

EFFECTS OF SYMPATHETIC INNERVATION AND TEMPERATURE ON THE PROPERTIES OF RAT HEART ADRENOCEPTORS

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1 The pharmacological characteristics of adrenoceptors at different temperatures were assessed on the basis of the effects of various α - and β -adrenoceptor agonists and antagonists on electrically-driven left atria and spontaneously-beating pairs of atria from rats.

2 Phenoxybenzamine (Pbz) potentiated inotropic responses of left atria to noradrenaline (NA) at 31°C, produced significantly less potentiation at 24°C and inhibited responses at 17°C; it had little effect on responses to CaCl₂. Both Pbz and phentolamine inhibited responses to phenylephrine more effectively at 17 than at 31°C. *N*-cyclohexylmethyl-*N*-ethyl- β -chloroethylamine hydrochloride (GD-131), a haloalkylamine with negligible α -adrenoceptor blocking activity, caused only potentiation of responses to NA at 17°C.

3 The presence of phentolamine during incubation with Pbz eliminated block of responses to NA and revealed a potentiation that was equivalent at all three temperatures tested. Phentolamine did not alter the block of responses to 5-hydroxytryptamine by Pbz. Protection of α -adrenoceptors by phentolamine during exposure to [³H]-Pbz significantly decreased the amount of label bound to the myocardium at 17°C, but did not alter binding at 31°C.

4 Inhibition of responses to NA by propranolol decreased with temperature, and the magnitude of the change increased with the concentration of propranolol. Compared to 31°C, the effect of the highest concentration of propranolol (4.0 μ M) was significantly decreased at 24°C, and the effects of all except the lowest concentration (0.04 μ M) were significantly decreased at 17°C.

5 The potency of isoprenaline decreased and that of phenylephrine increased at low temperatures, and their potency ratio was much lower at 17 than at 31°C for both the inotropic and chronotropic responses of spontaneously-beating atria. However, the ratio was unaffected by temperature in electrically-driven left atria. A similar difference between spontaneously-beating and driven preparations is apparent in the data of other workers, but its basis is not clear.

6 Atria from rats pretreated with 6-hydroxydopamine (6-OHDA) were sensitized to the effects of NA, and there was no increase in α -adrenoceptor properties at low temperatures. Little α -adrenoceptor activity could be demonstrated in chemically denervated atria at any temperature. 6-OHDA pretreatment did not alter the binding of [³H]-Pbz at 31°C, but decreased it significantly at 17°C. Pretreatment with reserpine caused some sensitization, but did not significantly alter the characteristics of the adrenoceptors or their responses to temperature.

7 It is concluded that the adrenoceptors of rat atria are affected by temperature in much the same way as those of frog hearts, although the transition from β - to α -adrenoceptor properties may begin at a slightly higher temperature. α -Adrenoceptor properties appear to require the presence of intact adrenergic nerves but not the presence of normal stores of neurotransmitter.

Introduction

Several studies have shown that the adrenoceptors mediating inotropic and chronotropic responses of frog hearts are β at higher and have a major α -component at lower temperatures (Kunos & Szentiványi, 1968; Buckley & Jordan, 1970; Kunos, Yong & Nickerson, 1973b, Harri, 1973; Tirri, Harri & Laitinen, 1974). The observations that reciprocal changes in the blocking effectiveness and the tissue

retention of α - and β -adrenoceptor antagonists occurred sharply between 17 and 23°C, and that alkylation of α -adrenoceptors by phenoxybenzamine at a low temperature prevented the appearance of β -adrenoceptors when the temperature was raised were interpreted to indicate that α - and β -adrenoceptors may be interconvertible forms of a single basic structure (Kunos & Nickerson, 1976). Recent studies

have suggested that adrenoceptors of the mammalian myocardium are similarly affected by temperature (Kunos, Vermes-Kunos, Boyd & Nickerson, 1973a; Benfey, Kunos & Nickerson, 1974; Amer & Byrne, 1975). The present observations on rat atria confirm the shift from β - towards α -adrenoceptor properties at low temperatures, and indicate that the α -adrenoceptor component in inotropic responses requires intact sympathetic innervation. An abstract of some of these findings has been published (Kunos *et al.*, 1973a).

