a Grass S88 stimulator at the following parameters: 0.1 Hz, 80 V, 2 ms. At these stimulation parameters the amplitude of contractions (twitch response) was maintained for at least 2 h. These contractile responses were abolished by 30 nM tetrodotoxin, indicating a nerve-mediated process (data not shown). After stabilization of twitches, inhibitory concentration-response curves were performed for agmatine or clonidine in the absence or presence of yohimbine (10 nM – 1  $\mu$ M), a competitive  $\alpha_2$ -adrenoceptor antagonist [5], incubated for a 20-min period before initializing the curves.

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. The negative logarithm of the concentration of the agonist inducing 50% of the inhibitory effect ( $pIC_{50}$ ) and the shift to the right, induced by yohimbine, of the concentration-response curve of the agonist (log DR) were calculated as previously described [2, 24].

### Expression of data and statistical analysis

All values are expressed as the means  $\pm$  SEM. Differences were termed significant at  $p < 0.05$ . Pharmacological parameters were analyzed by Student's *t*-test.

---

## Results

Agmatine inhibited twitch contractions in a dose-dependent manner in both Adra 2a and C57/BL mice,

**Tab. 1.** Drug-receptor parameters for agmatine and clonidine in vas deferens of knockout (Adra 2a) and control (C57BL/6) mice

	Parameters			
	pIC <sub>50</sub> value		log DR <sup>1</sup>	
	C57BL/6	Adra 2a	C57BL/6	Adra 2a
Clonidine	10.5 ± 0.22	9.1 ± 0.22*	1.7 ± 0.32**	2.2 ± 0.27**
Agmatine	3.75 ± 0.08	3.55 ± 0.04	0.04 ± 0.07	0.11 ± 0.05

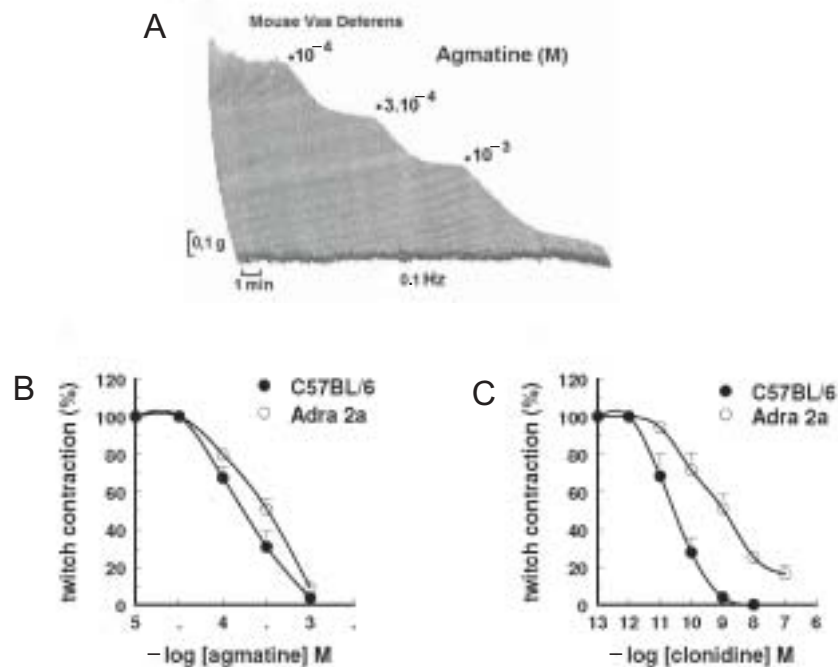
<sup>1</sup>Obtained from the shift induced by yohimbine (10<sup>-8</sup> M, for clonidine and 10<sup>-6</sup> M, for agmatine). \* Significantly lower from the corresponding value for control strain (C57BL/6). \*\* Significantly higher than the shift induced on the curves for agmatine

as shown in Figure 1B. Also, the corresponding pIC<sub>50</sub> value were not different from each other in both mice strains (Tab. 1). When clonidine was used instead of agmatine, it also inhibited the twitch contraction in a concentration dependent manner, as shown in Figure 1C. However, the vas deferens of Adra 2a mice was less sensitive than the respective controls, as indicated by the pIC<sub>50</sub> values that were 1.4 log units lower (Tab. 1). In addition, clonidine was not able to pro-

