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Invariable susceptibility to blockade by nifedipine of vasoconstriction to various α_2-adrenoceptor agonists in pithed rats

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The sensitivity of the increase in diastolic pressure brought about by the selective agonists of α_2-adrenoceptors, B-HT 920, B-HT 933, xylazine, UK-14,304, M-7, TL-99 and DP-6, 7-ADTN in pithed normotensive rats to blockade by the calcium entry inhibitor nifedipine has been investigated. To exclude any participation of vascular α_1- and β_2-adrenoceptors, as well as cardiac β_1-adrenoceptors, in the pressor responses, the study was made after treatment of the pithed rats with prazosin (0·1 mg kg^{-1}) and (−)-propranol (1 mg kg^{-1}). Without exception, the preferential agonists of α_2-adrenoceptors elicited vasoconstrictor responses which were susceptible to inhibition by nifedipine (0·03–1 mg kg^{-1}) in a dose-dependent manner regardless of the differences in intrinsic activity of the compounds. The pressor activity was almost completely abolished after 1 mg kg^{-1} of nifedipine. The results show that vasoconstriction induced in pithed rats by various selective stimulating agents of postjunctional vascular α_2-adrenoceptors is invariably and equally sensitive to attenuation by nifedipine. This susceptibility of α_2-adrenoceptor-mediated vasoconstriction to impairment by blockade of calcium entry is not dependent on the nature, the potency or the efficacy of the agonist.

The vasopressor responses in pithed normotensive rats to the selective agonists of α_2-adrenoceptors, B-HT 920 (2-amino-6-allyl-5,6,7,8-tetrahydro-4H-thiazolo-[4,5-d]-azepine) and M-7 (2-*NN*-dimethylamino-5,6-dihydroxytetralin), can be markedly suppressed by the calcium slow channel blockers nifedipine, verapamil and diltiazem (Van Meel et al 1981a, b; Cavero et al 1983; Gerold & Haeusler 1983). This observation was extended to other animal species treated with B-HT 920 (Van Meel et al 1982; Llenas & Massingham 1983; Timmermans et al 1983a) and was found to apply in-vivo to other calcium entry blockers (Van Meel et al 1983).

In contrast, the increase in diastolic pressure elicited in various animals by the selective agonists of α_1-adrenoceptors, methoxamine, phenylephrine and cirazoline, was almost unaffected by previous treatment with calcium entry inhibitors (Van Meel et al 1981a, b, 1982; Cavero et al 1983; Gerold & Haeusler 1983; Llenas & Massingham 1983; Timmermans et al 1983a). The differential influence of calcium entry blockers on α_1- and α_2-adrenoceptor-mediated vasoconstriction in-vivo, has led to the suggestion that this vasoconstriction triggered by stimulation of postjunctional α_2-adrenoceptors, is governed by an influx of extracellular calcium ions. On the other hand, the vasoconstrictor process initiated by α_1-adrenoceptor stimulation is not primarily determined by such an inward current of calcium ions.

Very recently, however, we demonstrated that the increase in diastolic pressure of pithed normotensive rats brought about by the highly selective α_1-adrenoceptor stimulants Sgd 101/75 (2-[2-methyl-indazol-4-imino]-imidazolidine) and St 587 ([2-chloro-5-trifluoromethylphenylimino]-2-imidazolidine), proved sensitive to blockade by nifedipine and verapamil (Timmermans & Van Meel 1983; Timmermans et al 1983b, c). The pressor effects to Sgd 101/75 were more susceptible to this inhibition than those to St 587, but not as great as those caused by the α_2-adrenoceptor agonist B-HT 920.

This variability in the inhibition by calcium entry blockade of pressor effects to α_1-adrenoceptor agonists prompted us to study whether such a differential sensitivity could also exist among the α_2-adrenoceptor agonists. To date, only B-HT 920 and M-7 have been examined from this aspect (see above), so we have assessed the influence of the calcium entry blocker nifedipine on the pressor effects in pithed normotensive rats of the currently available selective agonists of α_2-adrenoceptors, B-HT 933 (2-amino-6-ethyl-5,6,7,8-tetrahydro-4H-oxazolo-[4,5-d]-azepine), UK-14,304 (2-[8-bromoquinoxalyl-7-imino]imidazolidine), xylazine, TL-99 (2-*NN*-dimethylamino-6,7-dihydroxytetralin) and DP-6,7-ADTN (2-*NN*-di-*n*-propylamino-6,7-dihydroxytetralin). We also included B-HT 920 and M-7 in this study for comparison. The chemical structures of the agonists are shown in Fig. 1.

