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Phentolamine—an unexpected agonist in the rabbit

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Phentolamine (0.1–10 μM) caused an anomalous rightward shift of the relationship between the number of electrical field pulses and tachycardia in the rabbit isolated right atrium. Phentolamine was apparently acting as a presynaptic agonist on sympathetic nerve endings to inhibit transmitter release. The effect was prevented by benextramine treatment and antagonized 10 fold by yohimbine (1 μM) but not by prazosin (0.1 μM). In ganglion-blocked (mecamylamine) conscious or anaesthetized rabbits, phentolamine (3–1000 $\mu g\,kg^{-1}$) caused a dose-related rise in blood pressure that was antagonized by yohimbine (1 $mg\,kg^{-1}$). These pressor and inhibitory cardiac sympathetic nerve effects of phentolamine are not found in similar preparations from the guinea-pig or rat. Therefore, these rabbit-specific agonist effects of phentolamine at sites similar to α_2-adrenoceptors make this drug unsuitable as an α-adrenoceptor antagonist in rabbits.

Introduction Phentolamine is generally considered to be a competitive α_1- and α_2-adrenoceptor antagonist without any agonist activity. However, in the rabbit vas deferens the twitch response was inhibited by phentolamine but curiously not by phenoxybenzamine (Adebanjo & Ambache, 1979). Broadhurst *et al.* (1983) have offered the suggestion that phentolamine is an agonist at a presynaptic 'α_2-like' adrenoceptor in this rabbit preparation but not in the rat vas deferens.

We present evidence that phentolamine has agonist-like activity at rabbit presynaptic receptors on cardiac sympathetic nerve terminals and on vascular receptors that mediate vasopressor responses.

Methods Young rabbits (6–8 weeks old) were killed by a blow to the head. Hearts were removed and placed in a cool Krebs solution saturated with 95% O_2 plus 5% CO_2. Right atria were dissected free and placed in organ baths at 37°C for the measurement of atrial period from the surface electrogram (Angus & Harvey, 1981). Autonomic nerves were depolarized by applying electrical field pulses (2 ms, 100 mA) during the atrial refractory period to prevent arrhythmias. Bradycardia from vagal stimulation was prevented by atropine (1 μM). One hour after setting up the preparation, tachycardia was measured following 1, 2 and 4 field pulses (delivered one per refractory period). Phentolamine was added for 30 min before repeating the field pulses. After each set of field pulses the bathing solution was replaced and a higher concentration of phentolamine added.

The central ear artery and marginal ear vein were cannulated under local anaesthesia in adult rabbits (2–3 kg). Blood pressure and heart rate, triggered from the pressure signal, were monitored on a Grass polygraph (model 7). Ganglion blockade was induced with mecamylamine (10 $mg\,kg^{-1}$ i.v.) and some rabbits were lightly anaesthetized with alphaxalone/alphadolone (Alfathesin, Glaxo).

Results In rabbit right atria, the tachycardia (fall in period) to field stimulation was inhibited by phentolamine (0.1–10 μM) in a concentration-dependent manner (Figure 1a). In control experiments, the tachycardia to similar field pulses (1–4) over 6 periods of stimulation (each separated by 30 min) was not significantly shifted from the first period. In addition, exogenous noradrenaline concentration-response curves were not shifted to the right by phentolamine (1 μM).

Benextramine (10 μM, 60 min equlibration followed by 60 min wash) treatment of rabbit atria prevented the rightward shift by phentolamine (1 μM for 30 min). Yohimbine inhibited the phentolamine shift by about 10 fold, i.e. the degree of shift to phentolamine (10 μM) in the presence of yohimbine was equivalent to 1 μM in the absence of yohimbine.

The effect of phentolamine on the sympathetic terminals was not inhibited by prazosin (0.1 μM); sulpiride (1 μM); haloperidol (0.2 μM); mepyramine (1 μM)or cimetidine (100 μM) while the selective α_2-adrenoceptor antagonist idazoxan (RX781094, Reckitt & Coleman) had an agonist effect similar to phentolamine.

In 6 conscious and 2 anaesthetized ganglion-blocked rabbits, phentolamine (3–1000 $\mu g\,kg^{-1}$ i.v.) cumulative bolus injections caused a dose-dependent rise in blood pressure (30–50 mmHg) with little change in heart rate (Figure 1b). Yohimbine (1 $mg\,kg^{-1}$i.v.) given slowly over 5 min to anaesthetized, ganglion-blocked rabbits inhibited the effect of phentolamine. In rabbits without ganglion blockade, phentolamine (3–1000 $\mu g\,kg^{-1}$) did not evoke a pressor response. Similarly, 2 rabbits with prior sur-

