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α_1-ADRENOCEPTOR ACTIVATION CAN INCREASE HEART RATE DIRECTLY OR DECREASE IT INDIRECTLY THROUGH PARASYMPATHETIC ACTIVATION

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1 The chronotropic effects of α- and β-adrenoceptor agonists were investigated in the pithed rat.

2 The β-adrenoceptor agonist, isoprenaline, produced only a positive chronotropic response. α_1-Adrenoceptor agonists, phenylephrine and amidephrine, produced positive and negative chronotropic effects. Part of the response to phenylephrine was β-mediated.

3 A positive chronotropic response to amidephrine and phenylephrine was mediated directly through cardiac α_1-adrenoceptors and had a different time course from β-adrenoceptor-mediated responses.

4 A negative chronotropic response to α-agonists was potentiated by neostigmine and blocked by atropine, tetrodotoxin or hexamethonium as well as by α_1-adrenoceptor antagonists. This may indicate α_1-adrenoceptors on preganglionic parasympathetic nerves, stimulation of these receptors causing release of acetylcholine.

5 The α_2-adrenoceptor agonist, xylazine, produced a direct negative chronotropic effect on the heart, independent of α-adrenoceptors. No evidence was found for functional post-junctional α_2-adrenoceptors. At high doses xylazine stimulated cardiac α_1-adrenoceptors.

Introduction

α_1-Adrenoceptors can mediate a positive inotropic response (Wagner & Brodde, 1978; Shibata, Seriguchi, Iwadare, Ishida & Shibata, 1980; Benfey, 1980; Schumann, 1980; Boucher, 1981). In contrast, an α-adrenoceptor-mediated, positive chronotropic response has been demonstrated only in isolated atria from hypothyroid rats (Nakashima & Hagino, 1972; Kunos, 1977; Wagner & Brodde, 1978; Simpson & McNeill, 1980a). These and other studies (Weston, 1971; Bennett & Kemp, 1978) failed to find an α-mediated chronotropic response under 'normal physiological conditions'. However, in the pithed rat, amidephrine (a selective α_1-agonist) or noradrenaline, released from the cardioaccelerator nerves, can produce a prazosin-sensitive increase in heart rate (Flavahan & McGrath, 1981b).

We now describe a more detailed analysis of α_1-mediated chronotropic effects including those of phenylephrine, which was used in most previous studies as the 'selective α_1-adrenoceptor agonist'. Several interesting points emerged. When β-mediated effects were eliminated, two distinct α_1-adrenoceptor-mediated chronotropic responses remained: (1) the expected increase in heart rate and (2) an unexpected decrease in heart rate which pharmacological analysis showed to be due to release of acetylcholine from preganglionic parasympathetic nerves.

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.

Chronotropic responses to adrenoceptor agonists

The change in heart rate produced by each agonist was assessed in the absence of other drugs (control) or 5 min after injection of (a) an antagonist or (b) a combination of antagonists administered 5 min apart. The drugs employed as selective for particular adrenoceptors were: agonists – isoprenaline (β), xylazine (α_2; Docherty & McGrath, 1980a), amidephrine and phenylephrine (both α_1; Flavahan & McGrath 1981a,b), antagonists – propranolol (β),

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labetalol ($\alpha_1 + \beta$; Farmer, Kennedy, Levy & Marshall, 1972), WB4101 (α_1; Butler & Jenkinson, 1978), prazosin (α_1; Docherty & McGrath, 1980a), corynanthine and rauwolscine (α_1, α_2 respectively; Weitzell, Tanaka & Starke, 1979). Only one agonist was studied in each experiment and the effects of each antagonist (or combination of antagonists) were studied in separate experiments.

In addition to the construction of dose-response curves to the agonists, the time courses of the chronotropic responses to phenylephrine (100 μg/kg), amidephrine (100 μg/kg) or xylazine (10 mg/kg) were also analysed. The responses were measured every 5 s for the first 2 min after injection and thereafter every 30 s.

Chronotropic response to vagal stimulation

Reproducible negative chronotropic responses could be obtained to stimulation (50 pulses, 0.1 ms, 5 Hz) of the peripheral end of the cut right cervical vagus (Docherty & McGrath, 1980b). The effects of various pretreatments on the responses to vagal stimulation were assessed 5 min after injection.

Results are expressed as mean ± s.e.mean for groups of identical experiments. Statistical comparisons were made by Student's *t* test. Drugs were dissolved in 0.9% w/v NaCl solution (saline) except prazosin (distilled water) and rauwolscine (w/w ascorbic acid in distilled water). Doses quoted are of the salt. Drugs used were (−)-amidephrine hydrochloride (Mead Johnstone), atropine sulphate (BDH), corynanthine tartrate (Aldrich Chem. Co.), hexamethonium bromide (Koch-Light), (−)-isoprenaline *D*-bitartrate (Sigma), labetalol hydrochloride (AH5158, Allen & Hanburys), neostigmine bromide (Roche), phenylephrine hydrochloride (Sigma), prazosin hydrochloride (Pfizer), propranolol hydrochloride (Sigma), rauwolscine base (Inverni della Beffa), tetrodotoxin (Sigma), WB4101 hydrochloride (2-N(2',6'-dimethoxyphenoxyethyl aminoethyl) 1,4 benzodioxan hydrochloride, Ward Blenkinsop), xylazine hydrochloride (Bayer).