Methods

Preparation of isolated atria and experimental design

Male Sprague-Dawley rats (180–300 g) were lightly anaesthetized with ether and the hearts quickly removed. Left atria or spontaneously-beating pairs of atria were isolated and mounted in 20 ml organ baths containing modified Krebs-Henseleit solution of the following composition (mM): NaCl 115.3, KCl 4.6, CaCl₂ 1.8, MgSO₄ 1.1, NaHCO₃ 22.1, KH₂PO₄ 1.1 and glucose 11.1, (pH 7.43) bubbled with 5% CO₂ and 95% O₂. The temperature was controlled by a refrigerated, constant temperature circulating water bath (Lauda, Model K-2-R). Left atria were driven through small platinum electrodes by square-wave pulses of 3 ms duration at a voltage slightly above threshold (0.3–1.5 V) and a rate of 1 Hz (Grass stimulator, Model S88). Isometric contractions were recorded by force-displacement transducers (Grass, FT 83C) and a polygraph (Grass, Model 7). Rates of contraction were determined directly from the polygraph records.

The preparations were allowed to equilibrate for at least an hour at 31°C with frequent changes of the medium and the temperature was then set at 31, 24 or 17°C. Each preparation was used at only one temperature. Agonists were added cumulatively and the maximal response was determined in every experiment. The basal contractile amplitude often decreased significantly after the first concentration-response curve was determined. In such cases, a second concentration-response curve to the same agonist was obtained and used as control. All responses are expressed as a percentage of the maximal control increase in force of contraction or rate. Absolute values for force of contraction are also indicated for most series of experiments. The basal contractile amplitude increased and the absolute change in force in response to inotropic drugs decreased with decreasing temperature. Some preparations were insensitive to all agonists at 17°C, and remained insensitive despite extensive washing and repeated challenges with agonist. These are not included in the calculations of mean responses.

Atria treated with phenoxybenzamine (Pbz) or [³H]-phenoxybenzamine ([³H]-Pbz) were exposed to the drug (0.7–7.3 μ M) freshly diluted every 10 min from a stock solution in propylene glycol containing 0.01N HCl. After a 40 min exposure, the atria were washed with drug-free medium for 60–120 min and responses to the agonist retested. Preparations exposed to [³H]-Pbz were then removed for counting retained radioactivity. Exposure to propranolol was for 40 min and responses to noradrenaline (NA) were retested in the presence of the antagonist. Atria were first exposed to a concentration of 0.04 μ M propranolol. After retesting with NA and washout of the drugs, the same preparations were exposed to concentrations of 0.4 and 4.0 μ M propranolol in sequence, and responses to NA were retested at the end of each 40 min incubation period. The effects of phentolamine (2.6 μ M) on inotropic responses to phenylephrine were also tested after a 40 min exposure with the antagonist still in the bath.

Receptor protection experiments

Left atria were exposed to phentolamine (26.5 μ M) at 17, 24 or 31°C. After 10 min, [³H]-Pbz (7.3 μ M) was added to the medium, which was replaced every 10 min by fresh solution containing both drugs. After a 40 min exposure to both drugs, the preparations were washed for at least an hour to remove phentolamine and all unreacted [³H]-Pbz. Responses to NA were then retested and the preparations removed for counting radioactivity.

Pretreatment with 6-hydroxydopamine (6-OHDA) and reserpine

Rats with an initial body weight of 80–100 g were pretreated with 6-OHDA according to the schedule proposed by Thoenen & Tranzer (1968). Two doses of 50 mg/kg were given intravenously within 24 h, followed a week later by 2 \times 100 mg/kg. Experiments on isolated left atria were performed one week after the last dose of 6-OHDA. This dose schedule has been shown to reduce the NA content of rat hearts by more than 90%, and the resulting supersensitivity is qualitatively the same as that after other forms of sympathetic denervation (Haeusler, Haefely & Thoenen, 1969).

To deplete NA stores without destruction of sympathetic nerve endings, rats were pretreated with reserpine. Two treatment schedules were used: a single dose of 5.0 mg/kg given intraperitoneally 24 h before the experiment, or 0.5 mg/kg given daily for 7 days. Results with atria from rats on the two treatment schedules were almost identical and were pooled.

