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Adrenergic receptor subtype activation by (+)-, (−)- and (±)-norephedrine in the pithed rat

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The ability of (±)-norephedrine (phenylpropanolamine) and its component isomers, (+)- and (−)-norephedrine, to activate adrenergic receptor subtypes in the cardiovascular system of the urethane/chloralose-anaesthetized pithed rat has been investigated. At all adrenoceptor subtypes, (−)-norephedrine was the most potent agonist followed by (±)- then (+)-norephedrine. The greatest activity was observed at the α_1-receptor, with little activity observed at either β_1 or β_2-adrenoceptors. Reserpinization shifted the (−)-norephedrine dose-response curve slighty to the right, indicating that only a minor portion of its activity is due to the release of stored endogenous catecholamines. These results suggest that most of the cardiovascular activity of the compounds is through the direct activation of α_1-adrenoceptors.

(±)-Norephedrine (phenylpropanolamine) is present in many cough, cold and antiallergy products and appetite suppressants (Hoebel & Hernandez 1978). The racemate has been shown to exert mixed actions on adrenergic receptors, producing indirect and direct effects, depending on the site and the dose of the drug (Nickerson & Nomaguchi 1953; Trendelenburg et al 1962a, b). More recent reports have demonstrated that the drug binds to α_1-adrenoceptors, but with low affinity and low intrinsic activity (Minneman et al 1983). Those investigators concluded that it acts as a partial agonist at the α_1-adrenoceptor. Maher et al (1985) reported it to be largely a direct acting agonist on the cardiovascular system of the anaesthetized rat; moreover, Pentel et al (1985) were unsure whether (±)-norephedrine had any β_2-adrenoceptor activity.

Because of the confusion and of the clinical importance of the drug, we have investigated the cardiovascular profiles of the racemate and its isomers to see if they are directly or indirectly acting compounds, and which subtypes of adrenoceptors are stimulated in the cardiovascular system of the pithed rat.

METHODS

Normotensive male albino Sprague-Dawley rats (n = 130), 200–400 g, (Charles River Breeding Laboratories, Wilmington, MA), were housed individually in our facilities. Animals were acclimatized to the facilities for one week before experimentation. On the day of the experiment animals were anaesthetized with an intraperitoneal (i.p.) injection of a mixture of 500 mg kg^{-1} urethane and 50 mg kg^{-1} alpha-chloralose. The trachea, left carotid artery and right jugular vein were cannulated and rectal temperature was maintained at 37 °C. The animals were then pithed by inserting a steel rod into the spinal canal. Immediately following pithing the tracheal cannula was attached to a Harvard Apparatus dual phase control respirator pump (model 613) and the animal artificially ventilated at a frequency of 1 Hz with air in a volume of 2 mL/100 g of body weight. Systemic arterial blood pressure and heart rate were measured from the left carotid artery with a Statham P23 pressure transducer and recorded on a Grass Model 7D polygraph. The right jugular vein was cannulated for intravenous (i.v.) administration of the test compounds and pharmacological antagonists in a volume of 0·5 mL kg^{-1} and 1 mL kg^{-1}, respectively; phenoxybenzamine, the only exception, was given in a volume of 2 mL kg^{-1}. The preparation was allowed to equilibrate for at least 30 min before drug administration. All pressor responses were expressed as the change in mean arterial pressure (MAP) in mmHg. In the part of the study requiring catecholamine depletion to investigate direct vs indirect activity (α, β_1, β_2 responses), each rat was pretreated with reserpine (5 mg kg^{-1}, i.p.) 24 h before experimentation. This treatment abolished the pressor response to 5 mg kg^{-1} i.v. of the indirectly acting sympathomimetic, tyramine (data not shown). All other drug pretreatments were acute, at the doses indicated, and given 15 min before the study (Ruffolo & Yaden 1983).

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For the vasodepressor activity (β_2-activity) the above procedure was followed with the addition of cannulation of the left jugular vein for constant infusion of angiotensin II (150 ng kg^{-1} min^{-1}) with a Harvard Apparatus infusion pump (model 940). After a 15 min equilibration, angiotensin II infusion was begun to elevate mean systolic blood pressure to 50 ± 5 mmHg from the resting value. Phenoxybenzamine (3 mg kg^{-1} i.v.) was administered followed 15 min later by yohimbine (1 mg kg^{-1} i.v.), to antagonize completely vascular α-adrenoceptor vasoconstriction which could interfere with the vasodepressor response. Once the pressor response was stabilized (within 10 min), the vasodepressor effects (Ruffolo et al 1984; Ruffolo & Yaden 1983) of i.v. administered racemate and isomers were investigated. Drug-induced decreases in MAP were used as an index of β-activity.