Results

Effects of blocking drugs on heart rate

The resting heart rate (321 ± 1.9 min^{-1}, $\bar{x}$ ± s.e.mean, $n = 100$) was not significantly different between experimental groups. At the doses employed, atropine, prazosin, labetalol, WB4101, rauwolscine, tetrodotoxin and hexamethonium transiently decreased heart rate on injection but it had always returned to normal (± 5 min^{-1}) within 5 min. Similarly, corynanthine (10 mg/kg) and neostigmine (25 μg/kg) both decreased heart rate on injection but in these cases a significant reduction remained after 5 min (24 ± 7.8 min^{-1}, $n = 7$, and 29 ± 3.3 min^{-1}, $n = 10$, respectively).

Positive chronotropic responses to adrenoceptor agonists

In this section the response to each agonist was measured as the peak increase following injection.

Amidephrine (1–1000 μg/kg) produced dose-dependent increases in heart rate (Figure 1). Propranolol (1 mg/kg) reduced the response only to the highest dose of amidephrine (1000 μg/kg, $P < 0.05$, Figure 1a).

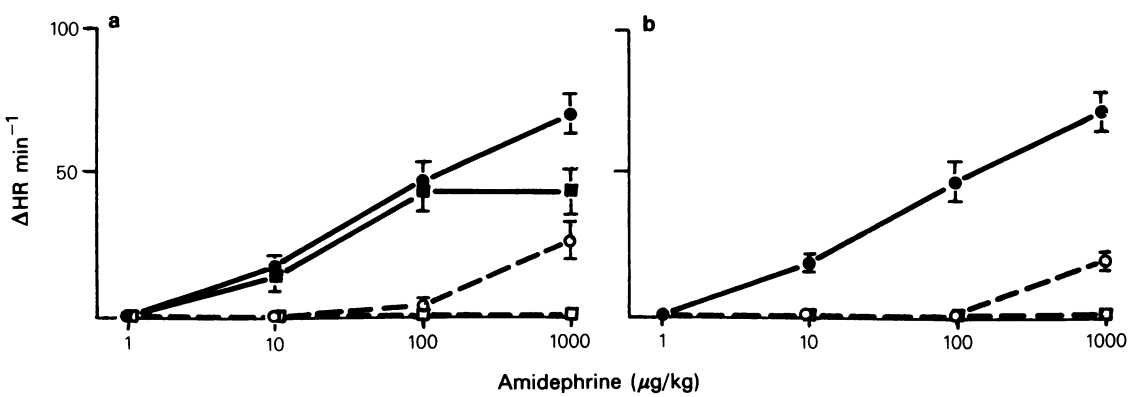

Figure 1 The effects of antagonists on the positive chronotropic response to amidephrine: (a) (●) control; (■) propranolol (1 mg/kg); (○) prazosin (1 mg/kg); (□) propranolol + prazosin (both 1 mg/kg). (b) (●) control; (○) WB4101 (1 mg/kg); (□) WB4101 + propranolol (both 1 mg/kg); ΔHR represents the increase in heart rate above the pre-drug control level. Bars indicate s.e.mean; ($n = 4-7$).

As observed previously (Flavahan & McGrath, 1981b), the positive chronotropic response to amidephrine was unaffected by rauwolscine (1 mg/kg) but the response to each dose was significantly reduced by prazosin (1 mg/kg) (Figure 1a, $P<0.001$).

WB4101 (1 mg/kg) significantly reduced the response to each dose of amidephrine (Figure 1b, $P<0.001$). After propranolol (1 mg/kg) plus WB4101 (1 mg/kg) or propranolol (1 mg/kg) plus prazosin (1 mg/kg) no chronotropic response to amidephrine was obtained (Figures 1b and 1a respectively).

Phenylephrine (1–1000 μg/kg) produced dose-dependent increases in heart rate (Figure 2). These responses were not significantly reduced after rauwolscine (1 mg/kg, Figure 2c).

After prazosin (1 mg/kg), the chronotropic response to a low dose of phenylephrine (10 μg/kg) was significantly reduced ($P<0.01$) but those to higher doses (100 or 1000 μg/kg) were not significantly changed (Figure 2a).

Corynanthine (10 mg/kg) had a qualitatively similar effect to prazosin, significantly reducing the chronotropic response to a low dose of phenylephrine (10 μg/kg, $P<0.01$, Figure 2b) but not to those to higher doses.

Propranolol (1 mg/kg) had no effect on the chronotropic response to a low dose of phenylephrine (10 μg/kg, Figure 2a–d) but significantly reduced the responses to higher doses (100 or 1000 μg/kg, $P<0.001$, Figure 2a–d).