Determination of retained radioactivity

Atria exposed to [³H]-Pbz were washed for 2 h,

including the test period with an agonist, before freeze-drying. The dry tissue was weighed and digested in 0.5 ml of NCS tissue solubilizer (Amersham/Searle) at 50°C; 14.5 ml of counting solution containing 4.0 g of 2,5-diphenyloxazole (PPO) and 0.05 g of 2-*p*-phenylene-bis(5-phenyloxazole) (POPOP) in 1.0 litre of reagent grade toluene was added. Radioactivity was measured in a liquid scintillation counter (Nuclear Chicago, Mark I), using the channels ratio technique and a quench calibration curve. Counting efficiency was in the range of 20–32%. Radioactivity is expressed as concentration of labelled drug in the dry tissue.

Drugs

[³H]-phenoxybenzamine hydrochloride was prepared by the method of Nikawitz, Gump, Kerwin & Ulliyot (1952), as detailed elsewhere (Yong & Nickerson, 1973). Other drugs used were phenoxybenzamine hydrochloride (Smith Kline & French), (–)-noradrenaline ((–)-arterenol bitartrate, K & K), (–)-phenylephrine hydrochloride (K & K), isoprenaline ((±)-isopropylnoradrenaline hydrochloride, K & K), phentolamine (Rogetine methanesulphonate, Ciba), (±)-propranolol (Inderal, Ayerst), 5-hydroxytryptamine (5-HT, serotonin creatinine sulphate hydrate, Calbiochem), 6-hydroxydopamine hydrobromide (Regis), reserpine (Serpasil, Ciba), and GD-131 (*N*-cyclohexylmethyl - *N* - ethyl - β - chloroethylamine hydrochloride, synthesized by Dr M.S. Yong). Most of the drugs were freshly diluted before each experiment in 0.9% w/v NaCl solution (saline) containing 0.01 N HCl. Concentrations are expressed as M in the bath.

Statistics

Differences between means were evaluated by Student's two-tailed *t* test for unpaired data or the *t* test for paired data, as appropriate, and differences with a *P* value of 0.05 or less were considered significant. Mean values are presented with their standard errors.

Results

Effects of α-adrenoceptor antagonists on inotropic responses to noradrenaline and phenylephrine at different temperatures

At all temperatures tested (17, 24 and 31°C), the baseline contractile amplitudes of electrically-driven left atria were transiently increased by Pbz (0.7–7.3 μM), but declined during the exposure period and were equal to or slightly less than the control values after washout of the drug. Pbz (7.3 μM)

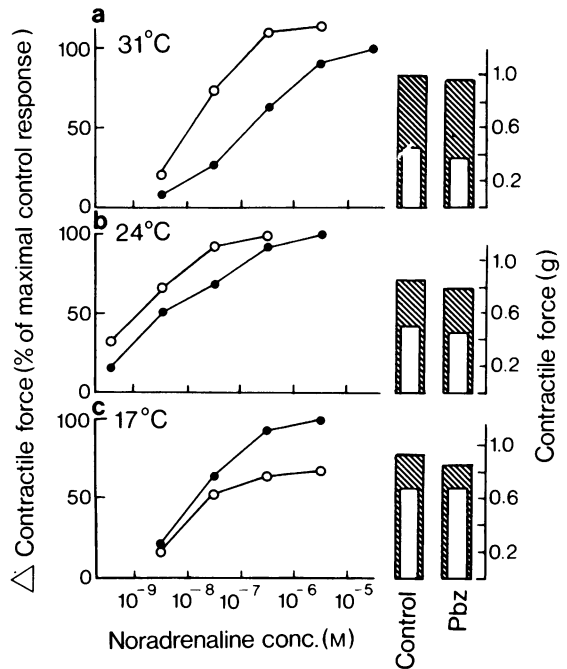


Figure 1 Effects of phenoxybenzamine (Pbz) on inotropic responses of rat left atria to noradrenaline (NA) at different temperatures. Control concentration-response curves (●); after 40 min exposure to Pbz (7.3 μM) (○). Open columns show basal contractile force and hatched columns the maximal force in response to NA. Number of experiments (a) 14 (31°C); (b) 8 (24°C); (c) 15 (17°C).