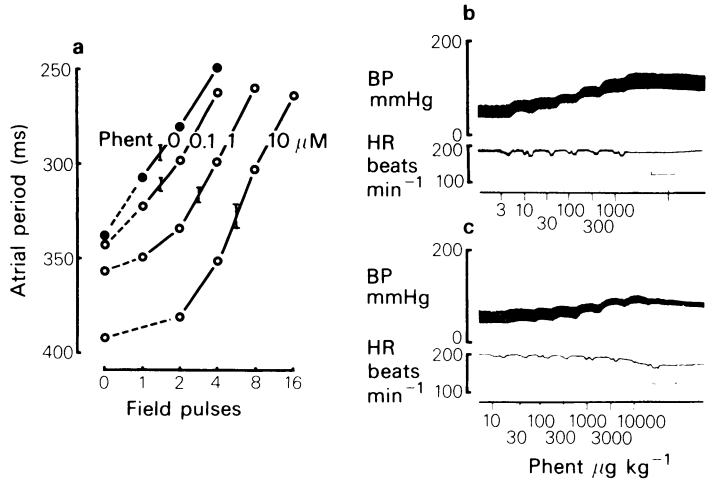

Figure 1 (a) Inhibition by phentolamine (Phent, 0.1–10 μM) of responses to field stimulation of guinea-pig right atria in the presence of atropine (1 μM) ($n = 5$). After the control curve was generated, phentolamine was equilibrated with the tissues in ascending concentrations (as indicated on figure) for 30 min before restimulation. Error bars are average s.e.mean from anaylsis of variance. (b and c) Blood pressure (BP) and heart rate (HR) responses of a ganglion-blocked, anaesthetized rabbit to cumulative bolus doses of phentolamine, before (b) and 15 min after yohimbine 1 mg kg $^{-1}$. An interval of 150 min was allowed between (b) and (c).

gical sino-aortic baroreceptor denervation did not respond with a pressor response to phentolamine. However, in rabbits pretreated with guanethidine (10 mg kg^{-1} over 60 min) and atropine (1 mg kg^{-1} plus 0.1 mg kg^{-1}min^{-1}) phentolamine (0.5 mg kg^{-1}i.v.) gave a marked pressor response.

Discussion In the rabbit right atrium phentolamine has an anomalous property of inhibiting the release of transmitter noradrenaline during nerve stimulation. That the site of action is a receptor on the nerve terminal follows from the lack of interaction of phentolamine with exogenous noradrenaline. This presynaptic phentolamine receptor is not prazosin-sensitive but is blocked by the irreversible α_2-adrenoceptor antagonist benextramine (Lew & Angus, 1983). Yohimbine had a lower affinity (pA_2 about 7) in the rabbit atrium compared with its affinity at α_2-adrenoceptors in other species (pA_2 7.5–8.4; Doxey *et al.*, 1977; Fuder, *et al.*, 1983; Lew & Angus, unpublished data). Thus the receptor is possibly not a normal α_1-or α_2-adrenoceptor.

The pressor effect of phentolamine is most clearly evident in conscious or anaesthetized rabbits where the baroreceptor reflexes and sympathetic tone have been abolished. Thus pharmacological ganglion blockade or a combination of guanethidine and atropine were necessary to prevent sympathetic withdrawal and vagal stimulation in response to the pressor action of phentolamine. Sino-aortic denervation caused a 45% increase in renal sympathetic nerve activity after 24 h, (Dorward, personal communication) and probably increased sympathetic nerve activity to vascular tissue as well. Under these conditions phentolamine presumably would tend to lower pressure through antagonism of postsynaptic neural α-adrenoceptors as well as tending to raise pressure through the agonist action.

The presynaptic inhibitory action of phentolamine on cardiac sympathetic nerves and the pressor effect appear to be specific to the rabbit as similar effects could not be observed in rat or guinea-pig. In guinea-pig right atria, phentolamine has no effect on tachycardia or ^{3}H-efflux in response to 1–4 field pulses under conditions where no autoinhibition occurs (Angus & Korner, 1980; Angus *et al.*, 1983). Similarly, phentolamine does not significantly alter the blood pressure in conscious ganglion-blocked guinea-pigs (Lew & Angus, unpublished). In the perfused rat heart, phentolamine did not inhibit ^{3}H-efflux in the absence of autoinhibition (Fuder *et al.*, 1983) and phentolamine does not raise the blood pressure in pithed rats (Drew, 1976; Timmermans & Van Zwieten, 1980).

In conclusion, there is now evidence to suggest that phentolamine has anomalous agonist activity in the rabbit at least in nerve terminals in the heart and vas deferens and at resistance blood vessels. These actions are in addition to the non-selective α_1- and α_2-adrenoceptor antagonism described for phentolamine in the rabbit and in other species. The

receptors mediating these agonist actions of phentolamine are blocked by benextramine and weakly by yohimbine. Care should be taken when using phentolamine as a pharmacological tool in the rabbit.

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