The responses to phenylephrine, which remained

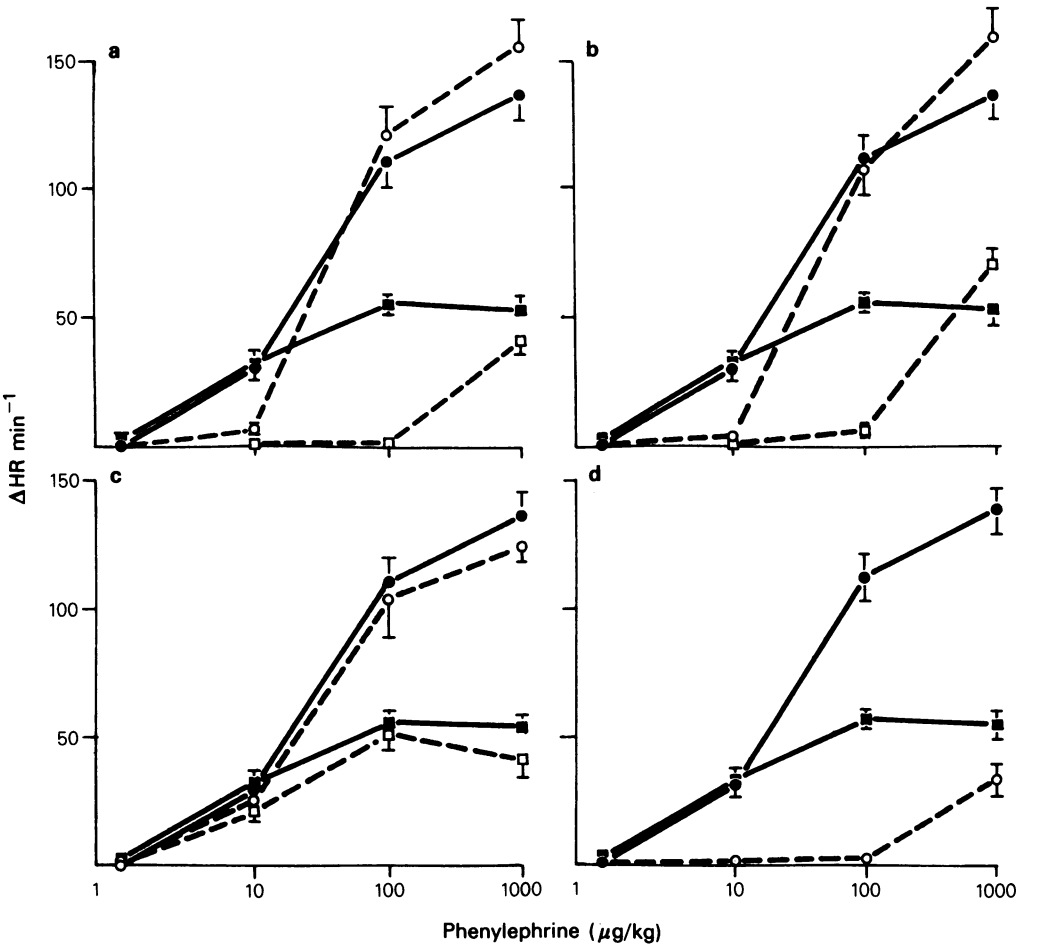

Figure 2 The effects of antagonists on the positive chronotropic response to phenylephrine. In each case: (●) control; (■), propranolol (1 mg/kg); (○) antagonist alone; (□) antagonist + propranolol (1 mg/kg), Antagonists studied were: (a) prazosin (1 mg/kg); (b) corynanthine (10 mg/kg); (c) rauwolscine (1 mg/kg); (d) labetalol (10 mg/kg). Bars indicate s.e.mean; ($n = 4-7$).

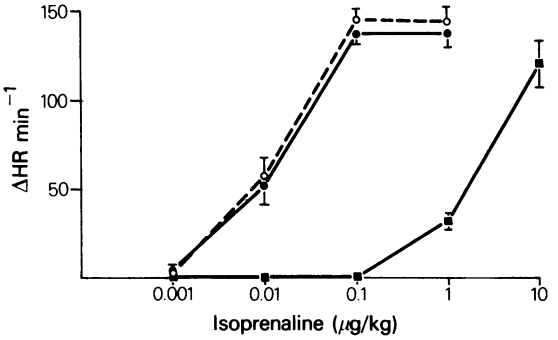

Figure 3 The positive chronotropic response to isoprenaline (control, ●) and the effect of prazosin (1 mg/kg) (○) or propranolol (1 mg/kg) (■). Bars represent s.e.mean; ($n = 5-6$).

after propranolol (1 mg/kg), were of similar magnitude to those of equal doses of amidephrine (compare Figures 1 and 2) and were significantly reduced by either prazosin (1 mg/kg, $P < 0.001$) or corynanthine (10 mg/kg, $P < 0.001$) but not by rauwolscine (1 mg/kg) (Figure 2a, b, and c respectively).

Labetalol (10 mg/kg) totally abolished the positive chronotropic response to phenylephrine (1–100 μg/kg, Figure 2d) although this dose produces no greater α_1-antagonism than prazosin (1 mg/kg) (authors, unpublished observations).

Isoprenaline (0.001–1 μg/kg) produced dose-dependent increases in heart rate that were unaffected by prazosin (1 mg/kg). Propranolol (1 mg/kg), however, caused more than a 100 fold shift to the right of the dose-response curve to isoprenaline (Figure 3).

Negative chronotropic effects

In this section the time course of the chronotropic response to each agonist was considered.