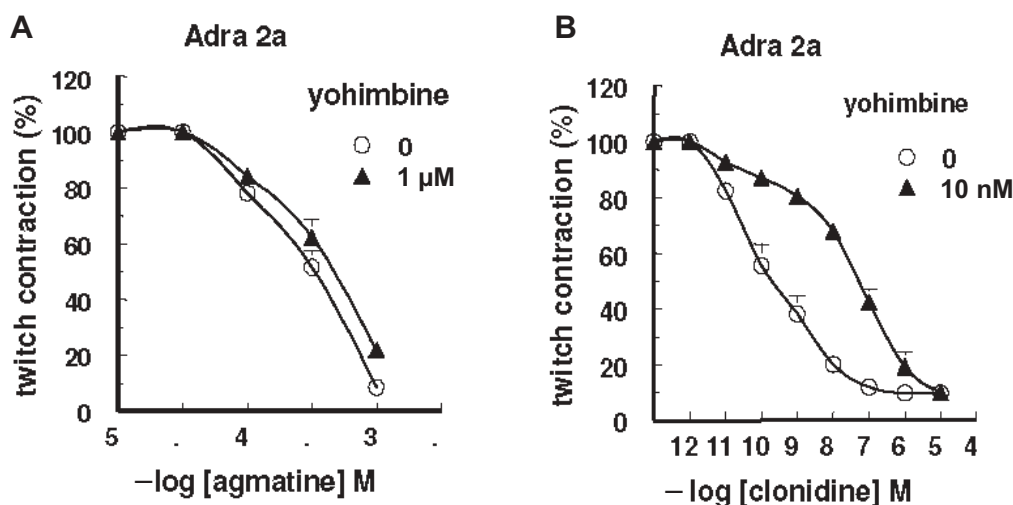
duce a complete inhibition of the twitch contractions, since a mean residual effect was still seen after the maximum concentration of this agonist (Fig. 1C). The competitive antagonist yohimbine produced, in both mouse strains, a rightward shift on the curve for clonidine (Fig. 2B and Tab. 1). However, the curves for agmatine were practically not shifted by yohimbine, even though the dose of the antagonist was increased from 10<sup>-8</sup> to 10<sup>-6</sup> M (Fig. 2A). A shift of the agmatine curve was also not observed in the corresponding controls, as judged from the respective log DR values (Tab. 1), indicating that agmatine was not interacting with adrenoceptors even in the non-transgenic mice.

### Discussion

Our experiments showed a clear difference between the effects of agmatine and clonidine in vas deferens from transgenic and control mice, indicating that both agonists have different mechanisms of action from each other. Such an observation is supported by the



**Fig. 1.** (A) Typical recording of the effects of agmatine on electrically induced contraction in mouse vas deferens. Mean cumulative concentration-response curves for agmatine (B) and clonidine (C) on electrically induced contractions in vas deferens of control (C57/BL) and knockout (Adra 2a) mice. Points represent the means ± SEM from at least 6 experiments, and are expressed as a percentage of the response in the absence of the drugs



**Fig. 2.** Mean cumulative concentration-response curves for agmatine (**A**) and clonidine (**B**) on electrically induced contraction in vas deferens of knockout (*Adra 2a*) mice. Experiments were performed in the absence or presence of yohimbine  $10^{-8}$  M (for clonidine) and  $10^{-6}$  M (for agmatine), after incubation for 20 min. Points represent the mean  $\pm$  SEM from at least 6 experiments, and are expressed as a percentage of the response in the absence of drugs

following results: (a) contrary to clonidine, the effect of agmatine was not altered when the drug was used in experiments with knockout mice, and (b) contrary to clonidine, the curves for agmatine were not significantly shifted by the competitive antagonist yohimbine. Since clonidine is a typical  $\alpha_2$ -adrenoceptor agonist, it was demonstrated here that the effect of agmatine in mice vas deferens was not due to interactions with  $\alpha_2$ -adrenoceptors in our experimental conditions.

The inhibitory effect of clonidine is known to be due to a reduction of noradrenaline released from electrically stimulated nerve terminals [23]. In control mice, the action of clonidine was clearly on the  $\alpha_{2A}$ -adrenoceptors, since a striking reduction of about 1.4 log units was observed for the  $pIC_{50}$  value for clonidine in knockout animals (Tab. 1). On the other hand, the fact that clonidine continued to inhibit the twitches, although with higher doses, indicates that it might also interact with other subtypes of  $\alpha_2$ -adrenoceptors [1]. This is corroborated by the finding that in the knockout mice the competitive antagonist yohimbine continued to cause a shift on the inhibitory curve for clonidine.

