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Evidence for α_2-Adrenoceptor Agonist Activity of Minoxidil*

NITIN SHARMA, ANITA A. MEHTA, DEV D. SANTANI AND RAMESH K. GOYAL

Department of Pharmacology, L. M. College of Pharmacy, Ahmedabad-380 009, India

Abstract

The present investigation was undertaken to study the mechanism of action of minoxidil using various smooth muscle preparations.

Minoxidil (4.7×10^{-6} M to 4.7×10^{-4} M) produced a concentration–dependent inhibition of field stimulation-evoked responses in rat anococcygeus muscle and vas deferens. The inhibition produced by minoxidil was antagonized by yohimbine (2.5×10^{-7} M). Minoxidil (1.4×10^{-5} M to 4.7×10^{-4} M) also produced a concentration-dependent relaxation in oestrogen-primed potassium chloride-depolarized rat uterus. These responses were blocked not only by yohimbine but also by glibenclamide (2.02×10^{-8} M).

Our results suggest that minoxidil possesses α_2-adrenoceptor agonist activity in addition to potassium-channel-opening activity.

Minoxidil, hydralazine, diazoxide and sodium nitroprusside have long been used as vasodilators. The vasorelaxant effect of minoxidil, like that of diazoxide and sodium nitroprusside, results primarily from opening of membrane potassium channels (Meisheri et al 1988). There is evidences that minoxidil may interfere with sympathetic nerve functions (DuChrome et al 1973). Several kinds of potassium channels with different characteristics and pharmacological sensitivities have been described in smooth muscles and neurons (Aronson 1992).

Potassium channels have been reported to be involved in the mechanical responses to various agonists in rat anococcygeus and vas deferens (Mehta et al 1995a) and rat uterus (Mehta et al 1995b). Clonidine and other agonists of α_2-adrenoceptors open potassium channels in neurons (Tatsumi et al 1990). Recently, hydralazine has been shown to have an effect on sympathetic transmission (Satia & Goyal 1993). The present investigation is an attempt to explore the possibility of presynaptic α_2-adrenoceptor agonistic and potassium-channel-opening activity of minoxidil using various smooth muscle preparations.

Materials and Methods

Rat anococcygeus muscle and vas deferens

Albino male Wistar rats, weighing 175–225 g, were stunned by a sharp blow to the head and killed by cutting the neck blood vessels. The abdominal wall was quickly opened and the anococcygeus muscle and vas deferens were isolated. The isolated anococcygeus muscle and vas deferens were passed through a pair of platinum electrodes and mounted in isolated organ baths containing 20 mL of physiological saline solution (PSS). The anococcygeus muscles were bathed in Kreb's bicarbonate of the following composition (mM): NaCl, 94·01; KCl, 4·69; KH_2PO_4, 1·7; $NaHCO_3$, 25·0; glucose, 10·75; $CaCl_2$, 2·5; $MgCl_2$, 1·2 (pH 7·2). The vasa deferentia were bathed in Kreb's Henseleit solution (vas deferens) of the following composition (mM): NaCl, 118; KCl, 4·7; $CaCl_2$, 2·5; $MgSO_4$, 1·2; $NaHCO_3$, 25·0; glucose, 11·1; KH_2PO_4, 1·2 (pH 7·2).

Correspondence: R. K. Goyal, Department of Pharmacology, L. M. College of Pharmacy, Ahmedabad-380 009, India.

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The PSS for both the tissues were maintained at 37 ± 1°C and continuously bubbled with Carbogen (95% O_2 and 5% CO_2). The stabilization period was 30 min, during which the PSS was changed at every 10-min interval. After stabilization of 30 min the preparations were stimulated electrically with square wave pulse of 5 V strength and pulse width 5 ms at a frequency of 20 Hz for 30 s. The responses to field stimulation were recorded on a Polygraph, Bio-devices, Ambala, India, using an F 0·2 force displacement transducer which exerted a basal tension equivalent to 1 g on the tissues.

