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BLOCKADE BY YOHIMBINE OF PRAZOSIN-RESISTANT PRESSOR EFFECTS OF ADRENALINE IN THE PITHED RAT

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In the pithed rat, following β-adrenoceptor blockade, the pressor effect of adrenaline can be blocked by phentolamine or by prazosin plus yohimbine but not by prazosin or yohimbine given alone. It is concluded that adrenaline produces its pressor effect by acting on two sets of post-junctional α-adrenoceptors, each of which is sensitive to phentolamine, one of which is sensitive to prazosin but resistant to yohimbine and the other of which is sensitive to yohimbine but resistant to prazosin.

Introduction In the pithed rat part of the pressor response to noradrenaline is resistant to prazosin (Drew & Whiting, 1979). In another study, the effects of prazosin and of yohimbine were tested against a range of synthetic α-adrenoceptor agonists. It was found that: (1) after prazosin the pressor effects of the agonists showed an order of potency similar to that found at pre-junctional α-adrenoceptors, and (2) xylazine and guanabenz, which were relatively 'selective' agonists at pre-junctional compared with post-junctional α-adrenoceptors, had pressor effects which were resistant to prazosin but were susceptible to yohimbine. Taken together, these results suggest two populations of post-junctional α-adrenoceptor, one of which is resistant to prazosin but has agonist and antagonist 'specificities' similar to pre-junctional α-adrenoceptors (Docherty, MacDonald & McGrath, 1979).

The purpose of the present work was to determine whether adrenaline, which exerts its physiological actions via the blood stream, could act on either or both of these post-junctional α-adrenoceptors.

Methods Wistar rats (250 to 275 g) were pithed by the method of Gillespie, MacLaren & Pollock (1970) and ventilated with O_2. Heart rate and carotid arterial pressure were monitored continuously. The right jugular vein was cannulated for drug injections (see also Docherty & McGrath, 1979).

The effects of antagonists on the peak diastolic pressor response to adrenaline (1 μg/kg) were tested. This dose of adrenaline produced reproducible, submaximal responses. Two or three such doses were administered to ensure reproducibility before administering further drugs.

In an initial set of experiments, the effects of α-adrenoceptor antagonists were tested. However, in the main set of experiments propranolol (1 mg/kg) was given to remove the β-adrenoceptor effects of adrenaline from both the heart and blood vessels. The α-adrenoceptor antagonists were then administered in doses and sequences described in the results. The 'response' to adrenaline was measured 5 min after each dose of antagonist. There was no time-dependent recovery from the cardiac or vascular effects of propranolol over the time involved in these experiments.

Results are expressed as mean ± s.e. mean for groups of identical experiments. Statistical comparisons were made with Student's *t* test.

Drugs were dissolved in distilled water except for adrenaline, which was dissolved in saline (0.9% w/v NaCl solution). Doses quoted are of the salt. Drugs used were adrenaline bitartrate (Sigma), phentolamine mesylate (CIBA), prazosin hydrochloride (Pfizer), propranolol hydrochloride (Sigma), yohimbine hydrochloride (Sigma).

Results *In the absence of propranolol* Phentolamine (1 mg/kg), prazosin (1 mg/kg), or yohimbine (1 mg/kg) could each reverse the pressor effect of adrenaline (1 μg/kg) to a depressor effect. In the absence of β-adrenoceptor blockade, therefore, either prazosin or yohimbine could apparently produce 'complete' adrenaline reversal and no 'prazosin-resistant' response could be found.

In the presence of propranolol Following propranolol (1 mg/kg), the pressor response to adrenaline (1 μg/kg) was significantly increased from 44.8 ± 1.3 mmHg to 74.7 ± 1.5 mmHg ($n = 15$, $P < 0.001$) (see also Figure 1). This response is still sub-maximal. The equivalent pressor response to xylazine (100 μg/kg), which is an α-adrenoceptor agonist without β-adrenoceptor agonism, was not potentiated by propranolol.

Phentolamine (1 mg/kg), prazosin (0.1 mg/kg) or yohimbine (1 mg/kg) significantly decreased the resulting response to adrenaline (1 μg/kg) by 78.9 ± 1.6%, 37.2 ± 4.5% and 55.6 ± 2.3% ($n = 5$) respectively. Phentolamine (0.1 mg/kg) had a smaller inhibitory effect than prazosin (0.1 mg/kg) but a subsequent dose of phentolamine (1 mg/kg) had a greater

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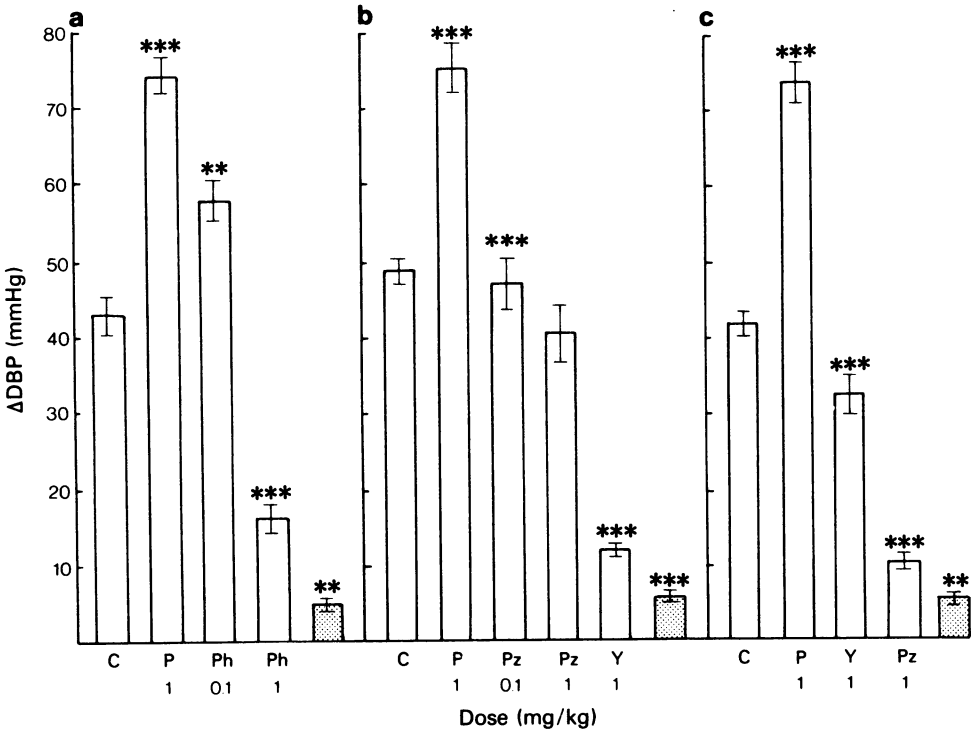