Therefore, it seems that a role for the  $\alpha_2$ -adrenoceptors on the effects of agmatine in sympathetic neurotransmission in vas deferens of rodents is still not uncovered, although other authors have already described agmatine's capacity for recognizing and interacting with these receptors. In the epididymal

end of rat vas deferens we recently suggested a double mechanism of action for the inhibitory effect by agmatine, and the pre-synaptic  $\alpha_2$ -adrenoceptors did not seem to account for the majority of the action of the amine. Nevertheless, agmatine did interact with these sites, since the amine was able to protect  $\alpha_2$ -adrenoceptors from the antagonistic action of phenoxybenzamine [22]. Moreover, there is the possibility that the nitroergic route activated by agmatine, which can account for the inhibitory effect, may result from an action on the  $\alpha_2$ -adrenoceptors [3, 6]. Along the same lines, it has already been observed [18] that agmatine does not alter the amplitude of electrically-induced contractions although it interacts with  $\alpha_2$ -adrenoceptors.

Considering the rodent *vasa deferentia*, the participation of the  $\alpha_2$ -adrenoceptors in controlling neurotransmission is broadly known [10, 23, 25], and, since anomalous action by the amine on the  $\alpha_2$ -adrenoceptors have been previously described [7, 15], we feel that the effects of agmatine on the sympathetic nervous system are complex.

Therefore, we conclude that  $\alpha_2$ -adrenoceptors are not fundamental for agmatine effects in sympathetic neurotransmission in mice vas deferens, as we have previously shown in the vas deferens of rats. Other mechanisms for the inhibition of neurotransmission by the amine remain to be investigated, and we think that imidazoline receptors and/or activation of the nitroergic route should be studied, since some concerns

on it have already pointed out, considering the rat vas deferens [6, 18, 22]. Nevertheless, we feel that specific attention should be given by the fact that such complex actions for agmatine on the  $\alpha_2$ -adrenoceptors, as reported in the present paper, has already been described by other Authors on the imidazoline system [14]. Thus, to our knowledge, agmatine still persists as an intriguing and versatile molecule.

#### Ac no ledgments:

Thanks are due to Ms. Theotila Reuter for her technical assistance and Ms. Shihane Mohamad for helping in the preparation of the manuscript. We also thank the Fundación Teófilo Hernando (Spain), FAPERJ (Brasil), and FAPESP (Brasil) for continued support.

