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α_1-ADRENOCEPTORS CAN MEDIATE CHRONOTROPIC RESPONSES IN THE RAT HEART

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In the pithed rat, amidephrine, a 'selective' α_1-adrenoceptor agonist, evokes a positive chronotropic response. This response can be antagonized by prazosin but not by propranolol or by rauwolscine. Similarly, part of the chronotropic response to cardioaccelerator nerve stimulation is resistant to blockade by propranolol but is sensitive to prazosin or WB 4101. We conclude that α_1-adrenoceptors can mediate a chronotropic response in the rat heart either to exogenous agonists or to the endogenous neurotransmitter noradrenaline.

Introduction In the mammalian heart, the positive chronotropic and inotropic responses to sympathetic nerve stimulation or to sympathomimetic drugs are considered to be mediated by β-adrenoceptors (Ahlquist, 1948). However, Govier (1967) reported an α-adrenoceptor-mediated inotropic action of phenylephrine in the guinea-pig and a similar effect has since been found in a number of species (Benfey & Varma, 1967; Nakashima, Maeda, Sekiya & Hagino, 1971; Wagner & Brodde, 1978; Schumann, 1980; Shibata, Seriguchi, Iwadare, Ishida & Shibata, 1980).

In contrast, there is little evidence for α-adrenoceptor-mediated chronotropic effects under normal physiological conditions. However, an α-adrenoceptor-mediated chronotropic response has been demonstrated in isolated atria from hypothyroid rats (Nakashima & Hagino, 1972; Wagner & Brodde, 1978; Simpson & McNeil, 1980).

We now show that amidephrine, a selective α_1-adrenoceptor agonist with little β-adrenoceptor agonistic activity (Stanton, Dungan & Lish, 1965; Buchthal & Jenkinson, 1970; Flavahan & McGrath, 1981), can evoke a positive chronotropic response. Furthermore, by the use of 'selective' antagonists it can be demonstrated that the response to cardioaccelerator nerve stimulation contains an α_1-adrenoceptor-mediated component.

Methods Male Wistar rats (250–275 g) were pithed by the method of Gillespie, MacLaren & Pollock (1970) and ventilated with O_2. Heart rate and right common carotid arterial pressure were monitored continuously. The right jugular vein was cannulated for drug injections. Experiments fell into two groups:

(1) The chronotropic responses to a range of doses of amidephrine were determined 5 min after injection of: prazosin (1 mg/kg); rauwolscine (1 mg/kg); propranolol (1 mg/kg); or prazosin (1 mg/kg) plus propranolol (1 mg/kg) given 5 min apart. Owing to the long duration of the responses to amidephrine, only 1 or 2 doses of it could be tested in each animal. Different antagonists were studied in separate experiments.

(2) Cardioaccelerator responses were produced by stimulation of the appropriate sympathetic outflow (T1) with single pulses (0.5 ms) at least 5 min apart. This procedure avoids activation of prejunctional α_2-adrenoceptors which modulate output of neurotransmitter (Docherty & McGrath, 1979; 1980).

Antagonists were given 5 min before a stimulus and the effect on the response was measured. In some experiments different antagonists were administered sequentially to determine the combined effect.

Results are expressed as mean ± s.e.mean for groups of identical experiments. Statistical comparisons were made either by Student's paired *t* test, or by Student's *t* test. Drugs were dissolved in 0.9% w/v NaCl solution (saline) except for prazosin (distilled water) and rauwolscine (w/w ascorbic acid in distilled water). Doses quoted are of the salt. Drugs used were (−)-amidephrine hydrochloride (Mead Johnston), prazosin hydrochloride (Pfizer), propranolol hydrochloride (Sigma), rauwolscine base (Inverni della Beffa) and WB 4101 hydrochloride (2-N([2′,6′-dimethoxy-phenoxyethyl] aminoethyl) 1,4-benzodioxan hydrochloride, Ward Blenkinsop).

Results The resting heart rate (320.7 ± 3.7 min^{-1}, $\bar{x}$ ± s.e.mean, $n = 21$) was not significantly different between experimental groups. At the doses employed each antagonist transiently decreased heart rate on injection but it had always returned to normal (± 5 min^{-1}) within 5 min.

(i) Amidephrine (1–1000 μg/kg) produced dose-dependent increases in heart rate (Figure 1a). These responses were not significantly reduced by rauwolscine (1 mg/kg). Propranolol (1 mg/kg) reduced the response only to the highest dose of amidephrine (1000 μg/kg, $P < 0.05$).