Materials and methods

Male normotensive Wistar rats (250–300 g) were pithed under hexobarbitone-sodium anaesthesia (150 mg kg^{-1} i.p.) by introducing a blunt steel rod into the vertebral canal via the orbit, as described by Gillespie et al (1970). Immediately thereafter, the pithed animals were subjected to forced respiration with room air via the cannulated trachea using a Braun-Melsungen pump with positive pressure. Rectal temperature was maintained at approximately 37 °C. Subsequently, catheters were inserted into the right jugular vein and common carotid artery for i.v. injection of drugs and measurement of arterial pressure, respectively (Statham P23 Db transducer). Arterial pressure was continuously displayed on

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a Hellige HE-19 pen recorder. The animals received heparin (about 150 IU kg^{-1} i.v.). After appropriate equilibration of cardiovascular parameters (about 20 min), the α_2-adrenoceptor agonists were administered i.v. as bolus injections (0·5 ml kg^{-1}). All pressor effects are expressed as increases in diastolic pressure (mm Hg). Dose-response curves were obtained by stepwise cumulative administration of the pressor agent, increasing the dose, about 3-fold with each successive injection, immediately after the maximal effect of the previous dose had been reached. One complete dose-pressor response curve was assessed per animal 15 min after i.v. pretreatment with prazosin (0·1 mg kg^{-1}) and (−)-propranolol (1 mg kg^{-1}) with or without addition of various doses of nifedipine. The calcium entry blocker was administered by infusion into the carotid artery to avoid direct cardiotoxicity.

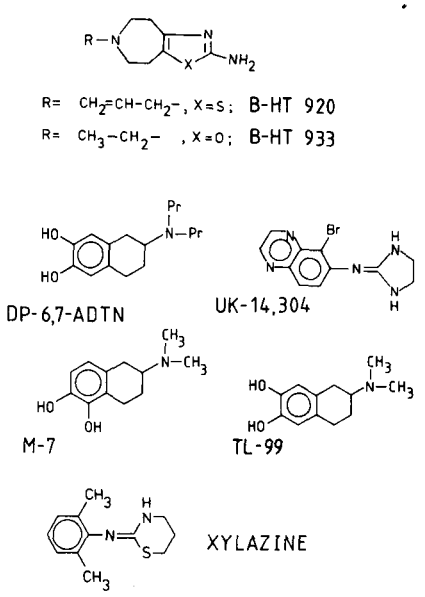

FIG. 1. Structural formulae of selective agonists of α_2-adrenoceptors used.

Drugs used were: B-HT 920 2 HCl and B-HT 933 2 HCl (Thomae); DP-6,7-ADTN HBr, M-7 HBr and TL-99 HBr (research Biochemicals Inc.); heparin (NOVO); hexobarbitone-sodium (Merck); nifedipine and xylazine HCl (Bayer); prazosin HCl and UK-14,304 D(+)-tartrate (Pfizer) and (−)-propranolol HCl (ICI).

Drugs were dissolved in 0·9% NaCl (saline) except for nifedipine (1 mg ml^{-1}) which was solubilized in 5% (wt vol^{-1}) glucose solution containing 10% (vol vol^{-1}) ethanol and 1% (vol vol^{-1}) Tween 80. Dilutions were made in saline. Doses quoted refer to the form given above.

Results

Before any drug treatment, the mean value ± s.e.m. of the basal diastolic pressure of the pithed rats was 39·2 ± 0·2 mm Hg (n = 162) and this was not significantly changed 15 min after prazosin (0·1 mg kg^{-1} i.v.) and (−)-propranolol (1 mg kg^{-1} i.v.) administered to exclude involvement of α_1- and β-adrenoceptors in the pressor effects of those agonists, which are not totally selective α_2-adrenoceptors (see Discussion). 15 min after nifedipine infusion, the diastolic pressure was reduced and for the 1 mg kg^{-1} dose it was lowered to 25·3 ± 0·8 mm Hg (n = 42).