#### References:

- Altman JD, Trendelenburg AU, MacMillan L, Bernstein D, Limbird L, Starke K, Kobilka BK, Hein L: Abnormal regulation of the sympathetic nervous system in alpha2A-adrenergic receptor knockout mice. *Mol Pharmacol*, 1999, 56, 154–161.
- Arunlakshana O, Schild HO: Some quantitative uses of drug antagonists. *Br J Pharmacol Chemother*, 1959, 14, 48–58.
- Bockman CS, Gonzalez-Cabrera I, Abel PW: Alpha-2 adrenoceptor subtype causing nitric oxide-mediated vascular relaxation in rats. *J Pharmacol Exp Ther*, 1996, 278, 1235–1243.
- Bylund DB, Blaxall HS, Iversen LJ, Caron MG, Lefkowitz RJ, Lomasney JW: Pharmacological characteristics of alpha 2-adrenergic receptors: comparison of pharmacologically defined subtypes with subtypes identified by molecular cloning. *Mol Pharmacol*, 1992, 42, 1–5.
- Doxey JC, Roach AG: Presynaptic alpha-adrenoreceptors; in vitro methods and preparations utilised in the evaluation of agonists and antagonists. *J Auton Pharmacol*, 1980, 1, 73–99.
- Garcez-do-Carmo L, Santos WC: L-NAME pretreatment partially inhibits the agmatine-evoked depression of the electrically induced twitch contraction of isolated rat vas deferens. *Life Sci*, 2006, 79, 854–860.
- Gonzalez C, Regunathan S, Reis DJ, Estrada C: Agmatine, an endogenous modulator of noradrenergic neurotransmission in the rat tail artery. *Br J Pharmacol*, 1996, 119, 677–684.
- Grillo MA, Colombatto S: Metabolism and function in animal tissues of agmatine, a biogenic amine formed from arginine. *Amino Acids*, 2004, 26, 3–8.
- Jurkiewicz NH, Garcez do Carmo L, Hirata H, da Costa Santos W, Jurkiewicz A: Functional properties of agmatine in rat vas deferens. *Eur J Pharmacol*, 1996, 307, 299–304.
- Langer S : 25 years since the discovery of presynaptic receptors: present knowledge and future perspectives. *Trends Pharmacol Sci*, 1997, 18, 95–99.
- Li G, Regunathan S, Barrow CJ, Eshraghi J, Cooper R, Reis DJ: Agmatine: an endogenous clonidine-displacing substance in the brain. *Science*, 1994, 263, 966–969.
- Link RE, Desai K, Hein L, Stevens ME, Chruscinski A, Bernstein D, Barsh GS, Kobilka BK: Cardiovascular regulation in mice lacking alpha2-adrenergic receptor subtypes b and c. *Science*, 1996, 273, 803–805.
- MacMillan LB, Hein L, Smith MS, Piascik MT, Limbird LE: Central hypotensive effects of the alpha2a-adrenergic receptor subtype. *Science*, 1996, 273, 801–803.
- Molderings GJ, Gothert M, von K gelgen I: Characterization of an antiproliferative effect of imidazoline receptor ligands on PC12 cells. *Pharmacol Rep*, 2007, 59, 789–794.
- Molderings GJ, Menzel S, Kathmann M, Schlicker E, Gothert M: Dual interaction of agmatine with the rat alpha2 -adrenoceptor: competitive antagonism and allosteric activation. *Br J Pharmacol*, 2000, 130, 1706–1712.
- O'Rourke MF, Iversen LJ, Lomasney JW, Bylund DB: Species orthologs of the alpha-2A adrenergic receptor: the pharmacological properties of the bovine and rat receptors differ from the human and porcine receptors. *J Pharmacol Exp Ther*, 1994, 271, 735–740.
- Picarelli P , Hyppolito N, Valle JR: Synergistic effect of 5-hydroxytryptamine on the response of rats's seminal vesicles to adrenaline and noradrenaline. *Arch Int Pharmacodyn Ther*, 1962, 138, 354–363.
- Pinthong D, Wright IK, Hanmer C, Millns P, Mason R, Kendall DA, Wilson VG: Agmatine recognizes alpha 2-adrenoceptor binding sites but neither activates nor inhibits alpha 2-adrenoceptors. *Naunyn Schmiedebergs Arch Pharmacol*, 1995, 351, 10–16.
- Raasch W, Schafer U, Chun J, Dominiak P: Biological significance of agmatine, an endogenous ligand at imidazoline binding sites. *Br J Pharmacol*, 2001, 133, 755–780.
- Reis DJ, Regunathan S: Is agmatine a novel neurotransmitter in brain . *Trends Pharmacol Sci*, 2000, 21, 187–193.
- Santos WC, Hernandez-Guijo JM, Ruiz-Nuno A, Olivares R, Jurkiewicz A, Gandia L, Garcia AG: Blockade by agmatine of catecholamine release from chromaffin cells is unrelated to imidazoline receptors. *Eur J Pharmacol*, 2001, 417, 99–109.
- Santos WC, Smaili SS, Jurkiewicz A, Picarro I, Garcez-do-Carmo L: Dual effect of agmatine in the bisected rat vas deferens. *J Pharm Pharmacol*, 2003, 55, 373–80.
- Starke K, Gothert M, Kilbinger H: Modulation of neurotransmitter release by presynaptic autoreceptors. *Physiol Rev*, 1989, 69, 864–989.
- Van Rossum JM: Cumulative dose-response curves. II. Technique for the making of dose-response curves in isolated organs and the evaluation of drug parameters. *Arch Int Pharmacodyn Ther*, 1963, 143, 299–330.
- Ventura S: Autoinhibition, sympathetic cotransmission and biphasic contractile responses to trains of nerve stimulation in the rodent vas deferens. *Clin Exp Pharmacol Physiol*, 1998, 25, 965–973.

#### Recei ed:

June 26, 2008 in revised form: February 27, 2009.