The β_1-positive chronotropic responses to the drug were evaluated by pretreating animals with phenoxybenzamine (3 mg kg^{-1} i.v.) and yohimbine (1 mg kg^{-1} i.v.). Because myocardial β_2-adrenoceptors are now known (Carlsson et al 1972; Broadley 1982; Brodde et al 1983) and could interfere with the quantitation of β-adrenoceptor-mediated chronotropic activity, the animals were also pretreated with the selective β_2-adrenoceptor antagonist ICI 118,551 (Ruffolo & Morgan 1984).

All results are expressed as MAP ± s.e.m. (n = 4–6 per group). Cumulative dose-response curves are drawn by linear regression and tested, wherever possible, for deviations from linearity by analysis of variance in regression (Difazio 1984; Wallenstein et al 1980). Slopes of the different cumulative dose-response curves were compared by test of parallelism (Tallarida & Murray 1981).

Drugs

Drugs were prepared daily in 0·9% NaCl (saline) or for phenoxybenzamine, propranolol, prazosin, yohimbine and ICI 118,551 in distilled, deionized water. Reserpine was prepared in dimethylsulphoxide (DMSO). Yohimbine HCl, reserpine HCl and angiotensin II were from Sigma Chemical Co. (St Louis, MO). Prazosin HCl was a gift from Pfizer Inc. (New York, NY); phenoxybenzamine from Smith Kline and French Laboratories (Philadelphia, PA); ICI 118,551 (erythro-DL-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol) from Imperial Chemical Industries (Cheshire, UK); (+)-norephedrine HCl was from Aldrich Chemical Co. and (±)-norephedrine HCl (phenylpropanolamine) and (−)-norephedrine HCl were from Rhoer Chemical Inc. (Long Island City, NY). The identity of the norephedrine compounds was established before use.

RESULTS

The pressor responses of (±)-, (+)- and (−)-norephedrine were evaluated in pithed rats pretreated with propranolol (3 mg kg^{-1} i.v.). Both (−)- and (±)-norephedrine when administered were potent pressor agents capable of elevating MAP by approximately 95 ± 10 and 70 ± 8 mmHg, respectively, at the highest dose tested (all responses are presented as changes in MAP; diastolic and systolic changes were identical in regard to significance in every case). The (+)-isomer only elevated MAP 19 ± 5 mmHg at the highest dose tested (10 mg kg^{-1}). The

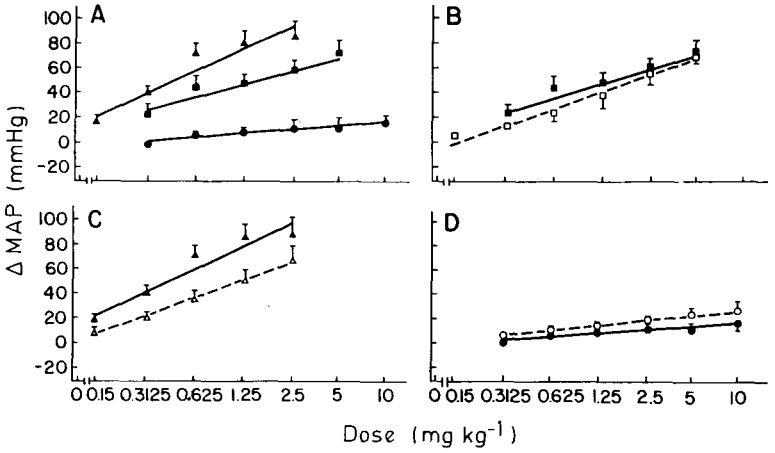

FIG. 1. Increases in mean arterial blood pressure (MAP) elicited by (●) (+)-, (■) (±)- and (▲) (−)-norephedrine administered i.v. to pithed normotensive rats. Pressor responses were obtained after i.v. pretreatment with propranolol and (B, C and D) reserpine (open symbols). Values represent mean ± s.e.m.