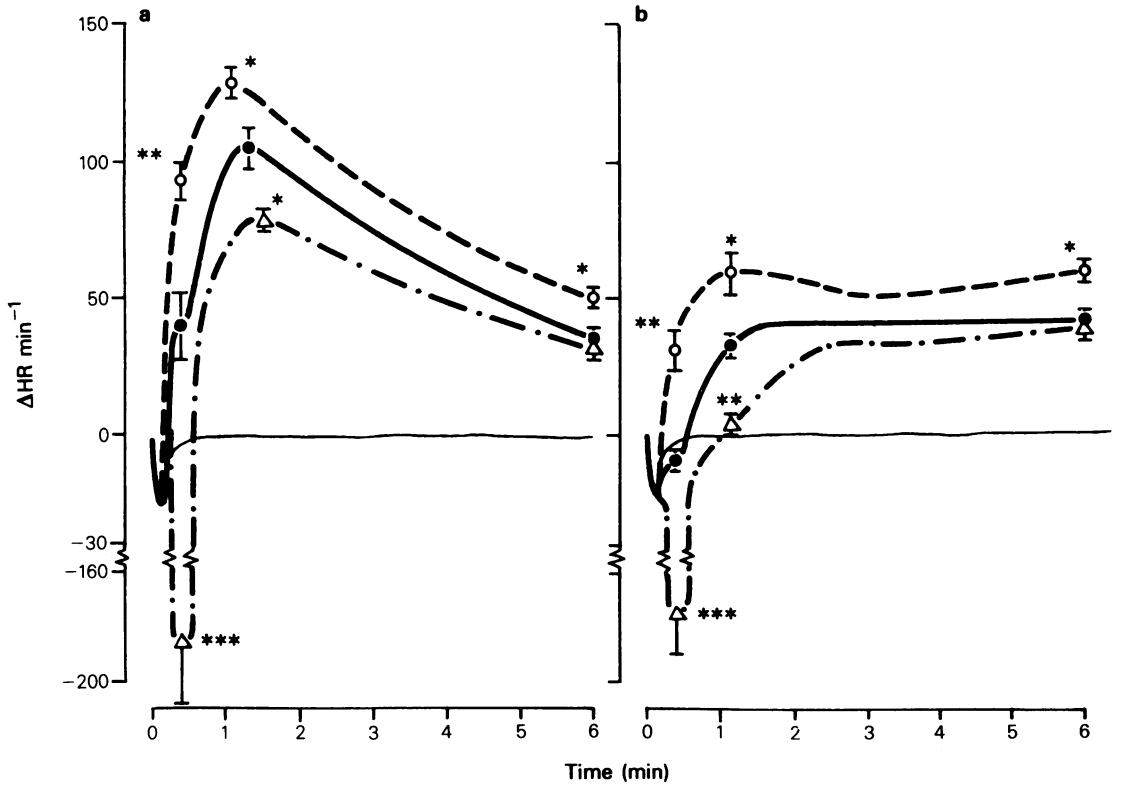

Figure 4 An analysis of the time course of the chronotropic response to (a) phenylephrine (100 μg/kg) or (b) amidephrine (100 μg/kg) injected at time zero. (●) Control; (○) after atropine (1 mg/kg); (△), after neostigmine (25 μg/kg); — saline. Note that the y-axis is discontinuous. The response following each pretreatment was compared with the corresponding drug-free control by Student's t test (only significant differences are indicated; $*0.05 > P > 0.01$; $**0.01 > P > 0.001$; $***P < 0.001$; $n = 4-6$. The initial transient decrease in heart rate is due to the bolus injection of the agonists and is also seen with saline. Bars represent s.e.mean.

Amidephrine and phenylephrine At high doses (100–1000 μg/kg) each agonist produced a rise in heart rate which did not rise smoothly (i.e. the rising phase of the response was double-peaked). This suggests an early effect which is opposing the rise in heart rate and which can produce a negative chronotropic effect during the positive chronotropic response.

For phenylephrine, this negative chronotropic response appeared in the mean data as an inflection in the rising phase of the positive chronotropic response. However, with amidephrine it occurred before the positive chronotropic response (Figure 4a and b respectively).

Cholinergic involvement

The negative chronotropic effect could result from the release of acetylcholine. To test this, the effects of atropine and neostigmine were examined.

Following atropine (1 mg/kg), no 'negative chronotropic' responses were observed with phenylephrine (Figure 4a) or amidephrine (Figure 4b) (both 100 μg/kg). Each agonist produced an earlier and more rapid rise in heart rate. The magnitude of the positive chronotropic response to each agonist was significantly greater throughout the response ($P<0.05$).

Neostigmine (25 μg/kg) markedly increased the negative chronotropic response to each agonist. Phenylephrine now produced an initial transient rise in heart rate followed by a decrease of $187 \pm 22.1\ min^{-1}$ ($\bar{x} \pm$ s.e.mean, $n=4$, Figure 4a). Amidephrine caused a reduction of $176 \pm 14.8\ min^{-1}$ ($n=4$) compared with $9.6 \pm 4.6\ min^{-1}$ ($n=5$) in the controls (Figure 4b). Following the decrease, each agonist produced a slower rise in heart rate than in controls. Consequently, if the rise in heart rate was measured at an early stage it was significantly less than controls: later (e.g. after 6 min) there was no significant difference.

In the presence of neostigmine, a higher dose of phenylephrine (1000 μg/kg), which in controls is relatively innocuous, caused cardiac arrest, while a lower dose (10 μg/kg), which normally produced only a positive chronotropic response, also produced a small negative chronotropic response.