The log dose–response curves for the agonists' effects on the maximal increase in diastolic pressure elicited in pithed normotensive rats are given in Fig. 2. B-HT 933 and B-HT 920 elicited a maximal pressor effect of 80 mm Hg, while for UK-14,304, TL-99 and M-7 it was 90 mm Hg, and for xylazine 70 mm Hg. It was less than 60 mm Hg for DP-6,7-ADTN. Within this series of agonists, the following order of potency to raise diastolic pressure was established: TL-99 > M-7 = DP-6,7-ADTN > UK-14,304 = B-HT 920 > xylazine > B-HT 933.

Irrespective of intrinsic activity and potency, the vasoconstrictor responses in pithed normotensive rats to the α_2-adrenoceptor agonists were markedly sensitive to inhibition by nifedipine which in doses of 0·03 to 1 mg kg^{-1} profoundly reduced both the slope and maximum of the log dose-pressor effect curves (Fig. 2A–F). There was no appreciable difference in the susceptibility of the pressor responses to the various α_2-adrenoceptor agonists to blockade by nifedipine. At 0·1 mg kg^{-1}, nifedipine significantly affected the log dose-vasoconstrictor effect curves of all agonists, whereas after 1 mg kg^{-1} pressor activity was almost completely abolished.

Discussion

The interference of nifedipine with the pressor activity of various α_2-adrenoceptor agonists has been quantified in the pithed normotensive rat. The agonists were selected to elicit in the rat pressor responses that are relatively sensitive to blockade by the α_2-adrenoceptor antagonists yohimbine and rauwolscine, but are relatively uninfluenced by the α_1-adrenoceptor blocking drug prazosin. Consequently, B-HT 920 (Van Meel et al 1981c; Kobinger & Pichler 1981), B-HT 933 (Timmermans & Van Zwieten 1980a, b), UK-14,304 (Cambridge 1981; Van Meel et al 1981; Ruffolo et al 1983), xylazine (Docherty & McGrath 1980), TL-99 (Hicks & Cannon 1980; Hicks & Waldron 1981; Clapham & Hamilton 1982), M-7 (Drew 1980; Clapham & Hamilton 1982) and DP-6,7-ADTN (Timmermans et al 1984) were used as they fulfilled this criterion. However, for xylazine and TL-99 (authors, unpublished), and for M-7 (Shepperson & Langer 1981; Timmermans et al 1983d) and DP-6,7-ADTN (Timmermans et al 1984), there was some (limited) participation of α_1-adrenoceptors, where-

