Effect of Moxonidine on Contractile Activity of Isolated Large Intestine in Mice: Role of α_2-Adrenoceptors and I_i-Imidazoline Receptors

L. P. Kozaeva, N. V. Korobov, and O. S. Medvedev

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We studied the ability of moxonidine to interact with α_2-adrenoceptors and I_i-imidazoline receptors in isolated mouse large intestine. Moxonidine caused contractions of longitudinal muscles in the large intestine, which depended on the dose of this preparation. Pretreatment with yohimbine (α_2-adrenoceptor antagonist with low affinity for I_i-imidazoline receptors) and efaroxan (I_i-imidazoline receptor antagonist with low affinity for α_2-adrenoceptors) abolished the effect of moxonidine. Antagonistic activity and relative selectivity of yohimbine and efaroxan suggest that the effects of moxonidine on mouse large intestine are realized via α_2-adrenoceptors.

Key Words: *moxonidine; agmatine; isolated mouse large intestine; α_2-adrenoceptors; imidazoline receptors*

Moxonidine is a hypotensive drug of central action. This imidazoline derivative is extensively used for the therapy of patients with arterial hypertension (similarly to clonidine). Moxonidine was first characterized as a selective agonist of presynaptic α_2-adrenoceptors [1]. Further studies showed that moxonidine is highly selective for I_i-imidazoline receptors. Moxonidine primarily binds to non-adrenergic recognition sites for clonidine (^{3}H) on neuronal membranes in the rostral ventrolateral zone of the medulla oblongata. In addition, moxonidine binds to membranes of chromaffin cells in the adrenal glands not carrying α_2-adrenoceptors [3]. The hypotensive effect of moxonidine administered into the rostral ventrolateral zone of the medulla oblongata correlates with its affinity for I_i-imidazoline receptors, but not for α_2-adrenoceptors [3]. Affinity of moxonidine for I_i-imidazoline receptors (K_i) slightly varies (1-2 nM) depending on animal species, tissues, and radioligand used [3]. Clonidine has similar characteristics. It should be emphasized that the coefficients of selectivity for moxonidine and clonidine calculated as the ratio between affinity for α_2-adrenoceptors and I_i-imidazoline receptors are 33 and 4, respectively [4].

Imidazoline receptors are present not only in the nervous system, but also in various organs and tissues (heart, kidneys, stomach, pancreas, liver, large intestine, placenta, and prostate) [6]. It was interesting to evaluate whether moxonidine can modulate functions of internal organs. Experiments were performed on the model of isolated mouse large intestine (MLI). Published data show that α_{2D}-adrenoceptors are involved in the regulation of muscle contractions in the intestine [2]. The model of MLI was used to study the influence of adrenoceptor agonists on the prostaglandin-mediated regulation of muscle tone in the large intestine [5].

In series I we evaluated possible mechanism underlying the stimulatory effect of moxonidine on smooth muscles. In series II the role of α_2-adrenoceptors and imidazoline receptors in the action of moxonidine was studied by pharmacological methods.

Department of Pharmacology, Faculty of Fundamental Medicine, M. V. Lomonosov Moscow State University. ***Address for correspondence:*** lali_k@mail.ru. Kozaeva L. P.

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MATERIALS AND METHODS

MLI segments (1 cm) were isolated from outbred animals weighing 20-30 g. They were placed in cells (10 ml) with Krebs solution at 32°C. The initial strain was 1 g. Air was continuously passed through the solution with MLI segments. Isometric contractions were recorded on a Rikadenki automatic recorder (Hugo Sachs Elektronik KG) using a K30 transducer (Hugo Sachs Elektronik KG). Preparations of MLI were maintained in Krebs solution for 1 h. Acetylcholine (ACh, 1×10^{-6} M) was introduced into the solution to determine sensitivity of smooth muscles. The test substances were dissolved in distilled water (5-15 μl) and administered into the washing solution. Each treatment was performed after 4-5-fold washing of isolated organs for 12-15 min. MLI segments were exposed to agonists for 2-3 min at 15-min intervals. Antagonists were administered 5 min before treatment with agonists. We constructed dose-response curves for moxonidine in the presence or absence of antagonists. Moxonidine activity was determined by pD_2 (negative common logarithm of a concentration causing half-maximal effect). The value of pA_2 for antagonists was estimated by the Schild's method. Since it was difficult to wash out moxonidine in high concentrations, we studied activity of agonists producing a 20%-maximal effect. The results were analyzed by Student's *t* test.

We used agmatine sulfate (Fluka), acetylcholine hydrochloride (Sigma), atropine sulfate (Sigma), moxonidine hydrochloride (Farmzashchita), yohimbine hydrochloride (Sigma), efaroxan hydrochloride (RBI), naloxone hydrochloride (Sigma), sodium diclofenac (AGIO), and dalargin (Russian Research-and-Production Complex for Cardiology, Russian Ministry of Health).

