J. Pharm. Pharmacol. 1992, 44: 231–234
Received July 29, 1991

Different Sites of Action for α_2-Adrenoceptor Antagonists in the Modulation of Noradrenaline Release and Contraction Response in the Vas Deferens of the Rat

L. G. HARSING* AND E. S. VIZI

Institute of Experimental Medicine, Hungarian Academy of Sciences, Szigony u. 43, H-1450 Budapest VIII, Hungary

Abstract—Rat vas deferens was prepared, loaded with [^{3}H]noradrenaline, and superfused to measure the release of tritium in resting conditions and in response to electrical field stimulation. The α_2-adrenoceptor antagonists yohimbine, CH-38083 (7,8-(methylenedioxi)-14α-hydroxyalloberbane HCl), and idazoxan increased the electrically induced release of tritium in a concentration-dependent manner, whereas noradrenaline and the α_2-adrenoceptor agonist xylazine exerted opposite effects. The inhibitory effect of noradrenaline on electrically induced tritium release was antagonized by yohimbine, CH-38083, and idazoxan. Of the α_2-adrenoceptor antagonists tested, yohimbine and CH-38083 reversed the xylazine-induced inhibition of tritium release, and idazoxan was found to be completely ineffective against xylazine. Idazoxan, yohimbine and CH-38083 antagonized the inhibitory effect of xylazine on electrical stimulation-induced contractions of the vas deferens, as was evidenced by the apparent pA_2 values. We conclude from the present experiments that noradrenaline and xylazine inhibit noradrenaline release by acting on distinct prejunctional α_2-adrenoceptors and that the receptor subtype that responds to xylazine is insensitive to idazoxan. In addition, inhibition by xylazine of contractility but not of noradrenaline release was antagonized by idazoxan, suggesting that besides noradrenergic neurotransmission, other motor transmitter systems (purinergic) may also be involved in the inhibition by α_2-adrenoceptor antagonists of mechanical responses in the rat vas deferens.

Several lines of evidence indicate the heterogeneous nature of α_2-adrenoceptors (Bylund 1988; Ruffolo et al 1988). This receptor heterogeneity has been demonstrated in different species (Neylon & Summers 1985; Alabaster et al 1986), with pre- and postsynaptic locations (Hieble et al 1988; but see Lonart et al 1989; Connaughton & Docherty 1990a) and in terms of presynaptic auto- and heteroceptors in the central nervous system (Raiteri et al 1983; Harsing & Vizi 1991). Heterogeneity of α_2-adrenoceptors was also shown by using agonists with phenylethylamine or imidazoline structures (Ruffolo et al 1977; Hicks et al 1985) or by demonstration of different pharmacological specificity and binding characteristics of α_2-adrenoceptor antagonists (Boyajian & Leslie 1987; Young et al 1989). A classification of α_2-adrenoceptors as α_{2A}- and α_{2B}-subtypes has been described on the basis of the different sensitivity of the receptor subtypes to prazosin (Bylund 1985). Recently we demonstrated that heterogenous α_2-adrenoceptors sensitive to noradrenaline and xylazine regulate noradrenaline release in the vas deferens of the mouse (Kapocsi et al 1987). In this paper the regulatory role of noradrenaline- and xylazine-sensitive α_2-adrenoceptor subtypes in [^{3}H]noradrenaline ([^{3}H]NA) release and in contractility was further examined in rat vas deferens by using selective α_2-adrenoceptor antagonists (yohimbine, CH-38083 and idazoxan).