Three responses of equal amplitude to field stimulation were recorded on rat anococcygeus muscle and vas deferens. These responses were re-elicited in the presence of graded concentrations of minoxidil (4.78×10^{-6} M to 4.78×10^{-4} M). The responses to field stimulation were then re-elicited in the presence of minoxidil along with yohimbine (2.56×10^{-7} M). The time interval between two successive stimuli was 5 min. The contact time for minoxidil and yohimbine was 3 min.

Oestrogen primed rat uterus

Young albino female Wistar rats, weighing 200–250g, were treated with diethylstilboesterol (100 μg/100 g; i.p.) 24 h before they were killed by a sharp blow to the head and cutting the neck blood vessels. The uterine horns were quickly dissected out and suspended in organ baths containing 20 mL de-Jalon solution maintained at 37 ± 1°C. The composition of solution in mM was: NaCl, 112·0; $CaCl_2$, 0·25; KCl, 4·69; glucose, 2·68; $NaHCO_3$, 5·95 (pH 7·2). The solution was continuously bubbled with air. The responses to drug were recorded on a polygraph using a force displacement transducer which exerted a basal tension equivalent to 1-g load on tissue. The tissue was allowed to equilibrate for 30 min during which the bathing solution was changed at every 10 min.

The effect of minoxidil was studied in preparations depolarized by the addition of 80 mM KCl. In the first series of experiments, depolarized uterine horns were exposed to graded concentrations of minoxidil (1.4×10^{-5} M to 4.7×10^{-4} M) added cumulatively and relaxation was recorded. The contact time for each concentration of minoxidil was 45 s. The depolarized preparations were then exposed to yohimbine (2.56×10^{-7} M), cimetidine (3.96×10^{-7} M) or glibenclamide (2.02×10^{-8} M). The

responses to minoxidil were re-elicited in the presence of these antagonists after 5 min.

Statistical analysis

Statistical analysis was performed using one-way analysis of variance. A probability of $P<0.05$ was taken as the level of statistical significance.

Results

Minoxidil (4.7×10^{-6} M to 4.7×10^{-4} M) produced a concentration-dependent inhibition of field stimulation-evoked contractions in rat anococcygeus muscle. Yohimbine (2.56×10^{-7} M), an α_2-adrenoceptor antagonist, produced a significant inhibition of the response to minoxidil (Fig. 1). Identical results were obtained with rat vas deferens. In vas deferens minoxidil also produced inhibition of field stimulation-evoked contractions.

Yohimbine (2.56×10^{-7} M) did not produce any effect on the contractions evoked by field stimulation. Our preliminary results indicated that a dose higher than 1×10^{-6} M produces potentiation of contractions obtained at lower frequency (<10 Hz). Minoxidil-induced inhibition was found to be prevented in the presence of yohimbine (Fig. 2). In oestrogen-primed and depolarized rat uterus, minoxidil (1.48×10^{-5} M to 4.7×10^{-4} M) produced a concentration-dependent relaxation. Yohimbine, glibenclamide and cimetidine in the doses used in the present investigation did not produce any effect per se on oestrogen-primed and depolarized rat uterus. The relaxant effect was significantly antagonized by yohimbine (2.56×10^{-7} M, Fig. 3). The responses to minoxidil were also blocked by glibenclamide (2.02×10^{-8} M, Fig. 4) but not by cimetidine (3.96×10^{-7} M).

Discussion

Rat anococcygeus muscle and vas deferens are densely innervated by adrenergic nerves and field stimulation causes release of noradrenaline (Gillespie & McGrath 1974). The release of noradrenaline can be inhibited by adrenergic neuron blocking agents like guanethedine and bretylium (Day 1962). Further, α_2-adrenoceptor agonists can also cause inhibition of the release of noradrenaline (Starke & Altmann 1973).