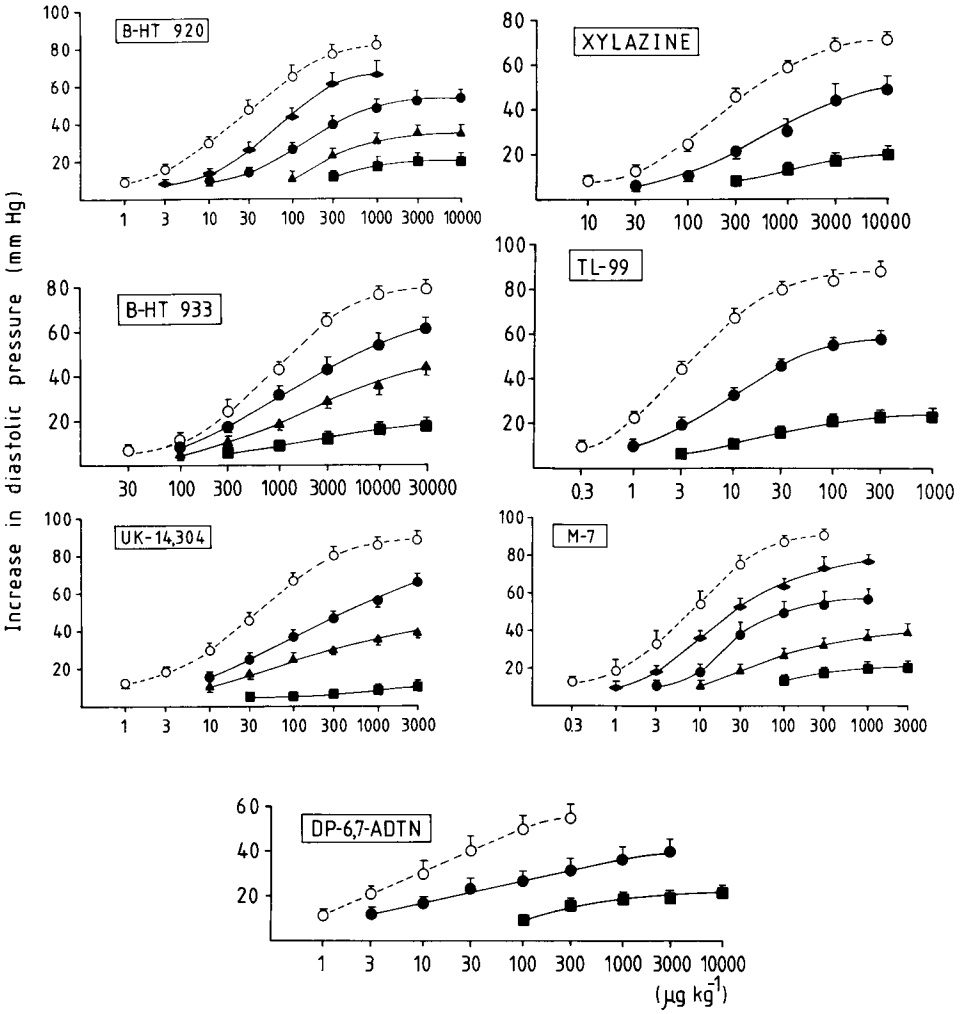

FIG. 2A–F. Log dose-response curves for the maximal increase in diastolic pressure caused by B-HT 920, B-HT 933, UK-14,304, xylazine, TL-99, M-7, DP-6,7-ADTN, applied i.v. to pithed normotensive rats treated with prazosin (0·1 mg kg^{-1}) and (−)-propranolol (1 mg kg^{-1}) 15 min after i.a. pretreatment with the vehicle (○ - - - ○) or 15 min after i.a. pretreatment with various doses of nifedipine. Key: 0·03 mg kg^{-1} (◆), 0·1 mg kg^{-1} (●), 0·3 mg kg^{-1} (▲) and 1 mg kg^{-1} (■). Symbols represent mean values ± s.e.m. (n = 6).

as M-7 can also stimulate vasodilatory β_2-adrenoceptors (Timmermans et al 1983d). To create identical conditions for all agonists under which the influence of nifedipine could be studied, pithed rats were pretreated with prazosin and (−)-propranolol.

We have shown here that independent of the chemical structure of the α_2-adrenoceptor agonist used, the increase in diastolic pressure elicited by its i.v. administration to pithed rats is invariably sensitive to inhibition by nifedipine infused at similar doses for each agonist. This result contrasts with our findings for selective agonists of α_1-adrenoceptors where the vasopressor effects exhibit a variability in their susceptibility to blockade by nifedipine and other calcium slow channel inhibitors (see Introduction).

It has been suggested that the high sensitivity of the vasoconstrictor effects of α_2-adrenoceptor agonists to inhibition by blockade of calcium entry, when compared with those of α_1-adrenoceptor stimulants, may be explained on the basis that the α_2-agonists generally evoke smaller responses than the α_1-agonists (Cauvin et al 1982, 1983). The differential sensitivity to calcium entry blockade between α_1- and α_2-adrenoceptor-mediated vasoconstriction is therefore a reflection of the degree of activation of the particular receptor. This has been interpreted as the susceptibility of vasocon-

striction to inhibition by calcium entry blockers, being related to the efficacy (intrinsic activity) of the agonists rather than to the α-adrenoceptor subtype involved (Bou et al 1983). However, it is obvious from the present study that differences in efficacy among the α_2-adrenoceptor agonists bear no relationship whatsoever to the potency exerted by nifedipine to attenuate the pressor effects.

In conclusion, the observation has been made that the vasoconstrictor responses in pithed rats to structurally different α_2-adrenoceptor selective agonists are invariably and equally susceptible to impairment by the calcium antagonist nifedipine. This finding distinguishes α_2-adrenoceptor agonists from α_1-adrenoceptor stimulants, the pressor effects of the latter being differentially affected by blockade of calcium entry. We suggest that vascular postjunctional α_2-adrenoceptors subserve a single vasoconstrictor mechanism primarily governed by an influx of extracellular calcium ions, whereas α_1-adrenoceptors are linked to processes dependent on, as well as independent of, such a calcium inward current.

B-HT 920/B-HT 933, nifedipine/xylazine, prazosin/UK-14,304 and (−)-propranolol were gifts of Thomae, Bayer, Pfizer and ICI, respectively.

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