Materials and Methods

Release of [^{3}H]NA

Wistar rats were decapitated and their vasa deferentia were prepared and loaded with [^{3}H]NA (370 kBq mL^{-1}) for 45 min at 37°C in oxygenated Krebs solution (NaCl 113, KCl 4·7, $CaCl_2$ 2·5, KH_2PO_4 1·2, $MgSO_4$ 1·2, $NaHCO_3$ 25 and glucose 11·5 mM) containing 3×10^{-4} M ascorbic acid and 3×10^{-5} M disodium EDTA. After the loading period, each vas deferens was transferred to a 4 mL organ bath and superfused at a steady rate of 1 mL min^{-1} for 120 min with Krebs solution containing ascorbic acid (3×10^{-4} M), disodium EDTA (3×10^{-5} M), cocaine (5×10^{-6} M), and prednisone (10^{-6} M) at 37°C. The effluent collected during the first 120 min washout period was discarded and subsequent 3 min fractions were collected. Electrical field stimulation was applied to evoke release of tritium (supramaximal voltage 10 V cm^{-1}, 2 Hz frequency, and 1 ms impulse duration for 3 min). Three field stimulations were applied at 30 min intervals in the 4th, 14th, and 24th fractions. The α_2-adrenoceptor antagonists were added to the superfusion medium 18 min before the 2nd electrical stimulation, and they were present during the 2nd and 3rd stimulations; xylazine and noradrenaline were introduced 18 min before the 3rd electrical stimulation. The outflow of radioactivity ([^{3}H]NA and ^{3}H-labelled metabolites) and the content of radioactivity in the vas deferens was measured by scintillation counting. The release of tritium was expressed as a fractional rate, i.e. as a percentage of the amount of radioactivity in the tissue at the time the release was determined. The effects of drugs on electrical stimulation-induced release of [^{3}H]NA were expressed by the calculated ratios of fractional release (S_2) and fractional release S_1 (S_2/S_1) and of fractional release S_3 and fractional release S_2 (S_3/S_2).

In previous experiments we showed by HPLC separation

* Correspondence: L. G. Harsing, Institute of Experimental Medicine, Hungarian Academy of Sciences, H-1450 Budapest, VIII, Szigony u. 43, Hungary.

that the major part of the tritium released from superfused vas deferens preloaded with [^{3}H]NA is in the form of [^{3}H]NA (Vizi et al 1985).

Calculation of pA_2 values

For determination of the α_2-adrenoceptor antagonist activity of yohimbine, CH-38083 and idazoxan, the whole vas deferens of the rat was prepared, suspended in an organ bath, and stimulated electrically (field stimulation via platinum electrodes, supramaximal voltage, 0·1 Hz frequency, 1 ms impulse duration as described by Vizi et al (1986)). Xylazine was added cumulatively to the isolated organ to inhibit the electrical stimulation-induced contraction. The pA_2 values of the antagonists were calculated from cumulative concentration response curves to xylazine in the presence and absence of antagonists (Arunlakshana & Schild 1959).

Statistics

One-way analysis of variance, *t*-test for two means, and paired *t*-test were used. The mean ± s.e.m. was calculated. When used, n indicates the number of experiments. $P < 0.05$ was considered significant.

Drugs

Racemic CH-38083 (7,8-(methylenedioxi)-14α-hydroxyalloberbane HCl) and idazoxan were synthesized by Drs Cs. Szantay and I. Toth, Central Chemical Research Institute, Hungarian Academy of Sciences, Budapest, Hungary (Vizi et al 1987), yohimbine HCl and noradrenaline HCl were obtained from Sigma Chemical Co., St Louis, MO, and xylazine was a gift from Bayer, Leverkusen, Germany. l-[7,8-^{3}H]Noradrenaline HCl (sp. act. 10·5 Ci mmol^{-1}) was purchased from the Radiochemical Centre, Amersham, UK. All other chemicals used were of analytical grade.

Results

Effect of α_2-adrenoceptor antagonists on noradrenaline and xylazine inhibition of [^{3}H]NA release

In control experiments the calculated ratios of fractional release S_2 over fractional release S_1 (S_2/S_1) and of fractional release S_3 over fractional release S_2 (S_3/S_2) were 1·03 ± 0·17 and 1·06 ± 0·08, respectively, not significantly different from unity (n = 7, $P > 0.05$).

Yohimbine, CH-38083 or idazoxan added to the superfusion medium 18 min before the 2nd stimulation increased the electrically induced release of tritium from the rat vas deferens preparation. These effects were concentration dependent in a range of 10^{-8}–10^{-6} M. Concentrations that increased the S_2/S_1 ratios to 50% above the control value were 1.6×10^{-7} for yohimbine, 3×10^{-8} for idazoxan, and 1.4×10^{-8} M for CH-38083. The α_2-adrenoceptor antagonists used in the above concentrations did not affect the basal efflux of radioactivity.