Figure 1 The effects of sequential administration of adrenoceptor antagonists on the peak diastolic pressor responses (ΔDBP) of pithed rats to adrenaline (1 μg/kg). The sequence and doses were varied in three groups of experiments (a–c). Control responses (C) and those obtained 5 min after propranolol (P), phentolamine (Ph), prazosin (Pz) and/or yohimbine (Y) are shown sequentially from left to right. The stippled column represents the response to administration of an equivalent volume of saline. Each response was compared with the previous response by Student's t test; $^{***}P < 0.001$, $^{**}0.01 > P > 0.001$. $n = 5$ in each group. Bars indicate s.e. mean.

effect than prazosin (1 mg/kg) or yohimbine (1 mg/kg) (Figure 1a–c).

Following prazosin (0.1 mg/kg), prazosin (1 mg/kg) produced no further significant decrease in the response but a subsequent dose of yohimbine (1 mg/kg) produced a significant further decrease (Figure 1b). However, after yohimbine (1 mg/kg), prazosin (1 mg/kg) significantly decreased the response (Figure 1c).

The small, residual response to adrenaline, which remained after yohimbine and prazosin (irrespective of order of administration) (Figure 1b and c) was not significantly different from that after phentolamine (1 mg/kg) (Figure 1a) ($P > 0.05$) but in each of these cases was significantly greater than the response to saline (Figure 1a–c).

Discussion If the β-adrenoceptor-mediated vasodilator effect of adrenaline was allowed to occur, any one of phentolamine, prazosin or yohimbine could produce reversal of the pressor response to adrenaline in the pithed rat. Following β-adrenoceptor blockade, however, phentolamine could almost abolish the pressor effect of adrenaline whereas prazosin or yohimbine could only reduce the response by approximately half; part of the response could, thus, be considered 'prazosin-resistant' and part 'yohimbine-resistant'.

To establish 'prazosin-resistance', a high dose of prazosin, 1 mg/kg, was employed; 0.013 mg/kg produced a 10 fold shift in the phenylephrine dose-pressor response curve in the pithed rat (Drew & Whiting, 1979). Furthermore, prazosin (0.1 mg/kg) produced its 'maximal' inhibition of the pressor response to adrenaline. Prazosin 1 mg/kg, which produced no further effect, is the highest practicable 'selective' dose since this is the threshold for antagonism at pre-junctional α-adrenoceptors (Docherty & McGrath, 1980). However, phentolamine (1 mg/kg) could produce greater inhibition than prazosin at 1 mg/kg although at the lower dose of 0.1 mg/kg, prazosin was more potent; this suggests that the 'prazosin-resistant' receptors are α-adrenoceptors.

This 'prazosin-resistant' response was susceptible to

yohimbine (1 mg/kg). Since yohimbine is relatively less potent than prazosin against phenylephrine, and more potent against xylazine or guanabenz, this suggests that the 'prazosin-resistant' effect of adrenaline is exerted against receptors similar to those activated by xylazine or guanabenz.

The observation that prazosin (1 mg/kg), which on its own could not reduce the response by more than half, could so effectively reduce the response remaining after yohimbine, supports the concept that the 'yohimbine-resistant' receptors are of the 'prazosin-sensitive' variety. However, this point cannot be further consolidated by the use of higher doses of yohimbine, since the latter would be 'non-selective' (Docherty & McGrath, 1979).

The proportion of the pressor response to adrenaline which was mediated by each receptor was approximately equal. Further quantification is not realistic due to the 'relative' nature of the 'selectivity' of the antagonists (Starke, Borowski & Endo, 1975). Nevertheless the net effect of prazosin plus yohimbine was equivalent to that of phentolamine, which does not differentiate between pre- and post-junctional α-adrenoceptors (Borowski, Starke, Ehrl & Endo, 1977) and, from the present results, also does not differentiate between 'prazosin-resistant' and 'yohimbine-resistant' post-junctional α-adrenoceptors.

It is concluded that the net pressor effect of adrenaline on the diastolic arterial pressure is a balance between pressor effects mediated by two different species of post-junctional α-adrenoceptor and a depressor response mediated by a post-junctional β-adrenoceptor. Removal of either one of the α-mediated effects allows the β-mediated effect to dominate. Removal of the β-mediated effect increases the size of the pressor response and allows the sequential removal of the two α-mediated effects. The physiological significance of these various receptors for adrenaline remains to be determined but the demonstration of their presence indicates a further basis for differences between the net effects of drugs possessing different profiles of activity at adrenoceptors.

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