RESULTS

Moxonidine caused dose-depended contractions of MLI segments (*n*=20, Fig. 1, *a*, 2, *a*). ACh and synthetic opioid peptide dalargin also produced dose-dependent effects. The cyclooxygenase inhibitor diclofenac in a concentration of 1×10^{-5} M completely blocked the effect of moxonidine. Atropine and naloxone (1×10^{-7} M) did not modulate the influence of moxonidine. Atropine and naloxone abolished the effects of ACh and dalargin, respectively. These results suggest that moxonidine induces contractions of MLI by inhibiting the non-cholinergic mechanism (via activation of α_2-adrenoceptors and, probably, I_i-imidazoline receptors). The inhibition of this non-cholinergic and non-adrenergic

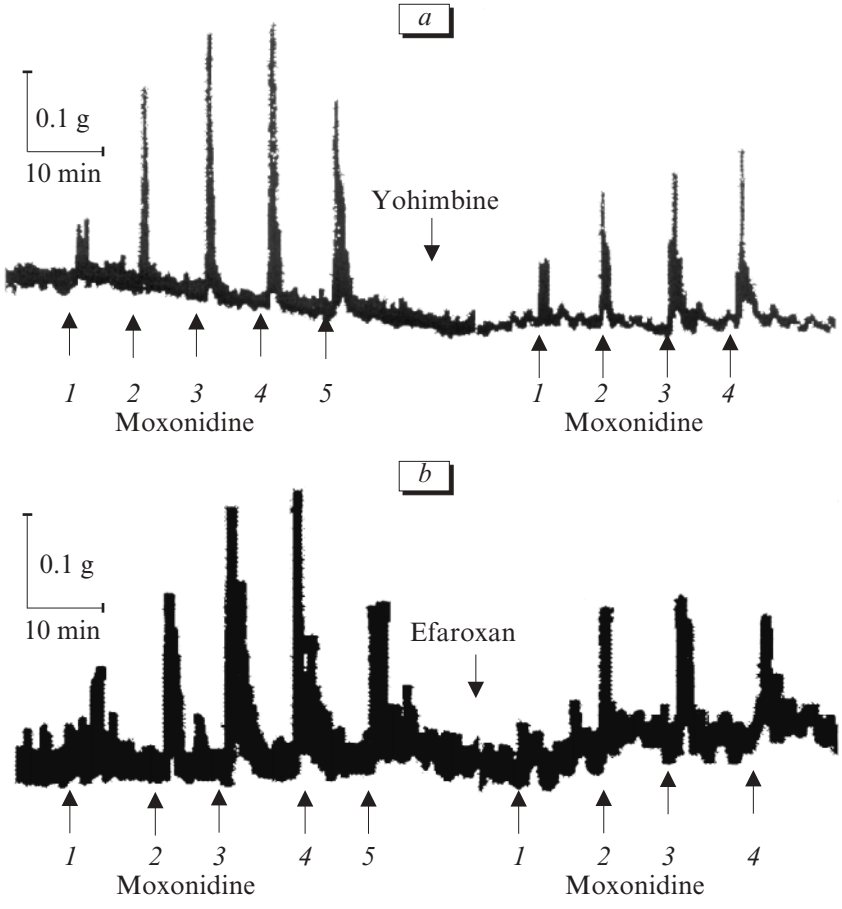

Fig. 1. Contractile response of mouse large intestine to moxonidine (1×10^{-7}-1×10^{-5} M) in the absence and presence of antagonists yohimbine (*a*) and efaroxan in a concentration of 1×10^{-8} M (*b*). Arrows: administration of moxonidine: 1×10^{-7} (*1*), 3×10^{-7} (*2*), 1×10^{-6} (*3*), 3×10^{-6} (*4*), and 1×10^{-5} (*5*).