Noradrenaline added 18 min before the 3rd stimulation significantly decreased the electrically-induced release of tritium from rat vas deferens: the S_3/S_2 ratio was 0·92 ± 0·08 in control experiments and 0·27 ± 0·08 in the presence of 10^{-7} M noradrenaline (n = 4 and 3, $P < 0.01$). Xylazine (10^{-6} M) also reduced the electrical stimulation-evoked tritium release: the ratio of S_3/S_2 was 0·95 ± 0·15 in control and 0·41 ± 0·10 in response to xylazine (n = 4 and 4, respectively, $P < 0.05$).

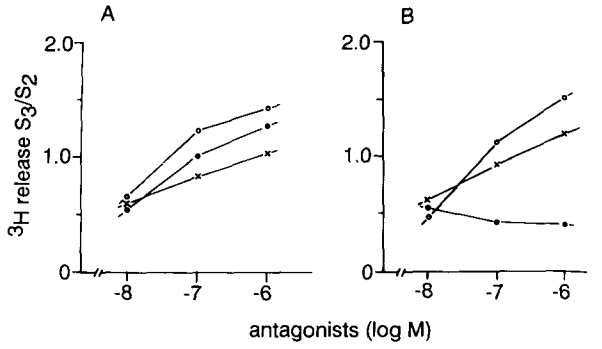

FIG. 1. Antagonism by α_2-adrenoceptor antagonists of noradrenaline- (A 10^{-7} M) and xylazine-inhibition (B 10^{-6} M) of tritium release elicited by electrical stimulation from superfused rat vas deferens preloaded with [^{3}H]NA. Ordinate: fractional release of tritium (expressed as S_3/S_2) measured in 3 min fractions of electrical stimulation (10 V cm^{-1}, 2 Hz, 1 ms for 3 min). Abscissa: molar concentrations of the α_2-adrenoceptor antagonists. ○ CH-38083, ● idazoxan, x yohimbine. The α_2-adrenoceptor antagonists were added 18 min before the 2nd electrical stimulation and noradrenaline or xylazine was added 18 min before the 3rd electrical stimulation; the drugs were maintained for the rest of the experiment. The ratio of fractional release of tritium was 0·27 ± 0·08 (n = 3) in the noradrenaline-treated group and 0·41 ± 0·10 (n = 4) in the xylazine-treated group, significantly different from the corresponding control values (0·92 ± 0·08 and 0·95 ± 0·15, n = 4, $P < 0.01$ and < 0.05, respectively). One-way analysis of variance: $F(9,39) = 5.04$, $P < 0.05$ in the noradrenaline-treated group and $F(9,39) = 3.99$, $P < 0.05$ in the xylazine-treated group. The maximum s.e.m. was less than 15% of the corresponding mean: the number of experiments, 4–6.

As shown in Fig. 1A yohimbine, CH-38083 and idazoxan antagonized the inhibitory effect of 10^{-7} M noradrenaline on tritium release evoked by electrical stimulation in the rat vas deferens preloaded with [^{3}H]NA. These effects of the α_2-adrenoceptor antagonists were concentration-dependent in the range of 10^{-8}–10^{-6} M. Yohimbine and CH-38083 (10^{-8}–10^{-6} M) also antagonized the inhibitory effect of 10^{-6} M xylazine on electrically-induced tritium release measured from rat superfused vas deferens (Fig. 1B). In contrast, idazoxan, which abolished the inhibitory effect of noradrenaline on stimulus-evoked release of tritium, failed to antagonize xylazine-induced inhibition (Fig. 1B).

α_2-Adrenoceptor antagonist activity against xylazine

Xylazine inhibited the electrical stimulation-induced contraction of rat isolated vas deferens with an IC50 value of

Table 1. pA_2 values and slopes of the Schild plots for α_2-adrenoceptor antagonists against xylazine in rat vas deferens.

Antagonists	pA_2	Slope
Yohimbine	7·79 ± 0·09	1·11 ± 0·30
CH-38083	8·84 ± 0·26	0·96 ± 0·09
Idazoxan	8·33 ± 0·39	0·91 ± 0·11

Rat isolated vas deferens was stimulated electrically (supramaximal voltage, 0·1 Hz frequency, 1 ms impulse duration). Cumulative concentration-inhibition curves to xylazine were constructed in the absence and presence of α_2-adrenoceptor antagonists. pA_2 values were determined according to Arunlakshana & Schild (1959); the antagonism was competitive in nature. Mean ± s.e.m., number of experiments = 3.