potentiated inotropic responses to noradrenaline (NA) at 31°C; concentration-response curves were shifted to the left (log dose-ratio -0.99 ± 0.12), but the maximal developed tension was unaltered (Figure 1a). A similar exposure to Pbz at 17°C inhibited responses to NA; the change in the mean EC_{50} was not statistically significant, but the maximal response was significantly decreased ($P < 0.02$) (Figure 1c). A concentration of 0.7 μM Pbz had similar but smaller effects. Although the effect of Pbz at 24°C was similar to that at 31°C, the shift to the left of the NA concentration-response curve was significantly smaller (log dose-ratio -0.53 ± 0.10 , $P < 0.02$) and the maximal developed tension was slightly reduced (Figure 1b). Inotropic responses to phenylephrine were inhibited by Pbz at both 17 and 31°C, but the block was significantly greater at the low than at the high temperature (Figure 2a & c). A similar temperature-dependent change in the blocking effect of phentolamine (2.6 μM) was also noted (Figure 2b & d). The basal amplitude was not significantly altered by phentolamine at either temperature but, unex-

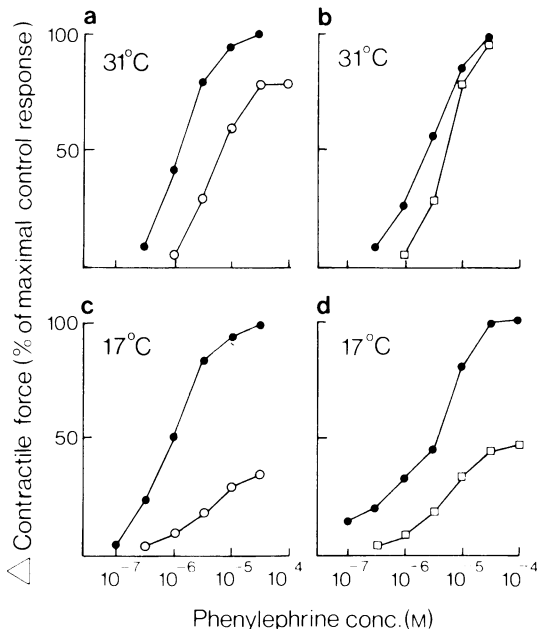


Figure 2 Inhibition of inotropic responses to phenylephrine by phenoxybenzamine (Pbz) in (a) and (c) and by phentolamine in (b) and (d) at 17 and 31°C. Control concentration-response curves (●); after exposure to Pbz (7.3 μM) (○); in the presence of phentolamine (2.6 μM) (□). Number of experiments: (a) 6; (b) 4; (c) 5; (d) 4.

pectedly, the maximal response to phenylephrine at 17°C was significantly decreased.

As observed in frog hearts, the Pbz blockade at 17°C developed slowly during the washout period and

the first NA concentration-response curve often showed some potentiation. Blockade then developed gradually and was stable after 1 hour. Figure 3 shows that almost complete block of the inotropic response of a left atrial preparation to NA at 17°C was associated with only a slight decrease in the contractile response to CaCl₂. In three experiments at 17°C, atria were exposed for 40 min to 10.4 μM GD-131, a haloalkylamine with negligible α-adrenoceptor blocking properties. This drug moderately potentiated inotropic responses to NA in all experiments.

Effects of phentolamine on block by and binding of [³H]-phenoxybenzamine in rat left atria

Exposure in the presence of phentolamine (26.5 μM) abolished the temperature dependence of the effects of Pbz (7.3 μM), and potentiation of response to NA at all three temperatures (Figure 4) was similar to that produced by Pbz alone at 31°C. This indicated that inhibition of responses to NA by Pbz at 17°C and the decrease in potentiation at 24°C were due to α-adrenoceptor blockade and that the potentiating effect of Pbz is largely independent of temperature. In three experiments at 17°C, Pbz (7.3 μM) almost completely blocked inotropic responses to 5-hydroxytryptamine (5-HT), and this effect was unaltered by exposure in the presence of phentolamine (26.5 μM) in three other preparations.

