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α-ADRENOCEPTOR BLOCKING PROPERTIES OF RAUBASINE IN PITHED RATS

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1 Raubasine was compared with yohimbine and corynanthine in pithed rats. Antagonist activity at α_1-adrenoceptors was evaluated on the pressor response to electrical stimulation of the spinal sympathetic outflow and to phenylephrine administration, both being reduced by raubasine in the dose range 1 to 4 mg/kg. Corynanthine was quantitatively similar, but yohimbine was not only less potent but also in doses of 0.125 to 0.5 mg/kg enhanced the effects of electrical stimulation.

2 Antagonist activity at α_2-adrenoceptors was determined against the inhibitory effects of clonidine on tachycardia induced by electrical stimulation of cardiac sympathetic nerves and against the pressor responses to B-HT-933 injection. Raubasine up to 4 mg/kg, like corynanthine, did not affect the pressor responses to B-HT-933 nor did it reduce the inhibitory effect of clonidine. By contrast yohimbine reduced the response to BHT-933 and antagonized clonidine as well as enhancing the tachycardia caused by electrical stimulation.

3 The results indicate that, *in vivo*, raubasine, like corynanthine, is a selective antagonist at α_1-adrenoceptors and that yohimbine is more potent in blocking α_2-than α_1-adrenoceptors.

Introduction

Raubasine is an adrenolytic drug. It reverses the hypertensive effect of adrenaline (Kroneberg, 1958) decreases that of noradrenaline and diminishes the contractile response of the nictitating membrane of the cat elicited by nerve stimulation (Schmitt & Gonnard, 1957). In the rat isolated thoracic aorta and vas deferens raubasine acts as a competitive antagonist of noradrenaline (Roquebert, Gomond & Demichel, 1981), preferentially blocking the post-synaptic α_1-adrenoceptors (Demichel, Gomond & Roquebert, 1981).

Studies revealing differences between pre-and postsynaptic α-adrenoceptors at sympathetic neuroeffector junctions have led to the classification of these receptors as α_2-and α_1-subtypes respectively (Langer, 1974). It was subsequently recommended that this classification should be used independently of the location of α-adrenoceptors but to accord with their relative affinity for agonists and antagonists (Berthelsen & Pettinger, 1977). For example clonidine and B-HT 933 preferentially activate (Kobinger & Pichler, 1977) and yohimbine preferentially blocks α_2-adrenoceptors (Weitzell, Tanaka & Starke, 1979). On the other hand, phenylephrine mainly activates (Starke, Endo & Taube, 1975) and corynanthine mainly blocks α_1-adrenoceptors (Weitzell *et al.*, 1979). In recent years α_2-adrenoceptors have been shown to exist postsynaptically, especially in the vascular system of rats (Docherty, McDonald & McGrath, 1979).

The purpose of the present investigation was to define further the effects of raubasine on α_1-and α_2-adrenoceptors of the cardiovascular system of pithed rats.

General Procedures

Male Wistar rats weighing 300–350 g were lightly anaesthetized with ether. After the trachea was cannulated, the animals were pithed by inserting a steel rod into the spinal canal via the right orbit. They were then immediatley ventilated with room air by a Harvard Ventilator (10 ml/kg, 50 strokes/min). Arterial blood pressure, expressed as the mean, was recorded from one carotid artery with a Statham P 23 Db pressure transducer and the arterial pulse was used to trigger a heart ratemeter. Blood pressure and heart rate were recorded on a physiograph MK III. The left femoral vein was cannulated for intravenous administration of drugs. All animals received atropine (1 mg/kg, i.v.) and the vagus nerves were severed in the neck. Five rats were used to determine the effect of each dose of a drug.

Tachycardia induced by electrical stimulation of the cardiac accelerator nerves from the spinal cord

The pithing rod was coated with enamel except for a 1 cm length 6 cm from the tip. The uncovered segment was thus situated at the spinal cord level

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C_7-Th_1. An indifferent electrode was placed subcutaneously in the dorsum and the animals were given (+)−tubocurarine (1 mg/kg, i.v.). The preganglionic nerves to the heart were electrically stimulated between the pithing rod and the indifferent electrode with monophasic square wave pulses (11.5 V, 1 ms, 0.5, 1, 3 and 6 Hz) delivered for 30 s and applied at 2 min intervals. The parameter measured was the maximal tachycardia elicited by electrical stimulation at each frequency. Frequency-response curves were obtained before and 5 min after intravenous injection of the α-adrenoceptor blocking drug (one dose per animal). Clonidine (50 μg/kg, i.v.) was then administered to all animals and frequency-response curves were repeated 5 min later.