The negative chronotropic response to phenylephrine was antagonized by hexamethonium (20 mg/kg) or tetrodotoxin (10 μg/kg) but not by

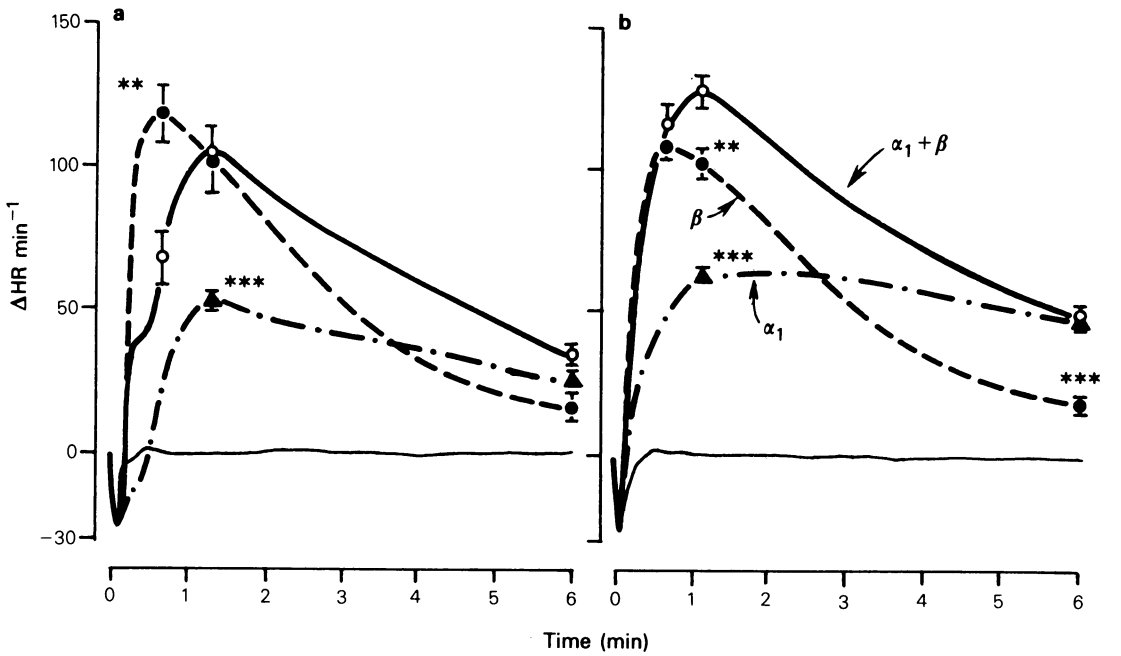

Figure 5 The effects of antagonists (all 1 mg/kg) on the time course of the chronotropic response to phenylephrine in (a) controls; (b) after pretreatment with atropine (1 mg/kg). Antagonists studied: (●) prazosin; (▲) propranolol; —— prazosin + propranolol; (○) control (a) or atropine (b). The greek letters in (b) refer to the adrenoceptors which mediate the effects of phenylephrine after each pretreatment (see text). The response following each pretreatment was compared with the corresponding control by Student's t test (only significant differences are indicated, $^{**}0.01>P>0.001$; $^{***}P<0.001$; $n=4$–6). The initial decrease in heart rate is due to the bolus injection of phenylephrine. Bars represent s.e.mean.

bilateral cervical vagotomy. This suggests that the response is initiated at the level of the parasympathetic ganglia.

α_1-Adrenergic involvement

The effects of selective adrenoceptor antagonists on the time courses of the positive and negative chronotropic responses to phenylephrine (100 μg/kg) were studied.

After propranolol (1 mg/kg), phenylephrine produced an initial decrease in heart rate followed by a rise which was smaller than in controls. Both components of this residual response were abolished by prazosin (1 mg/kg, Figure 5a). Thus, the biphasic response remaining after propranolol represents the α_1-mediated chronotropic effects of phenylephrine. The time course was now similar to that after amidephrine (compare Figures 5a and 4b).

In the absence of propranolol, prazosin (1 mg/kg) abolished the negative chronotropic response and modified the positive chronotropic response: the peak occured earlier but was less persistent than in controls. This response was abolished by propranolol and thus represents the β-mediated, positive chronotropic effect. After prazosin (1 mg/kg), the height of the peak increase produced by phenylephrine was not significantly different from controls. However, if the responses are compared at the time of the peak response to phenylephrine (following prazosin (1 mg/kg)), a significant increase is observed ($0.01 > P > 0.001$, Figure 5a). Like prazosin, corynanthine (10 mg/kg) or WB4101 (1 mg/kg) abolished the negative chronotropic response to phenylephrine or amidephrine and modified the rise in heart rate (to phenylephrine) in a qualitatively similar manner. This suggests the involvement of α_1-adrenoceptors in the negative chronotropic responses to phenylephrine and amidephrine.

In the presence of atropine (1 mg/kg), the effects of the adrenoceptor antagonists could be examined in the absence of the negative chronotropic response (Figure 5b). In these circumstances, prazosin did not affect the initial rate of rise in heart rate to phenylephrine. The response following atropine (1 mg/kg) plus prazosin (1 mg/kg) was similar to that after prazosin (1 mg/kg) alone. This suggests that prazosin and atropine block the same process in the negative chronotropic effect of phenylephrine, and would accord with the involvement of α_1- and muscarinic receptors at successive stages. Since prazosin did not block the negative chronotropic effect of stimulation of the vagus (see below), this implies that α_1-adrenoceptor activation occurs at or before the stage of muscarinic activation.

Comparison of α- and β-mediated positive chronotropic effects

The α- and β-mediated positive chronotropic effects of phenylephrine can be analysed only when the negative chronotropic response has been removed e.g. after atropine. Compared with controls, after atropine (1 mg/kg), there is a significant increase in the peak, positive chronotropic response to phenylephrine given alone ($P < 0.05$), or after propranolol (1 mg/kg, $P < 0.05$), but not after prazosin (1 mg/kg) (cf. Figures 5a and b).