In contrast to frog hearts (Kunos & Nickerson, 1976), the radioactivity retained by rat atria exposed to [³H]-Pbz was not significantly changed by temperature, and was even slightly higher at 31°C than at the lower temperatures (Figure 5, open columns). However, protection against Pbz block by phentolamine at 17°C was associated with a

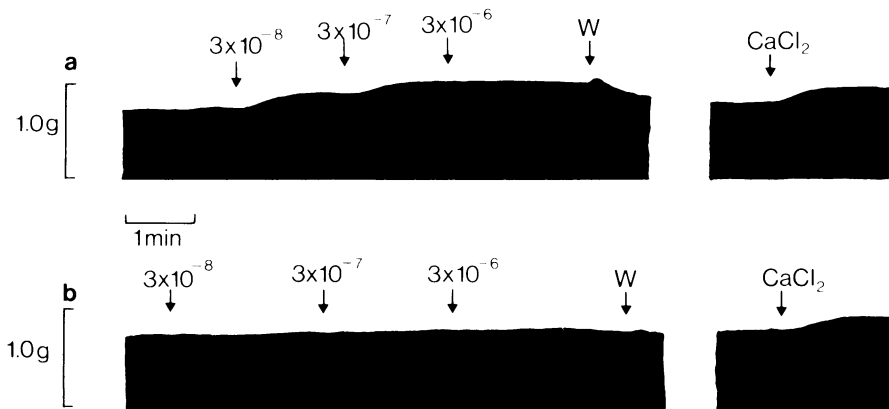


Figure 3 Block of inotropic responses to noradrenaline (NA) by [³H]-phenoxybenzamine (7.3 μM) without significant inhibition of the response to added CaCl₂ (2.25mM) in electrically-driven rat left atrium at 17°C. Stimulation: 1 Hz, 3 ms, 1.25 V. (a) Control; (b) 60 min after washout of phenoxybenzamine. Indicated concentrations of NA (M) administered at arrows. W indicates wash.

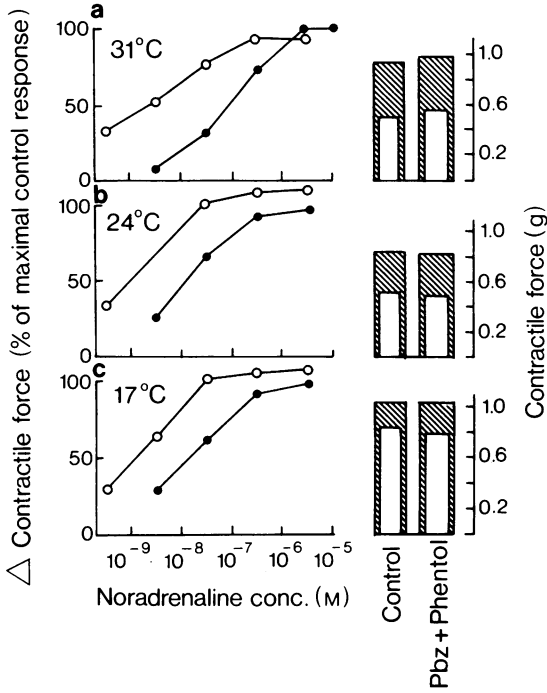


Figure 4 Effects of exposure to [³H]-phenoxybenzamine ([³H]-Pbz) in the presence of phentolamine on inotropic responses of rat left atria to noradrenaline (NA) at different temperatures. Control concentration-response curves (●); after exposure to [³H]-Pbz (7.3 μM) + phentolamine (26.5 μM) (○). Open columns show basal contractile force and hatched columns the maximal force in response to NA. Number of experiments: (a) 14 (31°C); (b) 8 (24°C); (c) 15 (17°C).

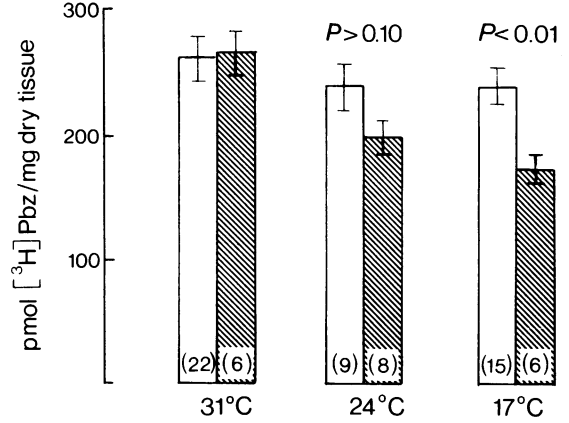


Figure 5 Retention of radioactivity by rat left atria after exposure to [³H]-phenoxybenzamine ([³H]-Pbz) (7.3 μM) alone (open columns) or in the presence of phentolamine (26.5 μM) (hatched columns). Numbers of experiments are shown in parentheses. Vertical lines show standard errors of means.

significant decrease in retained radioactivity, whereas at 31°C phentolamine did not alter the amount of label retained (hatched columns). At 24°C phentolamine caused an appreciable, but not statistically significant decrease in binding.