Hypertension induced by electrical stimulation of the sympathetic outflow from the spinal cord

In these experiments the pithing rod was coated with enamel except for an 8 cm length 2 cm from the tip, thereby enabling stimulation from Th_1-L_5. An indifferent electrode was placed subcutaneously in the dorsum and the animals were treated with (+)−tubocurarine (1 mg/kg, i.v.). The entire sympathetic outflow of the spinal cord was electrically stimulated with monophasic square wave pulses (2 ms, 10 V, 3 Hz) applied for 15 to 45 s at 5 min intervals. The parameter measured was the maximal increase in mean arterial pressure (mmHg) elicited by electrical stimulation. This increment in pressure was determined before and 5 min after intravenous injection of the drug (one dose per animal). Dose-response curves were established by plotting log dose of the drug as a function of inhibition (percentage of control) of the pressor response to stimulation. For each dose-response curve the dose of drug that inhibited the pressor response by 50% was determined.

Hypertension induced by phenylephrine and B-HT 933

The pressor effects (measured at the peak of the response) of phenylephrine and B-HT 933, regardless of the presence of the antagonist, were studied by administering no more than three different doses of the same agonist per rat, in random order. Log dose-response curves were established by plotting log dose of the drug as a function of the increase (mmHg) in blood pressure. Each point on the graph represented the mean of 5 observations and nine or ten rats were used for each curve. In other pithed rats, the α-adrenoceptor blocking agents (one dose per animal) were injected intravenously 10 min before the first agonist dose and the testing procedure was identical to that described above for untreated rats. The doses which increased blood pressure by 50 mmHg in the presence and absence of different doses of antagonists were calculated from the log dose-response curves.

Drugs

Drugs used were (−)-phenylephrine hydrochloride (Koch-Light); yohimbine hydrochloride, corynanthine hydrochloride (Sigma); clonidine hydrochloride (Boehringer Ingelheim); atropine sulphate (Rhone Poulenc); B-HT 933 (2-amino 6-ethyl-5, 6, 7, 8-tetrahydro-4 H-oxazolo (4, 5-d) azepine dihydrochloride) (Thomae); and raubasine (Fabre Laboratories). Drugs were dissolved in saline (0.91% w/v NaCl solution) except for raubasine which was dissolved in 1% tartaric acid solution (35 mg raubasine/10 ml tartaric acid solution) and further diluted with saline. Doses refer to the free bases.

Statistical analyses

Statistical analyses were performed with Student's *t* test, 95% level of significance.

Results

Raubasine, yohimbine and corynanthine antagonism towards clonidine inhibition of electrically induced tachycardia

The basal heart rate of pithed rats was 310 ± 10 beats/min (mean ± s.d., $n = 45$). Electrical stimulation of the cardiac accelerator nerve for 30 s at 0.5, 1, 3 and 6 Hz, caused frequency-dependent increase in heart rate. Clonidine (50 μg/kg i.v.) significantly reduced the tachycardia elicited by nerve stimulation in rats given saline (Figure 1c).

Yohimbine (0.125, 0.25, 0.50 mg/kg i.v.) caused no significant change in basal heart rate, but enhanced the positive chronotropic response to nerve stimulation at all frequencies and reduced or blocked the effect of clonidine (Figure 1a). However, neither raubasine nor corynanthine (0.5, 1, 2 mg/kg i.v.) affected the basal heart rate, the response to nerve stimulation or the inhibitory effect of clonidine (Figure 1b and c).

Antagonism of the hypertensive response to (−)-phenylephrine, B-HT 933 and sympathetic stimulation

The intravenous administration of (−)-phenylephrine or B-HT 933 induced dose-dependent increases in arterial pressure of pithed rats.