In the presence of atropine (1 mg/kg), propranolol (1 mg/kg) significantly decreases the response to

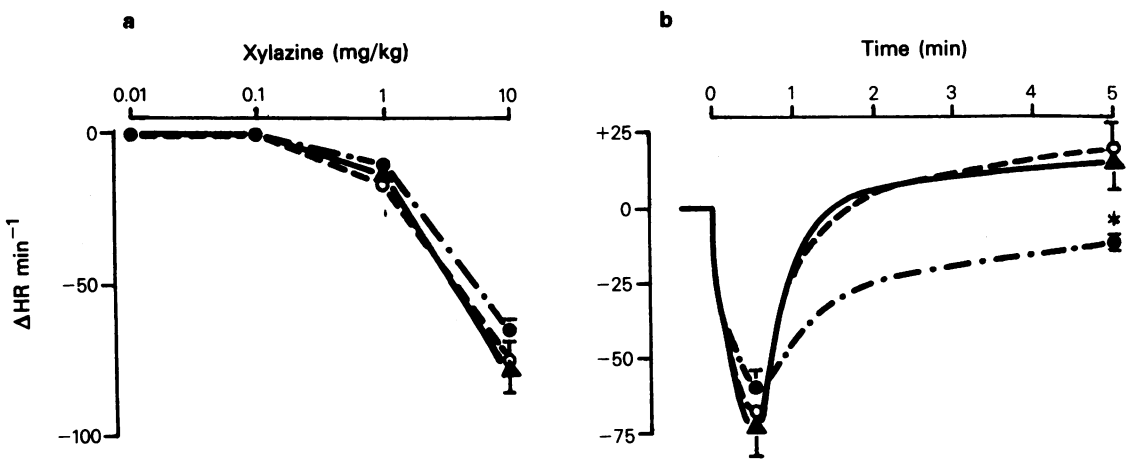

Figure 6 Effects of antagonists on (a) the dose-response curve for the peak negative chronotropic response to xylazine and (b) the time course of the response to xylazine (10 mg/kg). (▲) Control; (●) prazosin (1 mg/kg); (○) rauwolscine (1 mg/kg). The response following each pretreatment was compared with the corresponding drug-free control by Student's *t* test (only significant differences are indicated $*0.05 > P > 0.01$). Bars represent s.e.mean.

phenylephrine ($P<0.001$); the residual response is abolished by prazosin (1 mg/kg) and is thus the α_1-mediated response: prazosin (1 mg/kg), however, had no effect on the initial rising phase of the response but reduced the duration of the rise e.g. at all times after 30 s the response was smaller. This remaining response was abolished by propranolol and is, thus, the β-mediated component (Figure 5b).

The α_1-mediated component is a slower yet more persistent response than that mediated via β-adrenoceptors. Thus, the effect of phenylephrine is initially mainly on β-but changes to be predominantly on α_1-receptors as it continues.

Xylazine Xylazine was chosen as a relatively 'selective' α_2-agonist to test whether α_2-adrenoceptors could initiate either positive or negative chronotropic effects.

Low doses (1–100 μg/kg), which stimulate pre- and post-junctional α_2-adrenoceptors in the pithed rat (Docherty & McGrath, 1980a), produced no effect on heart rate. At higher doses, xylazine (1 and 10 mg/kg) produced an initial fall in heart rate followed by a small rise above baseline.

The negative chronotropic response was resistant to antagonism by prazosin (1 mg/kg), rauwolscine (1 mg/kg) (Figure 6), or atropine (1 mg/kg) and was not potentiated by neostigmine (25 μg/kg). It did not, therefore, resemble the response to α_1-agonists and apparently involved neither α_1-, α_2-, nor muscarinic receptors.

The small positive response was blocked by prazosin (1 mg/kg, $P<0.05$), but not by rauwolscine (1 mg/kg), uncovering a more prolonged negative chronotropic response (Figure 6b). This suggests that the negative chronotropic effect of xylazine is normally offset by a small prazosin-sensitive, α_1-adrenoceptor-mediated, positive chronotropic effect. 'Selectivity' of agonists is relative. In high concentrations, xylazine and other α_2-agonists can activate α_1-adrenoceptors in many other preparations *in vivo* and *in vitro* (Docherty & Starke, 1981; authors, unpublished observations).

Effect of antagonists on the negative chronotropic response to vagal stimulation

Vagal stimulation evoked a negative chronotropic response of $44.2 \pm 4.9\ \text{min}^{-1}$ ($\bar{x} \pm$ s.e.mean, $n = 15$). This response was abolished by atropine (1 mg/kg) or tetrodotoxin (10 μg/kg). It was significantly decreased to $23.8 \pm 3.6\%$ ($n = 3$) of control by hexamethonium (20 mg/kg) but was unaffected by prazosin (1 mg/kg). Neostigmine (25 μg/kg) potentiated the response to vagal stimulation by a factor of 2.2 ± 0.2 ($n = 5$).

Discussion

These results demonstrate that activation of α_1-adrenoceptors can produce two distinct and physiologically opposing effects on heart rate.

One of these, an increase in heart rate, is presumably exerted directly on the cardiac cells in the pacemaker region and is in addition to the well-established β-adrenoceptor-mediated, positive chronotropic response.