$2{\cdot}4 \pm 0{\cdot}1 \times 10^{-8}$ M (n = 11). The pA_2 values were determined according to Arunlakshana & Schild (1959) to express the antagonist potency of yohimbine, CH-38083 and idazoxan against xylazine in rat vas deferens (Table 1). The rank order of antagonist potency on α_2-adrenoceptors was CH-38083 > idazoxan > yohimbine. The nature of antagonism was competitive, as slopes of Schild plots for yohimbine, CH-38083 and idazoxan did not differ statistically from unity (Table 1).

Discussion

It is generally accepted that the release sites of noradrenaline are equipped with α_2-adrenoceptors and noradrenaline is able to inhibit its own release via stimulation of these receptors (Starke et al 1989). Accordingly, noradrenaline and xylazine decreased, and the α_2-adrenoceptor antagonists yohimbine, CH-38083 and idazoxan, increased the electrical stimulation-induced release of [^{3}H]NA from rat superfused vas deferens. These changes are probably due to autoreceptor-mediated presynaptic inhibition. α_2-Adrenoceptor antagonists, however, exerted different effects: yohimbine and CH-38083 antagonized both noradrenaline- and xylazine-induced inhibition of [^{3}H]NA release, whereas idazoxan antagonized the inhibitory effect of noradrenaline, but not that of xylazine. This finding suggests the involvement of two different α_2-adrenoceptors in the presynaptic inhibition of noradrenaline release in rat vas deferens: one population of receptors is sensitive to noradrenaline, and the other to xylazine. Recently we found that, as with rat vas deferens, prejunctional α_2-adrenoceptors with selective sensitivity to noradrenaline and xylazine also exist in mouse vas deferens and in longitudinal muscle strip preparation of the guinea-pig ileum (Kapocsi et al 1987; Harsing et al 1988). Although in the present experiments yohimbine and CH-38083 were apparently not able to differentiate between the two sites, idazoxan exerted an antagonist effect on noradrenaline- but not on xylazine-sensitive receptors. This finding provides further support for the concept that α_2-adrenoceptors are heterogeneous in nature, as already demonstrated (Bylund 1985, 1988; Ruffolo et al 1988; Young et al 1989; Connaughton & Docherty 1990b). The relationship between noradrenaline- and xylazine-sensitive α_2-adrenoceptors shown in this paper and other α_2-adrenoceptor subtypes widely discussed in the literature (Bylund 1985; Ruffolo et al 1988), however, remains to be elucidated.

α_2-Adrenoceptors present in the vas deferens not only modulate the release of noradrenaline but also regulate motor transmission. It has been shown that α_2-adrenoceptor agonists with phenylethylamine and imidazoline structures, which inhibit the twitch response, interact at different sites of α_2-adrenoceptors or at different receptor subtypes (Ruffolo et al 1977; Langer & Shepperson 1982; Mottram 1982). α_2-Adrenoceptor antagonists distinguish between the actions mediated by the two sites: idazoxan antagonized the clonidine-induced inhibition of twitch response of the rat vas deferens but was much less effective against the inhibitory effect of 6-fluoro-noradrenaline (Hicks et al 1985). In our experiments, all three α_2-adrenoceptor antagonists reversed the xylazine-induced inhibition of the twitch response of the rat vas deferens evoked by electrical stimulation, whereas only yohimbine and CH-38083 but not idazoxan reversed the xylazine-induced inhibition of [^{3}H]NA release. The apparent discrepancy between the modulation of [^{3}H]NA release and mechanical responses indicates that noradrenergic neurotransmission in the rat vas deferens is probably not exclusively involved in the field stimulation-induced contractility.