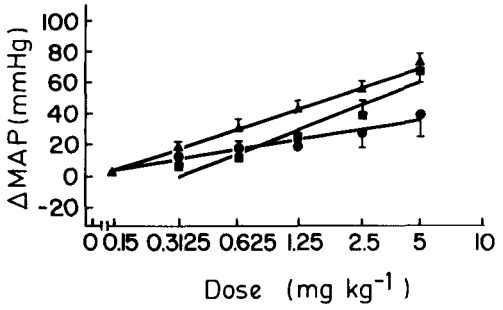

FIG. 2. Increases in mean arterial blood pressure (MAP) elicited by (●) (+)-, (■) (±)- and (▲) (−)-norephedrine administered i.v. to pithed normotensive rats. Pressor responses were obtained after i.v. pretreatment with propranolol and yohimbine. Values represent mean ± s.e.m.

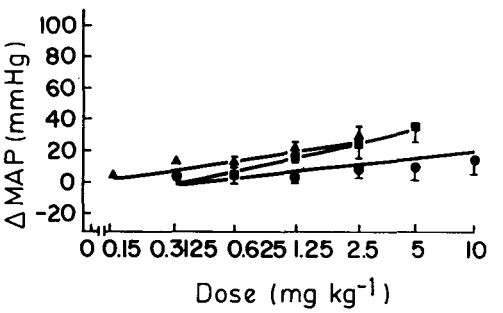

FIG. 3. Increases in mean arterial blood pressure (MAP) elicited by (●) (+)-, (■) (±) - and (▲) (−)-norephedrine administered i.v., to pithed normotensive rats. Pressor responses were obtained after i.v. pretreatment with propranolol and prazosin. Values represent mean ± s.e.m.

pressor effects of (±)- and (+)-norephedrine were not significantly affected ($P > 0.05$) by 24 h pretreatment with reserpine (5 mg kg^{-1} i.p., Fig. 1B, D), suggesting that the vasoconstriction was mediated by direct activation of postsynaptic vascular α-adrenoceptors. However, the (−)-norephedrine pressor response was significantly affected by reserpine pretreatment ($P < 0.05$; Fig. 1C) suggesting that it acts in part as a mixed agonist.

Pressor responses elicited by an α-adrenoceptor agonist in pithed rats may result from activation of a mixed population of postsynaptic vascular α_1- and/or α_2-adrenoceptors (Timmermans et al 1979; Langer & Massingham 1980). The pressor responses of the compounds were therefore evaluated after pretreatment of the preparation with propranolol and with either the α_1-adrenoceptor selective antagonist prazosin (0·1 mg kg^{-1} i.v.) or the α_2-selective antagonist yohimbine (1 mg kg^{-1} i.v.) to establish the contribution made by each adrenoceptor subtype to the pressor response observed in Fig. 1A. These doses of prazosin and yohimbine produce relatively selective antagonism of postsynaptic vascular α_1- and α_2-adrenoceptors, respectively, with little crossover between the α-adrenoceptor subtypes (Ruffolo & Yaden 1983; Ruffolo & Morgan 1984).

The results of selective antagonism by prazosin or yohimbine on the pressor response to the racemate and isomer are presented in Figs 2 and 3. The rank order of potency was: (−)- > (±)- > (+)-norephedrine. The pressor responses were mediated predominantly by postsynaptic vascular α_1-

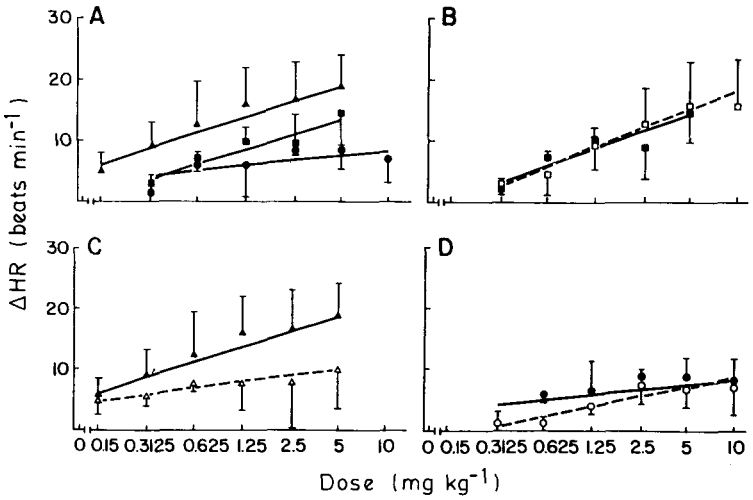

FIG. 4. Increases in β_1-mediated positive chronotropic responses elicited by (●) (+)-, (■) (±)- and (▲) (−)-norephedrine i.v., to pithed rats. Fig. 4A–D chronotropic responses were obtained after i.v. pretreatment with phenoxybenzamine, yohimbine, ICI 118,551 and (B, C and D) reserpine (open symbols). Values represent mean ± s.e.m.

adrenoceptors as evidenced by the fact that their respective vasopressor effects (Figs 2, 3) were antagonized by prazosin and only to a lesser extent by yohimbine.