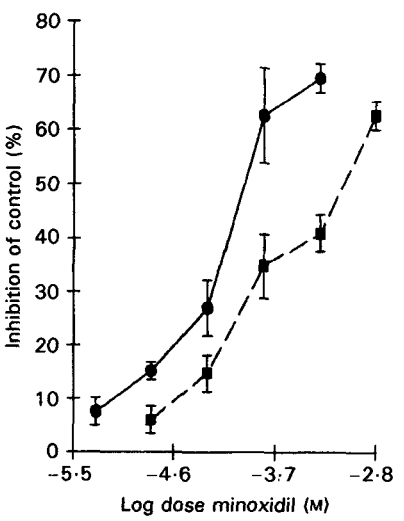

FIG. 1. Effect of minoxidil and its interaction with yohimbine on field-stimulation-evoked responses in rat anococcygeus muscle. Minoxidil control (●), minoxidil + yohimbine (■). Each point depicts the mean of six experiments and the bar represents ± s.e.m.

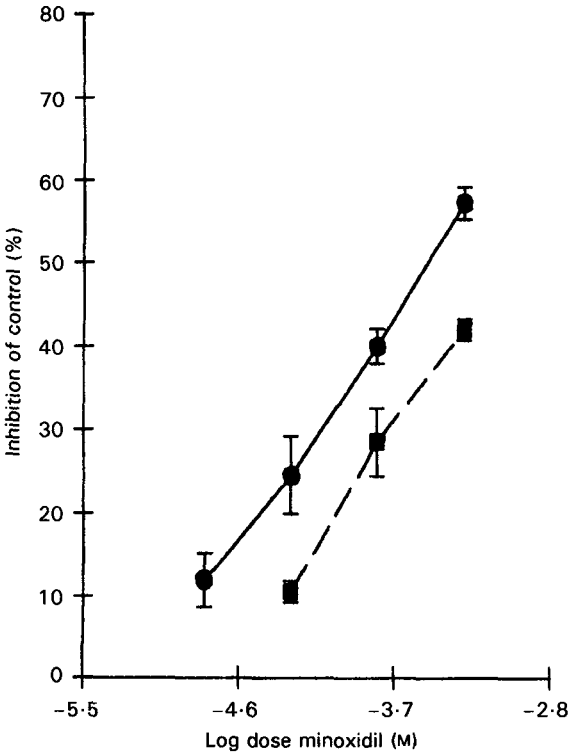

FIG. 2. Effect of minoxidil and its interaction with yohimbine on field-stimulation-evoked responses in rat vas deferens. Minoxidil control (●), minoxidil + yohimbine (■). Each point depicts the mean of six experiments and the bar represents ± s.e.m.

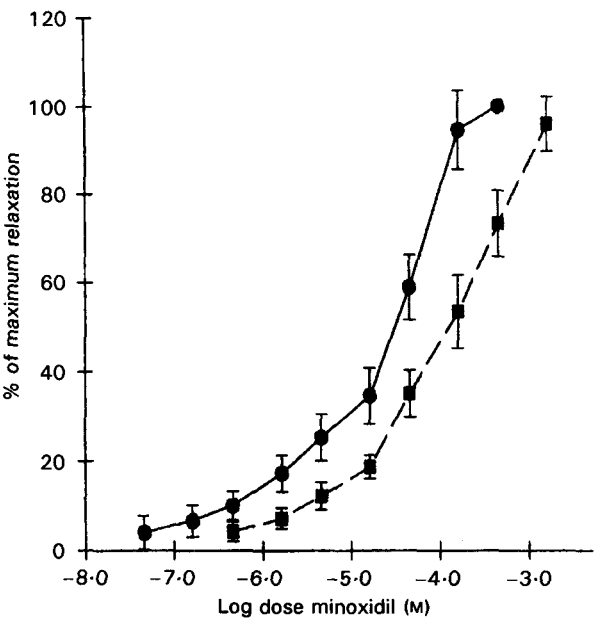

FIG. 3. Effect of minoxidil and its interaction with yohimbine on oestrogen-primed rat uterus. Minoxidil control (●), minoxidil + yohimbine (■). Each point depicts the mean of six experiments and the bar represents ± s.e.m.