The other, a negative chronotropic effect, was unexpected. This expressed itself as a rapid fall in heart rate on injection and a more prolonged attenuation of any positive chronotropic response being produced concurrently. On pharmacological analysis this negative effect was revealed as an indirect effect involving parasympathetic activation. These two opposite effects seem to arise from receptors which are identical to each other and to α_1-adrenoceptors in other preparations; in respect of both agonist and antagonist potency (Docherty & McGrath, 1980a; Flavahan & McGrath, 1981a).

The absence of an α_2-adrenoceptor-mediated, chronotropic effect of xylazine suggests that there are no functionally important, postjunctional, chronotropic α_2-adrenoceptors in the rat heart. Xylazine shares with other α_2-agonists a direct negative chronotropic effect independent of α-adrenoceptors (J.R. Docherty, personal communication; Kobinger, Lillie & Pichler, 1979).

The pharmacological separation of the two α_1-mediated effects was possible because the negative chronotropic effect could be blocked by atropine. In the presence of atropine, it was possible to isolate the α_1-adrenoceptor-mediated, positive chronotropic response of α_1-agonists and to compare this with the β-mediated response. With amidephrine, the response after atropine was abolished by prazosin and can be considered as purely α_1. In the case of phenylephrine it was, in addition, necessary to block its β-adrenoceptor-mediated effect. Once this was accomplished, the remaining prazosin-sensitive, α_1-adrenoceptor mediated, positive chronotropic response to phenylephrine was similar in size to, but of shorter duration than, that to equal doses of amidephrine. For comparison, a 'purely β' positive chronotropic effect could be produced by isoprenaline or. after prazosin, by phenylephrine.

The maximal increase in heart rate which could be established via α_1-adrenoceptors was smaller than that via β-adrenoceptors. This suggests a different mechanism engendered by each receptor at the subcellular level, as has been suggested for inotropic effects (Osnes, Refsum, Skomedal & Oye, 1978; Wagner & Schumann, 1979), or that different pacemaker cells possess different populations of receptors. It also appears from the responses to

phenylephrine that the β-adrenoceptor-mediated rise in heart rate is more rapid but less persistent than the α_1-mediated response which, again, might reflect different mechanisms. However, this may arise from the lack of equilibration in the initial stages of the response to a bolus injection. The dose-response relationship in Figure 2 indicates that the β-adrenoceptor effect of phenylephrine has a higher threshold than has the α_1-adrenoceptor effect. Thus the initial effect of the bolus will be mainly β while α_1 will dominate as the concentration at the effector cells falls. A similar phenomenon occurs with pre- and post-junctional α_1 and α_2-adrenoceptors in other organs in the pithed rat (Docherty & McGrath 1979).

Earlier *in vitro* studies on isolated, spontaneously beating, rat atria failed to demonstrate an α_1-adrenoceptor-mediated chronotropic response to phenylephrine under 'normal' physiological conditions (Weston, 1971; Nakashima & Hagino, 1972; Kunos, 1977; Wagner & Brodde, 1978; Bennett & Kemp, 1978; Simpson & McNeill, 1980a). This response may, however, have been obscured by the β-adrenoceptor-mediated, positive chronotropic and α_1-adrenoceptor-mediated, negative chronotropic effects of phenylephrine.

We have shown that the α_1-adrenoceptor-mediated, positive chronotropic response is clear if the β-adrenoceptors are blocked. Indeed, a propranolol-resistant, positive chronotropic response to phenylephrine has been demonstrated *in vitro* (Bennett & Kemp, 1978, Simpson & McNeill, 1980a) and was antagonized by phenoxybenzamine (Simpson & McNeill, 1980a). However, in the absence of propranolol, α-antagonists failed to shift the concentration-response curve for the positive chronotropic response to phenylephrine (Weston, 1971; Nakashima & Hagino, 1972; Kunos, 1977; Bennett & Kemp, 1978). This accords with the present study which shows that α-antagonists inhibit the responses only to low doses of phenylephrine and that no change is seen at the ED_{50} level (Figure 2a, b). This is due to the β-adrenoceptor antagonist effect of phenylephrine and its α_1-adrenoceptor-mediated, negative chronotropic effect, each of which counteracts the action of α-antagonists. In fact, the ability to demonstrate α_1-mediated, positive chronotropic responses in hypothyroidism (Nakashima & Hagino, 1972) may be the result of a reduction in either of these latter effects.

Methoxamine failed to evoke a positive chronotropic response *in vitro* (Simpson & McNeill, 1980a) which tended to support a lack of functional, post-junctional α_1-adrenoceptors. However, methoxamine produces negative inotropic and chronotropic responses in the pithed rat, independent of α-adrenoceptors, and is, thus, unsuitable for studying α_1-adrenoceptor-mediated chronotropic effects (authors, unpublished observations).