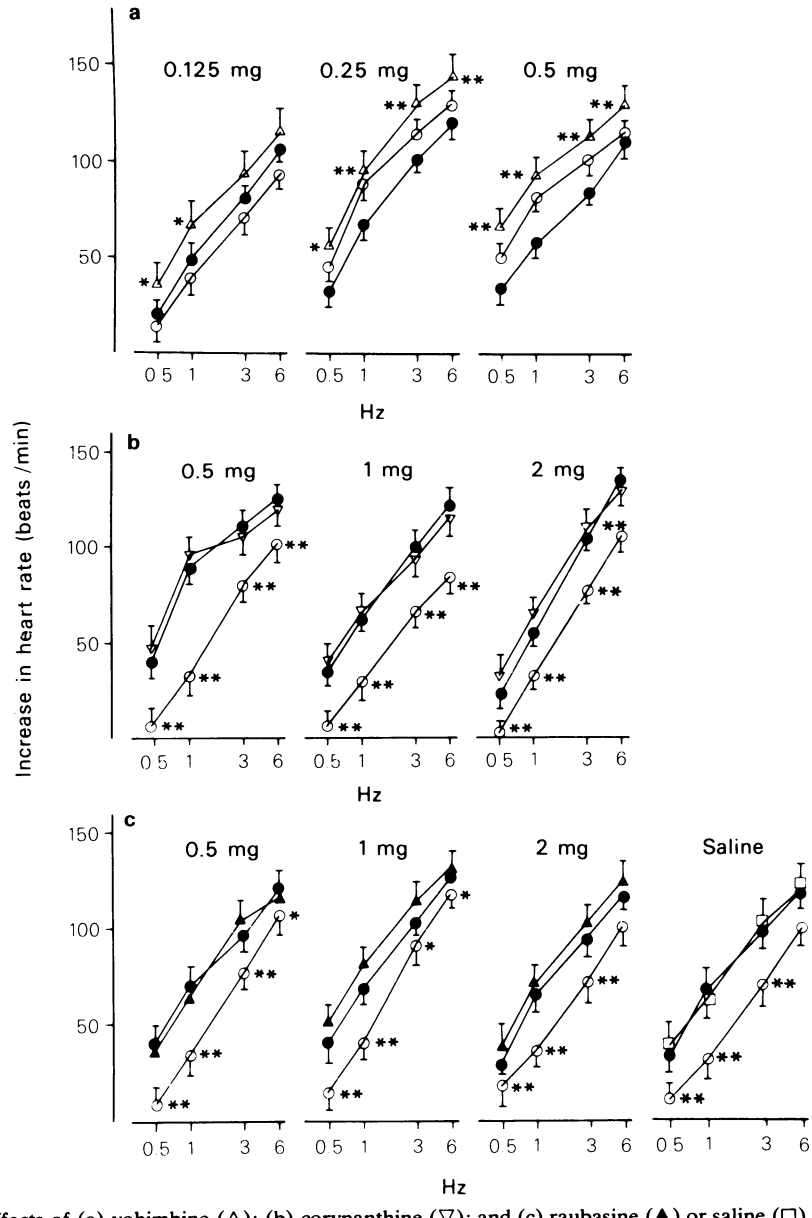

Figure 1 Effects of (a) yohimbine (△); (b) corynanthine (▽); and (c) raubasine (▲) or saline (□) on heart rate response to cardiac accelerator nerve stimulation at increasing frequencies in pithed rats. (●) Control before injection of tests drugs; (○) clonidine (50 μg/kg i.v.) after injection of test drugs. The significance of differences from the control is represented by asterisks (* $P<0.05$; ** $P<0.01$). Each family of curves was constructed from results in 5 rats.

After injections of 1, 2, or 4 mg/kg i.v. of raubasine, yohimbine or corynanthine, basal pressure was not significantly changed. Raubasine, like corynanthine and yohimbine, reduced the effects of (−)-phenylephrine at all doses tested, producing dose-dependent shifts of the dose-response curves to the right and approximately in parallel. By contrast the administration of the same doses of raubasine and corynanthine produced no significant rightward shift of the curves with B-HT 933. However, yohimbine (1, 2, 4 mg/kg, i.v.) produced significant rightward shifts of the pressor response to B-HT 933, its an-

Table 1 Doses of agonist which increase mean blood pressure in pithed rats by 50 mmHg, in the presence and absence of different doses of antagonist

Antagonist		*Agonist*			
		Phenylephrine		*B-HT 933*	
		(Dose, μg/kg)	(Dose-ratio)	(Dose, mg/kg)	(Dose-ratio)
Raubasine					
	0	4 ± 1		0.24 ± 0.02	
	1	16 ± 2**	4	—	
	2	26 ± 3**	6.5	0.22 ± 0.03	
	4	45 ± 5**	11.2	0.35 ± 0.05	1.5
Yohimbine					
	0	4.1 ± 1		0.24 ± 0.02	
	1	4.8 ± 1	1.2	3 ± 0.1 **	12.5
	2	14 ± 2**	3.4	4.2 ± 0.1 **	17.5
	4	24 ± 2**	5.8	—	
Corynanthine					
	0	3.9 ± 1		0.24 ± 0.04	
	1	13 ± 2**	3.3	—	
	2	27 ± 5**	6.9	0.25 ± 0.05	1
	4	74 ± 10**	19	0.32 ± 0.04	1.3

Each value is mean ± s.e.mean ($n = 5$).
Significant differences from corresponding controls: $^*P < 0.05$; $^{**}P < 0.01$.

tagonistic effect being more pronounced against B-HT 933 than against (−)-phenylephrine. The agonist doses which increased mean arterial pressure by 50 mmHg, in the presence and absence of different doses of antagonists, were interpolated from the dose-response curves and are shown in Table 1. Calculated agonist dose-ratios are also included.