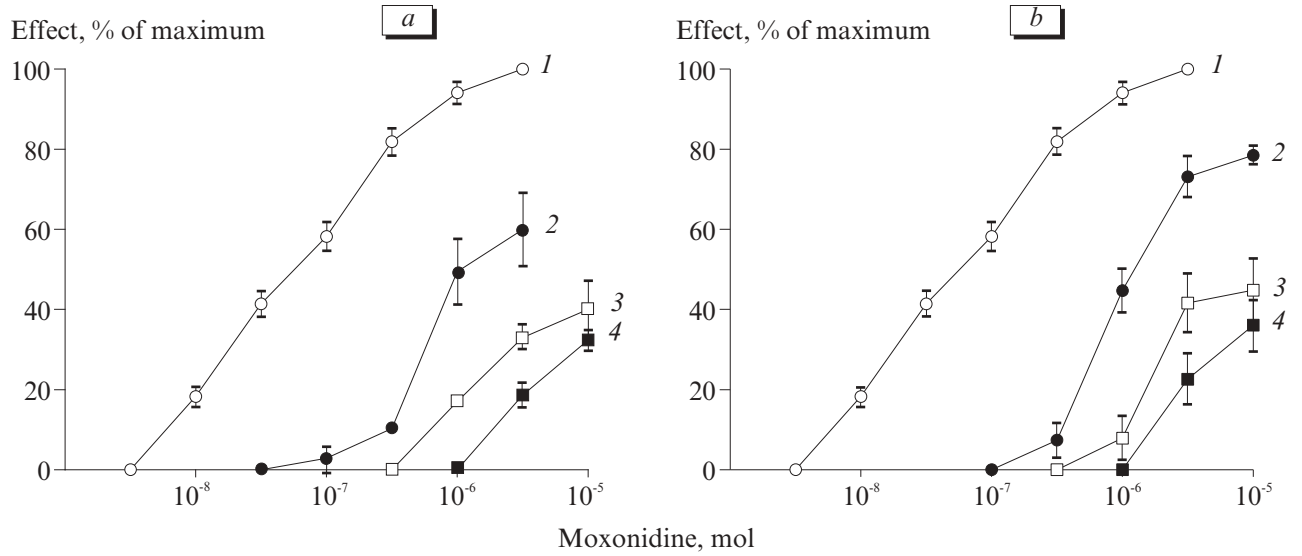

Fig. 2. Dose-response curves for moxonidine in the absence or presence of yohimbine (*a*) and efaroxan (*b*) in various concentrations: 1×10^{-9} (*1*), 3×10^{-9} (*2*), 1×10^{-7} (*3*), and 3×10^{-8} M (*4*). Each point represents the mean of at least 4 experiments.

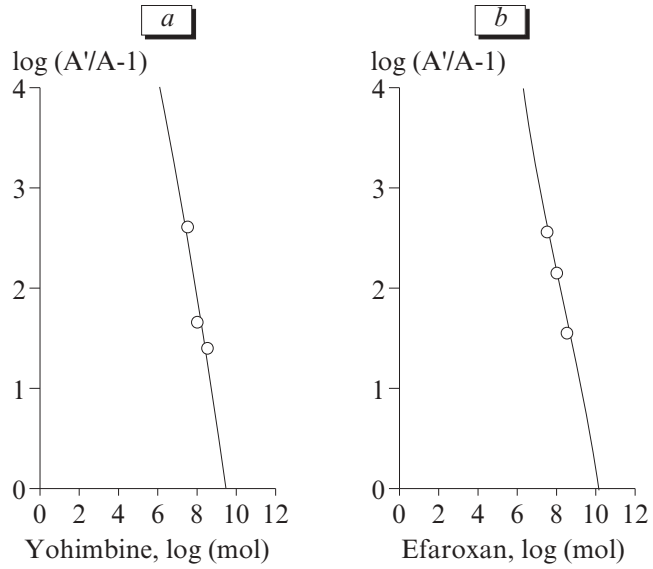

Fig. 3. pA_2 curves for antagonists yohimbine (*a*) and efaroxan (*b*) (Schild analysis).

mechanism activates another mechanism for the regulation of muscle tone in MLI (prostaglandin synthesis) [5]. This suggestion is confirmed by the ability of diclofenac to abolish the effect of moxonidine. Recent studies showed that moxonidine affects the synthesis of prostaglandins in isolated perfused rat heart [7].

Endogenous imidazoline receptor ligand agmatine (1×10^{-6}-1×10^{-4} M had no effect on contractility of smooth muscles. Agmatine did not modulate the action of moxonidine.

Pretreatment with yohimbine and efaroxan reduced the strength of contractions induced by moxonidine (Fig. 1, *b*).

Yohimbine and efaroxan produced a rightward shift of the dose-response curve for moxonidine (Fig. 2).

The interaction between these substances is most likely to be competitive. It was impossible to construct complete dose-response curve due to difficulties in washing out of moxonidine in high concentrations.

Yohimbine and efaroxan had different pA_2 values (Fig. 3). Affinity of efaroxan for I_i-imidazoline receptors is much higher than that of yohimbine (K_i 0.15 and 5000 nmol, respectively). However, these substances have the same affinity for α_2-adrenoceptors (K_i 5.6 and 5.8 nmol, respectively) [4]. These data suggest that the effect of moxonidine on MLI is realized via its interaction with α_2-adrenoceptors, but not with I_i-imidazoline receptors.

Moxonidine blocks the inhibitory effect of noncholinergic and non-adrenergic mechanism and activates prostaglandin synthesis in MLI. This effect of moxonidine is probably associated with its influence on α_2-adrenoceptors.

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