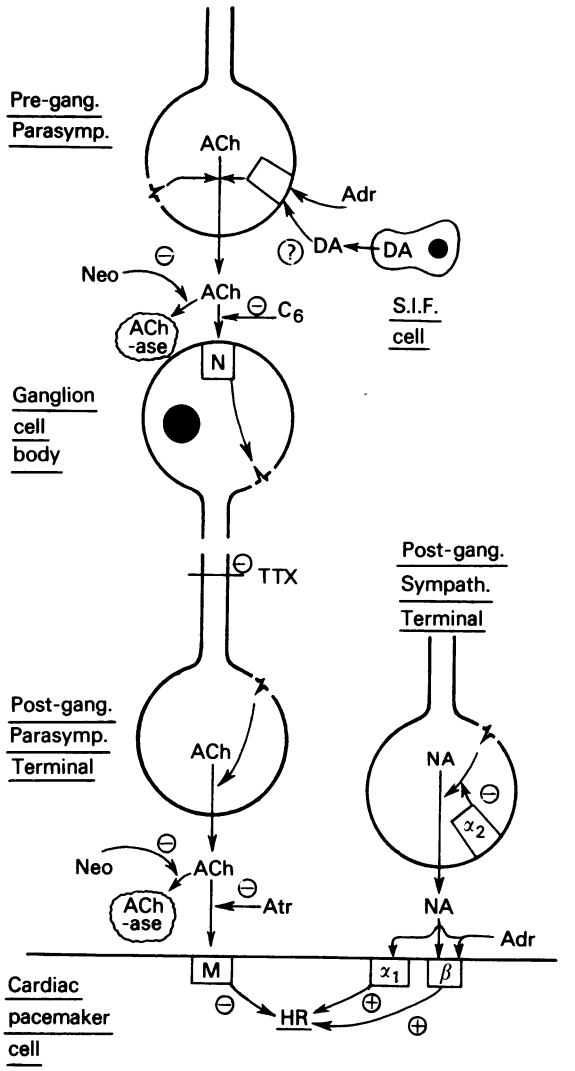

Figure 7 Summary of the sites of action of drugs used to locate the α_1-mediated effects on heart rate. ACh acetylcholine; ACh-ase, Acetylcholinesterase; Adr, adrenaline; Atr, atropine; DA, dopamine; NA, noradrenaline; Neo, neostigmine; TTX, tetrodotoxin; C_6, hexamethonium. Cholinoceptors: M, muscarinic; N, nicotinic.

Neither α- nor β-antagonism alone is as effective as is their combination in restraining the adrenergic influence on the heart rate. For example, labetalol was more effective than either prazosin or propranolol (see also Simpson & McNeill, 1981b).

Is this α_1-adrenoceptor-mediated increase in heart rate likely to be of physiological significance and relevant to the action of 'adrenotrophic' drugs or is it simply a high-dose, pharmacological curiosity? The doses of α_1-adrenoceptor agonists required to initiate

the positive chronotropic response are the same as those necessary to elevate blood pressure. Moreover, part of the positive chronotropic response to cardioaccelerator nerve stimulation and to injected adrenaline is susceptible to prazosin (Flavahan & McGrath, 1981b, and unpublished observations). Thus, a physiological role for α_1-adrenoceptors in the positive chronotropic response to nerve-released or circulating catecholamines seems possible and, this being the case, 'adrenotrophic' drugs may interfere with this physiological process or themselves activate the receptors.

The quantitative contribution of α seems less than β since the maximum increase in heart rate is less and since only 20% of the response to cardioaccelerator nerve stimulation or to adrenaline is prazosin-sensitive (Flavahan & McGrath 1981b; unpublished observations). However, different physiological conditions may alter the relative contribution of each receptor subtype. For example, cholinergic nerve activation may attenuate α_1- and β-adrenoceptor-mediated, positive chronotropic responses to different degrees. α_1-Adrenoceptor-mediated inotropic responses are more resistant to cholinergic stimulation than are those mediated via β-adrenoceptors (Endoh & Motomura, 1979). Local metabolic conditions may also alter the contribution from each receptor as suggested for post-junctional vascular α_1- and α_2-adrenoceptors (Flavahan & McGrath, 1981c).

The negative effect of α_1-adrenoceptor agonists could be potentiated by neostigmine and blocked by atropine, hexamethonium or tetrodotoxin. This suggests that the response is mediated through activation of the parasympathetic ganglion cell body. This response was also interrupted by α_1-antagonists which did not attenuate ganglionic transmission. Thus, α_1-adrenoceptor stimulation must occur at a stage prior to nicotinic activation. We suggest that α_1-adrenoceptors are located on the parasympathetic, preganglionic nerves. Activation of these receptors causes the release of acetylcholine which then stimulates the ganglion cell body via nicotinic receptors culminating in a negative chronotropic response (Figure 7).

Prejunctional 'α_1-like' adrenoceptors that mediate the release of acetylcholine are found on somatic nerve endings (Malta, McPherson & Raper, 1979; Bowman, 1980). These two groups of α_1-adrenoceptors, located on both parasympathetic and somatic nerve terminals, may, therefore, have common properties in their mechanism of acetylcholine release.

Phenylephrine evokes an α-mediated, negative chronotropic response in canine, isolated, blood-perfused atria which is thought to be mediated via stimulation of parasympathetic nerve fibres (Chiba, 1977). Although a negative chronotropic effect has not been reported previously for rat hearts, α-antagonists increase the positive chronotropic response to phenylephrine in rat isolated atria (Bennett & Kemp, 1978; Simpson & McNeill, 1980a). Presumably this results from antagonism of the α_1-mediated, negative chronotropic response to phenylephrine.

These prejunctional, ganglionic α_1-adrenoceptors are distinct from prejunctional α_2-adrenoceptors whose activation results in a decrease in transmitter output (Gillespie, 1980). The physiological role, if any of these prejunctional α_1-adrenoceptors is unclear. Their anatomical location indicates the possibility of activation by circulating adrenaline or locally released dopamine from SIF (small intensely fluorescent) cells (Libet, 1970). The effect might be facilitation of parasympathetic activity.

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