Clonidine, an α_2-adrenoceptor agonist, has been reported to inhibit the release of noradrenaline in rat anococcygeus muscle (Doxey & Easingwood 1978). In the present study minoxidil produced a concentration-dependent inhibition of the field-stimulation-evoked responses of rat anococcygeus muscle and rat vas deferens. It was also found that yohimbine, a specific pre-

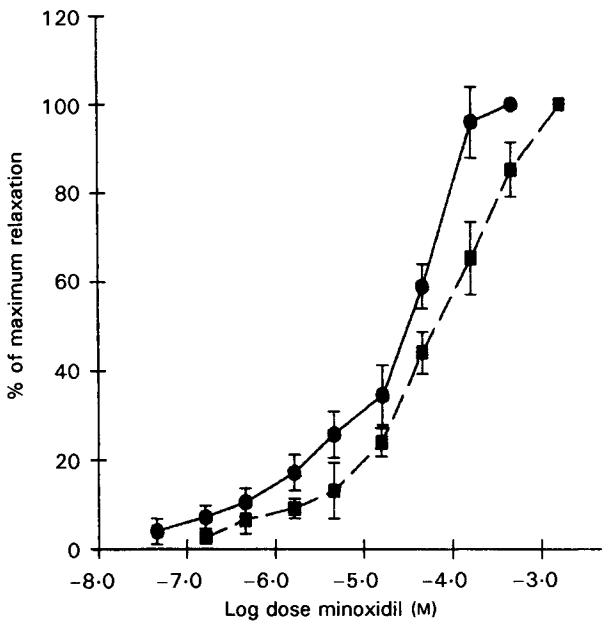

FIG. 4. Effect of minoxidil and its interaction with glibenclamide on oestrogen-primed rat uterus. Minoxidil control (●), minoxidil + glibenclamide (■). Each point depicts the mean of six experiments and the bar represents ± s.e.m.

synaptic α_2-adrenoceptor antagonist, produced inhibition of minoxidil-induced inhibition of responses to field stimulation in rat anococcygeus muscle as well as in rat vas-deferens.

The subclassification of α_2-adrenoceptors has been a matter of debate because of their heterogenicity in location as well as functional properties. α_2-Adrenoceptors may also cause release of noradrenaline in the CNS. An extensive review has recently been published in this matter by Ruffolo et al (1991). It has been reported that α_2-adrenoceptors are present post-synaptically in rat and cat tissues (Drew & Whiting 1985). Oestrogen-primed rat uterus is also reported to contain α_2-adrenoceptors (Goyal et al 1983). It was reported that clonidine causes the stimulation of α_2-adrenoceptors and H_2-histamine receptors, leading to the release of noradrenaline from endogenous stores which finally acts on β-adrenoceptors to cause relaxation of rat uterus (Goyal et al 1983). Results of the present study on the rat uterus also provide evidence for α_2-adrenoceptor agonist activity of minoxidil. In oestrogen-primed and depolarized rat uterus, minoxidil produced a concentration-dependent relaxation. Yohimbine produced a rightward parallel shift of the concentration-response curve. Oestrogen-primed rat uterus has been found to be relaxed by histamine (McNeill & Verma 1975) as well as by adrenergic agents (Goyal & Verma 1981). Histamine acts on pre-synaptic H_2-receptors to cause release of noradrenaline which in turn stimulates β-adrenoceptors to cause relaxation of depolarized rat uterus (Goyal & Dave 1988). In the present study we found that cimetidine did not alter the minoxidil-induced relaxation. It is possible that minoxidil act through the release of noradrenaline which ultimately is responsible for the relaxation of rat uterus.

Meisheri et al (1988) have shown that vasorelaxant effect of minoxidil results primarily from opening of membrane potassium channels. Mehta et al (1995a) reported the presence of potassium channels associated with adrenergic receptors, and their involvement in mechanical responses to various agonists, in rat anococcygeus and vas deferens. It has been shown that potassium-channel openers cause relaxation of depolarized rat uterus (Mehta et al 1995b). The relaxant response to potassium-channel openers are blocked by potassium-channel blockers like glibenclamide (Mehta et al 1995b). The association between the stimulation of α_2–adrenoceptors and opening of potassium channels has been reported in several neurons (Christie & North 1988; Tatsumi et al 1990; Ocana & Baeyens 1993). In the present study, the relaxation produced by minoxidil was also significantly inhibited by glibenclamide. The results of experiments on rat anococcygeus muscle and vas deferens support the association between potassium channels and α_2-adrenoceptors.