Effects of propranolol on inotropic responses of left atria to noradrenaline at different temperatures

The effects of propranolol on inotropic responses to NA at 17, 24 and 31°C are shown in Table 1. The inhibitory effects of all except the lowest concentration (0.04 μM) decreased significantly with decreasing temperature, and this change was apparent at 24 as well as at 17°C with the highest concentration of propranolol (4.0 μM).

Table 1 Effects of propranolol on inotropic responses of electrically-driven left atria to noradrenaline

Temp.	Propranolol concentration (μM)			
	0	0.04	0.4	4.0
	<i>pD</i> ₂ *	<i>Log dose-ratio</i> †		
31°C (8)	7.40 ± 0.16	0.76 ± 0.11	1.92 ± 0.25	2.99 ± 0.27
24°C (7)	7.83 ± 0.10	0.69 ± 0.06	1.54 ± 0.07	2.05 ± 0.09**
17°C (5)	7.66 ± 0.13	0.72 ± 0.14	1.27 ± 0.16*	1.82 ± 0.12**

* Means and standard errors of negative log of noradrenaline EC₅₀'s. † Means and standard errors of the log of the ratio of noradrenaline EC₅₀'s in the presence and in the absence of propranolol. Asterisks indicate significance of difference from corresponding value at 31°C (**P* < 0.05; ***P* < 0.01). Numbers in parentheses show number of experiments.

Sensitivity of atria to adrenoceptor agonists at different temperatures

The pD_2 values (negative logarithm of the molar EC_{50}) for the inotropic effects of isoprenaline and phenylephrine were determined from control concentration-response curves of spontaneously-beating pairs of atria and of electrically-driven left atria. pD_2 values for chronotropic responses were also determined in the former preparations. Sensitivity to isoprenaline was significantly lower and that to phenylephrine significantly higher at 17 than at 31°C for both the inotropic and chronotropic responses of spontaneously-beating preparations (Table 2). This resulted in a considerable decrease in the potency ratio of the two amines at the lower temperature. However, in electrically-driven left atria the inotropic potencies of both amines increased with decreasing temperature, and there was no change in the potency ratio.

Responses of left atria from rats pretreated with 6-hydroxydopamine

Haloalkylamines can inhibit the uptake of NA into adrenergic nerve endings (Iversen, Salt & Wilson, 1972), which could contribute to the potentiation of myocardial responses to NA by these drugs. The receptor protection experiments presented above showed that the potentiating effect of Pbz is present at all temperatures, but is masked by its α -adrenoceptor blocking action at 17°C. Therefore, destruction of sympathetic nerve endings by 6-OHDA might reduce

potentiation and reveal the blocking action of Pbz more clearly.

Pretreatment of rats with 6-OHDA significantly increased the sensitivity of left atria to NA. The mean EC_{50} was decreased more at 17 than at 31°C, 42- vs. 20-fold; responses to phenylephrine were potentiated much less. In contrast to control preparations, exposure to Pbz had no initial inotropic effect in atria from 6-OHDA pretreated rats, which probably reflected depletion of endogenous NA. Exposure to [3H]-Pbz (7.3 μM) at 31°C only slightly potentiated inotropic responses to NA and the somewhat greater maximal tension increase was probably related to the slightly lower baseline amplitude after Pbz (Figure 6a). Unexpectedly, Pbz did not significantly alter the NA concentration-response curve of atria from pretreated animals at 17°C. The blocking action observed in control preparations at this temperature was completely absent (Figure 6b). Pbz blockade of responses to phenylephrine at both temperatures (see Figure 2) was also nearly abolished by 6-OHDA pretreatment.