The β_1-adrenoceptor-mediated effects were investigated by measuring chronotropic responses in pithed rats pretreated with phenoxybenzamine, yohimbine and the selective β_2-adrenoceptor antagonist ICI 118,551 (Bilski et al 1983) at 5 mg kg^{-1} i.v. The β_1-positive chronotropic responses are shown in Fig. 4A. The rank order of potency was: (−)- > (±)- ≥ (+)-norephedrine. The positive chronotropic responses of (±)- and (+)-norephedrine were not significantly affected ($P > 0{\cdot}05$) by 24 h pretreatment with reserpine (5 mg kg^{-1} i.p., Fig. 4B, D), indicating that the responses were mediated by the direct activation of β_1-adrenoceptors. However, the β_1-response to (−)-norephedrine was significantly affected by reserpine pretreatment ($P < 0{\cdot}05$, Fig. 4C), suggesting that it acts as a mixed agonist at the β_1-adrenoceptor as was seen at the α-adrenoceptors.

β_2-adrenoceptor-mediated vasodilation was determined by measuring the decrease in MAP in pithed rats during constant angiotensin II infusion. The results indicate that at this adrenoceptor subtype the depressor responses of racemate and isomers are very small and the rank order of potency was similar to that observed at the α- and β_1-adrenoceptors (data not shown).

DISCUSSION

The pharmacological profiles of racemate and isomers of norephedrine differ markedly at the α-adrenoceptor subtypes in the cardiovascular system of the pithed rat. All three compounds were effective vasoconstrictors capable of mediating dose-dependent increases in systolic, diastolic and MAP, (−)-norephedrine being the most potent (Fig. 1). The mechanism of the pressor response was similar for the other compounds. All three compounds increased MAP mainly by direct activation of α_1-adrenoceptors, but a proportion of their activity was also due to a direct activation of α_2-adrenoceptors. Moreover, the pressor response of (−)-norephedrine may also be due to an activation of a mixed population of α-adrenoceptors by direct and indirect mechanisms (but largely direct).

The racemate was the most interesting compound. The direct activation and selectivity of it at α-adrenoceptors is consistent with previous findings (Nickerson & Nomaguchi 1953; Trendelenburg et al 1962a, b). An apparent anomaly exists as it does not have any observed indirect activity at the β_1-adrenoceptor subtype as reported by Carlsson et al (1972). Moreover, since the (−)-isomer has some minor indirect activity, the possibility of some undetected non-significant indirect activity by the racemate cannot be ruled out. All three compounds possess the ability to lower blood pressure in reserpine-, phenoxybenzamine- and yohimbine-pretreated pithed rats with increased vascular tone induced by a constant infusion of angiotensin II. These depressor responses were relatively small, similarly, the magnitude of the positive chronotropic response for all the compounds was small but, greater than that seen at the β_2-adrenoceptors. Therefore, their differential effects on the cardiovascular system of the pithed rat may be preferably explained by differences in their potencies at α-adrenoceptors, mainly the α_1-subtype.

It should be mentioned that ether or hexobarbitone-sodium are the most appropriate anaesthetic agents in pithed animals because they are more superficial and shorter acting. We used a mixture of urethane-chloralose which is longer acting and possesses some cardiodepressant activity but it is nonetheless commonly used (Conlay et al 1981). Additionally, we chose chloralose and urethane because we planned to compare cardiovascular profiles of these compounds in pithed and non-pithed rats.

In summary, it can be concluded that the three compounds tested are capable of activating each type of adrenoceptor studied. The main drug response of the three compounds was seen at the α_1-adrenoceptor subtype. The β-response is significantly less than that observed at the α_1-adrenoceptors. The racemate and (+)-isomer are direct acting while the (−)-isomer is mixed, but largely direct acting.

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