In conclusion, our data provides evidence for α_2-adrenoceptor agonist activity of minoxidil in addition to its potassium-channel-opening activity.

References

Aronson, J. K. (1992) Potassium channels in nervous tissue. Biochem. Pharmacol. 43: 11–14

Christie, M. J., North, R. A. (1988) Agonists at μ-opioid, M_2–muscarinic and $GABA_b$-receptors increase the same potassium conductance in rat lateral parabrachial neurones. Br. J. Pharmacol. 95: 896–902

Day, M. D. (1962) Effects of sympathomimetic amines on the blocking action of guanethedine, bretylium and oxylocholine. Br. J. Pharmacol. 18: 421–439

Doxey, J. C., Easingwood, R. E. (1978) Profile of α-adrenoceptor antagonists in the pithed rat. Br. J. Pharmacol. 63: 401–402

Drew, G. M., Whiting, S. B. (1985) What do antagonists tell us about α-adrenoceptors. Clin. Sci. 68: 15–19

DuChrome, D. W., Freyburger, W. A., Graham, B. E., Carlson, R. B. (1973) Pharmacological properties of minoxidil: a new hypotensive agent. J. Pharmacol. Exp. Ther. 184: 662–670

Gillespie, J. S., McGrath, J. C. (1974) The effect of pithing and nerve stimulation on the depletion of noradrenaline by reserpine in rat anococcygeus muscle and vas-deferens. Br. J. Pharmacol. 52: 585–590

Goyal, R. K., Dave, K. C. (1988) Mechanism of noradrenaline release by histamine in the estrogen primed rat uterus. Ind. J. Pharmacol. 20: 135–142

Goyal, R. K., Verma, S. C. (1981) Studies on the effects of adrenaline, noradrenaline and phenylephrine on the estrogen primed rat myometrium. Ind. J. Pharmacol. 13: 261–269

Goyal, R. K., Dave, K. C., Verma, S. C. (1983) The inhibitory effect of clonidine on the oestrogen-primed rat isolated uterus. J. Auton. Pharmacol. 3: 213–217

McNeill, J. H., Verma, S. C. (1975) Histamine H_2-receptors in rat uterus. Res. Chem. Pathol. Commun. Pharmacol. 11: 639–644

Mehta, A. A., Goyal, R. K., Dave, K. C. (1995a) Evidence for the involvement of calmodulin and K channels in contractile responses to various agonists in rat vas deferens and anococcygeus muscle. Pharmacol. Commun. 5: 267–276

Mehta, A. A., Dave, K. C., Goyal, R. K. (1995b) Involvement of potassium channels in the relaxant responses to various agonists in estrogen primed rat uterus. Ind. J. Physiol. Pharmacol. 39: 140–144

Meisheri, K. D., Cipkus, L. A., Taylor, C. T. (1988) Mechanism of action of minoxidil sulfate-induced vasodilatation: a role for increased K^+ permeability. J. Pharmacol. Exp. Ther. 245: 751–760

Ocana, M., Baeyens, J. M. (1993) Differential effects of potassium channel blockers on antinociception induced by α_2-adrenoceptor, $GABA_b$- and K-opioid receptor agonists. Br. J. Pharmacol. 110: 1048–1054

Ruffolo Jr, R. R., Nichols, A. J., Stadel, J. M., Le, P. (1991) Structure and functions of α-adrenoceptors. Pharmacol. Rev. 43: 475–508

Satia, M. C., Goyal, R. K. (1993) Evidence for α_2-adrenoceptor agonistic activity of hydralazine. Ind. J. Pharmacol. 25: 125–129

Starke, K., Altmann, K. P. (1973) Inhibition of adrenergic neurotransmission by clonidine, an action of pre-junctional α-receptors. Neuropharmacology 12: 339–347

Tatsumi, H., Costa, M., Schimerlik, M., North, R. A. (1990) Potassium conductance increased by noradrenaline, opioids, somatostatin, and G-proteins. Whole-cell recording from guinea pig submucous neurons. J. Neurosci. 10: 1675–1682