The amount of radioactivity retained after exposure to [3H]-Pbz (7.3 μM) at 31°C was almost identical in atria from 6-OHDA pretreated and control animals (Figure 7). However, the absence of α -adrenoceptor blockade at 17°C in the atria from 6-OHDA pretreated rats was associated with a significantly lower retention of label than that of control atria exposed at the same temperature. This was analogous to the reduction in binding observed when α -adrenoceptors were protected from [3H]-Pbz block by

Table 2 Sensitivity of spontaneously-beating pairs and electrically-driven left atria to isoprenaline and phenylephrine at different temperatures

	Temp.	Isoprenaline	Phenylephrine	Log potency-ratio
Left atria (inotropic)	31°C	9.29 ± 0.12 ^a (34) **	5.50 ± 0.06 (24) *	3.79
	17°C	9.78 ± 0.11 (12)	5.99 ± 0.23 (10)	3.79
(a) Inotropic	31°C	9.69 ± 0.22 (6) *	5.52 ± 0.10 (5) **	4.17
	17°C	8.80 ± 0.28 (9)	5.94 ± 0.09 (10)	2.86
(b) Chronotropic	31°C	9.49 ± 0.21 (11) **	5.64 ± 0.09 (16)	3.85
	17°C	8.55 ± 0.24 (10)	5.87 ± 0.10 (10)	2.73

^a Means and standard errors of pD_2 's.

Asterisks indicate significance of difference between values at the two temperatures (* $P < 0.05$; ** $P < 0.01$). Numbers in parentheses show numbers of experiments.

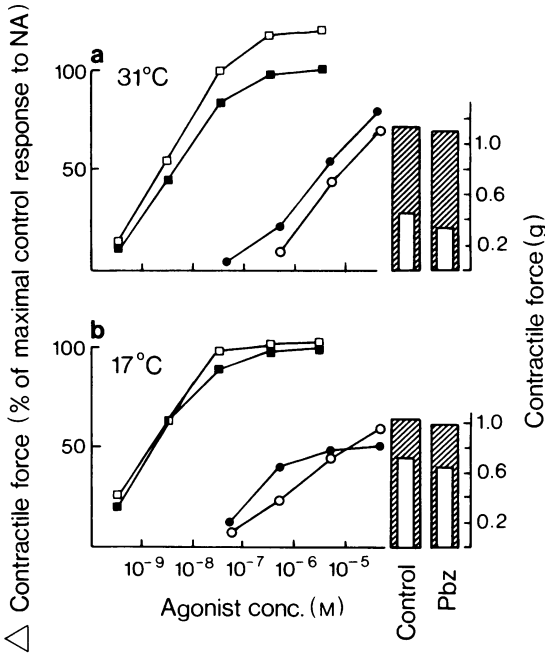


Figure 6 Effects of [³H]-phenoxybenzamine ([³H]-Pbz) on inotropic responses of left atria from rats pretreated with 6-hydroxydopamine to noradrenaline (NA) and phenylephrine at 17 and 31°C. Control concentration-response curves for NA (■) and for phenylephrine (●); after [³H]-Pbz (7.3 μM), NA (□) and phenylephrine (○). Open columns show basal contractile force and hatched columns the maximal force in response to NA. Number of experiments: (a) 10 (31°C); (b) 9 (17°C).

phentolamine (see Figure 5).

Pretreatment with 6-OHDA also abolished the decrease in the effect of propranolol noted when the temperature of control preparations was reduced. Cumulative doses of propranolol caused slightly greater shifts to the right of the NA concentration-response curves of 6-OHDA pretreated (Table 3) than those of control preparations (Table 1) at 31°C. However, the propranolol block was at least as great

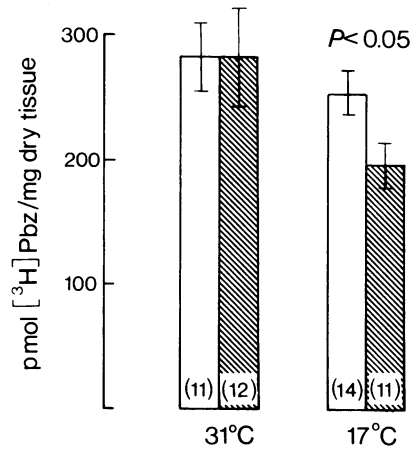


Figure 7 Retention of radioactivity by left atria from control (open columns) and 6-hydroxydopamine pretreated (hatched columns) rats after exposure to [³H]-phenoxybenzamine ([³H]-Pbz) (7.3 μM). Numbers of experiments are shown in parentheses. Vertical lines show standard errors of means.

at 17 as at 31°C, significantly greater for 0.4 μM, and was clearly greater for all concentrations than that produced in control atria at 17°C. Thus, the inotropic effect of NA in atria from 6-OHDA pretreated rats appeared to be mediated only by β-adrenoceptors at both test temperatures.