Stimulation of the sympathetic outflow from the spinal cord of pithed rat (Gillespie & Muir, 1967) is known to increase the arterial pressure solely by the release of noradrenaline from sympathetic nerve endings in resistance blood vessels with little or no contribution from increased heart rate (Yamaguchi & Kopin, 1979).

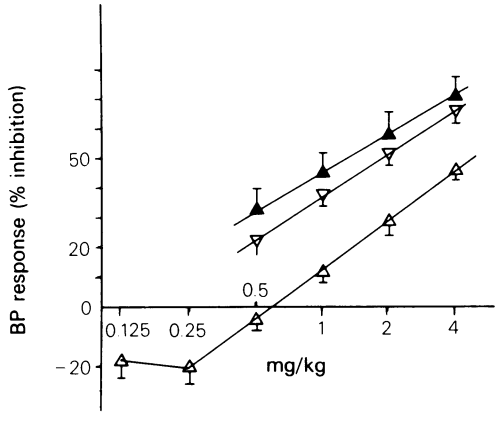

Figure 2 Effect of various doses of raubasine (▲), yohimbine (△) and corynanthine (▽) on the rise in mean blood pressure elicited by stimulation of sympathetic outflow from the spinal cord of pithed rats. Each point is mean of values from 5 rats; vertical lines show s.e.mean.

After constant control pressor responses to stimulation had been obtained, the administration of raubasine or corynanthine (0.5, 1, 2, 4 mg/kg, i.v.) produced a marked dose-dependent inhibition of the response (Figure 2). Low doses (0.125, 0.25 mg/kg, i.v.) of yohimbine significantly potentiated the stimulation-induced pressor response (indicated in the dose-response curves as negative inhibition), but at 1 and 2 mg/kg intravenously yohimbine significantly reduced it (Figure 2). The values for 50% inhibition of the pressor responses were 1.3 mg/kg raubasine, 1.8 mg/kg corynanthine and 4.6 mg/kg yohimbine.

Discussion

Clonidine inhibits the chronotropic responses to cardiac sympathetic nerve stimulation in rats (Armstrong & Boura, 1973) by the activation of presynaptic α_2-adrenoceptors thereby reducing transmitter release (see reviews by Starke, 1977). In the present study, yohimbine enhanced the cardiac response to nerve stimulation and antagonized the inhibitory effects of clonidine on the responses (Figure 1a). These effects substantiate the view that presynaptic α-adrenoceptors in this preparation are of the

α_2-subtype (see Introduction). By contrast, raubasine and corynanthine did not significantly modify stimulation-induced tachycardia nor the inhibitory effects of clonidine (Figure 1b and c), implying little antagonist activity at α_2-adrenoceptors.

Yohimbine reduced but raubasine and corynanthine did not change the pressor responses induced by B-HT 933 in pithed rats. This compound has been shown to be a selective agonist at α_2-adrenoceptors (Pichler, Placheta & Kobinger, 1980), so these results further confirm the selectivity of action of these drugs.

Reductions in the pressor response to stimulation of sympathetic outflow from the spinal cord of pithed rats were used as an index of postsynaptic α-adrenoceptor blockade. In untreated animals, pressor responses are directly proportional to the logarithm of the stimulation rate and to increases in serum noradrenaline levels (Yamaguchi & Kopin, 1979) and are the result of activation of α_1-adrenoceptors in the region of the vascular neuroeffector junctions (Yamaguchi & Kopin, 1980). Raubasine and corynanthine produced a marked dose-dependent inhibition of the pressor responses (Figure 2). Low doses of yohimbine potentiated and higher doses reduced the pressor responses, which confirm the findings of Docherty & McGrath (1980) and suggest that low doses of yohimbine block presynaptic α_2-adrenoceptors thereby inducing an increase of noradrenaline release. At high doses yohimbine blocks the postsynaptic α-adrenoceptors.

(−)-Phenylephrine is considered to be a potent α_1-adrenoceptor stimulant (Starke *et al.*, 1975). Raubasine, corynanthine and yohimbine reduced the pressor response to (−)-phenylephrine. Of the three drugs, only yohimbine also antagonized the pressor response to B-HT 933, and was more effective against this agent than against phenylephrine (see dose-ratios in Table 1).

The results of the present study on the α_1- or α_2-adrenoceptor blocking activity of raubasine enable us to conclude that this drug, like corynanthine, acts selectivity on α_1-adrenoceptors in pithed rats. Its potency is comparable to that of corynanthine.

Concerning yohimbine and corynanthine, our results agree with those of Weitzell *et al.* (1979), Docherty & McGrath (1980) and Timmermans & Van Zwieten (1980). Yohimbine is more potent in blocking α_2- than α_1-adrenoceptors and corynanthine selectively blocks α_1-adrenoceptors.

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