Prazosin (1 mg/kg) significantly attenuated the re-

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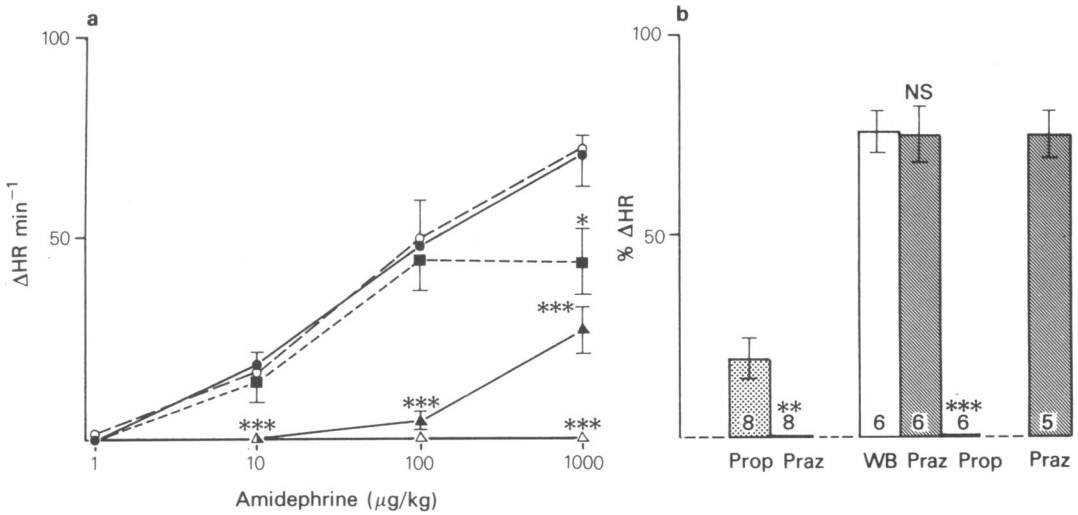

Figure 1 The effects of antagonists on chronotropic responses to (a) amidephrine, (b) cardioaccelerator nerve stimulation. (a) Control (●); prazosin (1 mg/kg) (▲); propranolol (1 mg/kg) (■); rauwolscine (1 mg/kg) (○); prazosin (1 mg/kg) plus propranolol (1 mg/kg) (△). ΔHR represents the increase in heart rate above the pre-drug control level. The response following each antagonist was compared with the corresponding drug-free control by Student's t test (only significant differences are indicated; $^{*}P<0.05$; $^{***}P<0.001$; $n=5-7$). (b) The effect of sequential administration of antagonists on the response to cardioaccelerator nerve stimulation (T1, single pulse, 0.5 ms). ΔHR is expressed as a percentage of the pre-drug control. Sets of histograms separated by broken lines refer to different groups of experiments. Prop = propranolol; Praz = prazosin; WB = WB 4101. Within each group, each column was compared with the preceding column, paired t test: NS no significant difference; **, $0.01>P>0.001$; $^{***}P<0.001$; n for different groups shown within columns. Bars indicate s.e.mean.

sponse at every dose of amidephrine ($P<0.001$). After prazosin (1 mg/kg) plus propranolol (1 mg/kg) no chronotropic response was obtained to amidephrine (1–1000 μg/kg).

(ii) Nerve stimulation Figure 1b presents the results of three groups of experiments showing the effects of antagonists, given alone or sequentially, on the response to a single pulse. The responses are expressed as a percentage of the drug-free control. The magnitude of the response to a single pulse was (28.8 ± 1.4 min^{-1}, $n=19$).

After propranolol (1 mg/kg) a response remained ($19.3\pm5.1\%$ of control) which could be abolished by prazosin (1 mg/kg) (no detectable response, $n=8$).

After WB 4101 (1 mg/kg) the response was significantly reduced ($75.4\pm5.3\%$ of control) and subsequent administration of prazosin (1 mg/kg) had no further effect; addition of propranolol (1 mg/kg) now abolished the response.

After prazosin (1 mg/kg) the response was significantly reduced ($74.8\pm5.8\%$ of control) and was similar to that obtained following WB 4101 ($P>0.9$).

Discussion Amidephrine (<100 μg/kg) produces a positive chronotropic response in the pithed rat which can be antagonized by prazosin but not by rauwolscine or by propranolol. The receptors involved, therefore, display agonist and antagonist specificities similar to those of the α_1-adrenoceptors found in vascular smooth muscle of the rat (Docherty & McGrath, 1980; Flavahan & McGrath, 1981). The chronotropic response to a higher dose of amidephrine (1000 μg/kg) contained a β-adrenoceptor-mediated component which could be demonstrated with or without prazosin.

The effects of the antagonists on the nerve-mediated response suggest that this α_1-adrenoceptor can be activated also by the neurotransmitter, noradrenaline. Part of the response ($19.3\pm5.1\%$) was resistant to a dose of propranolol which produced a 100 fold shift in the dose-response relationship for isoprenaline (unpublished observations). However, this residual response was susceptible to prazosin suggesting that it was mediated by α_1-adrenoceptors. WB 4101 (Butler & Jenkinson, 1978; MacDonald & McGrath, 1980) or prazosin each decreased the

single pulse response by a similar amount but these effects were not additive. However, after these α-adrenoceptor antagonists, propranolol was able to abolish the response as would be expected if only a β-adrenoceptor component remained. This indicates that the cardioaccelerator response to sympathetic nerve stimulation is mediated predominantly by β-adrenoceptors but that there is a significant component from α_1-adrenoceptors.

Earlier *in vitro* studies (Weston, 1971; Nakashima & Hagino, 1972; Wagner & Brodde, 1978; Simpson & McNeil, 1980) implied that α-adrenoceptor-mediated chronotropic effects in the rat are either absent or unimportant in the normal physiological state. In the pithed rat, however, by using relatively 'selective' agonists and antagonists, it has been possible to demonstrate such a receptor, classify it as α_1- and implicate it in responses to blood-borne agents or to the neurotransmitter.

These observations have significance for the study of adrenergic mechanisms in the heart, particularly the involvement of prejunctional α_2-adrenoceptors which, hitherto, have been assumed to be the only α-adrenoceptor associated with cardioaccelerator neurotransmission (Docherty & McGrath, 1979; 1980). If such receptors occur in man this also has implications for combined therapy with α- and β-adrenoceptor blocking drugs.

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