Hicks et al (1985) concluded that the role of endogenous noradrenaline in the vas deferens is predominantly inhibitory, reducing the release of an excitatory neurotransmitter. Electrophysiological studies indicated that the release of the motor transmitters in the rat vas deferens is inhibited by activation of presynaptic α_2-adrenoceptors (Illes & Starke 1983). Electrical stimulation evokes biphasic contractile response of the vas deferens, suggesting the involvement of more than one motor transmitter: the fast twitch response may be mediated by the release of adenosine triphosphate (ATP) and the slow phase is induced by noradrenaline release (Amobi & Smith 1988; Major et al 1989). It has been suggested that ATP functions as a cotransmitter of noradrenaline in the rat vas deferens, and that the main source of the neuronally released ATP in this tissue is the sympathetic nerve terminals (Lew & White 1987; Vizi & Burnstock 1988). It is not known, however, whether ATP, which mediates twitch response, originates from noradrenaline-containing vesicles or from some other sites within the varicosities. Alternatively, ATP may also be released from purinergic nerves that are nonadrenergic and noncholinergic in nature (Burnstock 1972). In fact, pharmacological manipulations differently affect noradrenergic transmission and ATP release in the vas deferens, suggesting different localizations, release mechanisms, and regulation for noradrenaline and ATP (Fredholm et al 1982; Kirkpatrick & Burnstock 1987). Although there is no direct neurochemical evidence for the regulatory role of α_2-adrenoceptors in the release of ATP, we speculated that noradrenaline, if once released, activates prejunctional α_2-adrenoceptors, inhibiting not only its own release but the release of ATP and the twitch response as well. Accordingly, yohimbine, CH-38083, and idazoxan may reverse the xylazine-induced inhibition of the field stimulation-evoked contractions of the vas deferens by blocking α_2-adrenoceptors involved in the regulation of ATP release. α_2-Adrenoceptors that mediate release of noradrenaline and ATP and the concomitant twitch response may be different, as suggested by the fact that idazoxan antagonized xylazine-induced inhibition of the contraction but was ineffective on xylazine-induced noradrenaline release inhibition.

References

Alabaster, V. A., Keir, R. F., Peters, C. J. (1986) Comparison of potency of α_2-adrenoceptor antagonists in vitro: evidence for heterogeneity of α_2-adrenoceptors. Br. J. Pharmacol. 88: 607–614

Amobi, N. I. B., Smith, I. C. H. (1988) Adrenergic modulation of non-adrenergic twitches in the rat vas deferens. J. Auton. Pharmacol. 8: 141–152

Arunlakshana, O., Schild, H. O. (1959) Some quantitative uses of drug antagonists. Br. J. Pharmacol. 14: 48–58

Boyajian, C. L., Leslie, F. M. (1987) Pharmacological evidence for alpha-2 adrenoceptor heterogeneity: differential binding properties of [^{3}H]rauwolscine and [^{3}H]idazoxan in rat brain. J. Pharmacol. Exp. Ther. 241: 1092–1098

Burnstock, G. (1972) Purinergic nerves. Pharmacol. Rev. 24: 509–581

Bylund, D. B. (1985) Heterogeneity of α_2 adrenergic receptors. Pharmacol. Biochem. Behav. 22: 835–843

Bylund, D. B. (1988) Subtypes of α_2-adrenoceptors: pharmacological and molecular biological evidence converge. Trends Pharmacol. Sci. 9: 356–361

Connaughton, S., Docherty, J. R. (1990a) No evidence for differences between pre- and postjunctional α_2-adrenoceptors in the periphery. Br. J. Pharmacol. 99: 97–102

Connaughton, S., Docherty, J. R. (1990b) Functional evidence for heterogeneity of peripheral prejunctional alpha2-adrenoceptors. Ibid. 101: 285–290

Fredholm, B. B., Fried, G., Hedqvist, P. (1982) Origin of adenosine released from rat vas deferens by nerve stimulation. Eur. J. Pharmacol. 79: 233–243

Harsing, L. G., Vizi, E. S. (1991) Evidence that two stereochemically different alpha-2 adrenoceptors modulate norepinephrine release in rat cerebral cortex. J. Pharmacol. Exp. Ther. 256: 44–49

Harsing, L. G., Lonart, G., Vizi, E. S. (1988) Berbanes: search for novel alpha-2 adrenoceptor antagonists. Pol. J. Pharmacol. 40: 697–708

Hicks, P. E., Langer, S. Z., Macrae, A. D. (1985) Differential blocking actions of idazoxan against the inhibitory effects of 6-fluoronoradrenaline and clonidine in the rat vas deferens. Br. J. Pharmacol. 86: 141–150

Hieble, J. P., Sulpizio, A. C., Nichols, A. J., Willette, R. N., Ruffolo, R. R. (1988) Pharmacological characterization of SKF 104078, a novel alpha-2 adrenoceptor antagonist which discriminates between pre- and postjunctional alpha-2 adrenoceptors. J. Pharmacol. Exp. Ther. 247: 645–652

Illes, P., Starke, K. (1983) An electrophysiological study of presynaptic α-adrenoceptors in the vas deferens of the mouse. Br. J. Pharmacol. 78: 365–373

Kapocsi, J., Somogyi, G. T., Ludvig, N., Serfozo, P., Harsing, L. G., Woods, R. J., Vizi, E. S. (1987) Neurochemical evidence for two types of presynaptic α_2 adrenoceptors. Neurochem. Res. 12: 141–147

Kirkpatrick, K., Burnstock, G. (1987) Sympathetic nerve-mediated release of ATP from the guinea-pig vas deferens is unaffected by reserpine. Eur. J. Pharmacol. 138: 207–214

Langer, S. Z., Shepperson, N. B. (1982) Differences between noradrenaline and clonidine on the α_2-adrenoceptor mediated inhibition of the response of the rat vas deferens. Br. J. Pharmacol. 77: 319P

Lew, M. J., White, T. D. (1987) Release of endogenous ATP during sympathetic nerve stimulation. Ibid. 92: 349–355

Lonart, G., Harsing, L. G., Vizi, E. S. (1989) Failure of selective antagonists (CH-38083 and idazoxan) to distinguish between prejunctional and postjunctional α_2-adrenoceptors. J. Auton. Pharmacol. 9: 149–158

Major, T. C., Weishaar, R. E., Taylor, D. G. (1989) Two phases of contractile response in rat isolated vas deferens and their regulation by adenosine and α-receptors. Eur. J. Pharmacol. 167: 323–331

Mottram, D. R. (1982) Pharmacological evidence for high and low affinity sites on prejunctional α_2 adrenoceptors. Br. J. Pharmacol. 77: 534P

Neylon, C. B., Summers, R. J. (1985) (3H)-Rauwolscine binding to α_2-adrenoceptors in the mammalian kidney: apparent receptor heterogeneity between species. Ibid. 85: 349–359

Raiteri, M., Maura, G., Versace, P. (1983) Functional evidence for two stereochemically different alpha-2 adrenoceptors regulating central norepinephrine and serotonin release. J. Pharmacol. Exp. Ther. 224: 679–684

Ruffolo, R. R., Turowski, B. S., Patil, P. N. (1977) Lack of cross-desensitization between structurally dissimilar α-adrenoceptor agonists. J. Pharm. Pharmacol. 29: 378–380

Ruffolo, R. R., DeMarinis, R., Wise, M., Hieble, J. P. (1988) Structure-activity relationships for alpha-2 adrenergic receptor agonists and antagonists. In: Limbird, L. E. (ed.) The Alpha-2 Adrenergic Receptors. The Humana Press Inc., Clifton, NJ, pp 115–185

Starke, K., Gothert, M., Kilbinger, H. (1989) Modulation of neurotransmitter release by presynaptic autoreceptors. Physiol. Rev. 69: 864–989

Vizi, E. S., Burnstock, G. (1988) Origin of ATP release in the rat vas deferens: concomitant measurement of (^{3}H)noradrenaline and (^{14}C)ATP. Eur. J. Pharmacol. 158: 69–77

Vizi, E. S., Somogyi, G. T., Harsing, L. G., Zimanyi, I. (1985) $(Ca^{2+})_o$-Independent release of noradrenaline and its role in negative feedback modulation. Proc. Natl. Acad. Sci. USA 82: 8775–8780

Vizi, E. S., Harsing, L. G., Gaal, J., Kapocsi, J., Bernath, S., Somogyi, G. T. (1986) CH-38083 a selective, potent antagonist of alpha-2 adrenoceptors. J. Pharmacol. Exp. Ther. 238: 701–706

Vizi, E. S., Toth, I., Harsing, L. G., Szabo, L., Somogyi, G. T., Szantay, Cs. (1987) Berbanes, a new class of α_2 adrenoceptor antagonists. J. Med. Chem. 30: 1355–1359

Young, P., Berge, J., Chapman, H., Cawthorne, M. A. (1989) Novel alpha2-adrenoceptor antagonists show selectivity for α_{2A} and α_{2B}-adrenoceptor subtypes. Eur. J. Pharmacol. 168: 381–386