Responses of left atria from rats pretreated with reserpine

The results with atria from 6-OHDA pretreated rats indicated that adrenergic innervation may be necessary for the shift from β- to α-adrenoceptor properties in rat atria at low temperature. This effect of innervation might be due to the neurotransmitter or to some other influence of the nerves. A tentative differentiation of these possibilities was sought by testing atria from rats pretreated with reserpine to deplete endogenous NA. Reserpine pretreatment moderately increased sensitivity of left atria to both

Table 3 Effects of propranolol on inotropic responses to noradrenaline in electrically-driven left atria from rats pretreated with 6-hydroxydopamine

Temp.	Propranolol concentration (μM)			
	0	0.04	0.4	4.0
31°C	8.76 ± 0.26	1.07 ± 0.24	2.08 ± 0.18	—
17°C	9.51 ± 0.24	1.65 ± 0.33	2.97 ± 0.26*	3.83 ± 0.30

* Significantly different (P < 0.05) from ratio at 31°C. Four experiments at each temperature.

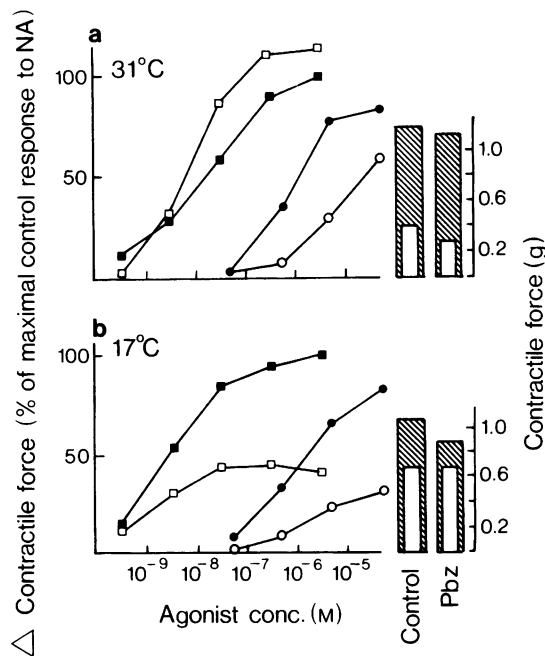


Figure 8 Effects of phenoxybenzamine (Pbz) on inotropic responses of left atria from rats pretreated with reserpine to noradrenaline (NA) and phenylephrine at 17 and 31°C. Control concentration-response curves for NA (■) and for phenylephrine (●), after Pbz (7.3 μM), NA (□) and phenylephrine (○). Open columns show basal contractile force and hatched columns the maximal force in response to NA. Number of experiments: (a) 5 (31°C); (b) 13 (17°C).

NA and phenylephrine, but did not alter the temperature-dependence of Pbz blockade (Figure 8). Pbz (7.3 μM) potentiated the inotropic response to NA (log dose-ratio -0.45 ± 0.18) and partially blocked that to phenylephrine at 31°C. The same exposure to Pbz at 17°C inhibited responses to NA, and blocked responses to phenylephrine more effectively than at the higher temperature. At both temperatures, the block produced by Pbz was more pronounced, and the potentiation at 31°C was significantly smaller than in atria from control animals (see Figures 1 and 2). These results indicate that the α -adrenoceptor component of the inotropic response to sympathomimetic amines at 31°C and the increase in this component when the temperature is reduced to 17°C are not abolished and tend to be enhanced by depletion of endogenous NA.

Discussion

The experimental results presented above are in agreement with and extend previous observations made on frog isolated hearts (Kunos & Szentiványi, 1968; Buckley & Jordan, 1970; Kunos *et al.*, 1973b; Harri, 1973; Kunos & Nickerson, 1976). The observed changes in the effectiveness of drugs acting on α - and β -adrenoceptors can be interpreted to indicate an interconversion of β - to α -adrenoceptors as the temperature of a mammalian heart preparation was lowered. In both frog and rat preparations the greatest change in adrenoceptor properties occurred below 24°C, but the upper limit of receptor transition appears to be somewhat higher in the mammalian heart. In the present experiments on rat atria there was an increase in the effect of α - and a decrease in the effect of β -adrenoceptor antagonists when the temperature was reduced from 31 to 24°C.

Pbz is known to react with nonspecific sites (Harvey & Nickerson, 1954) as well as with several receptors, and the change in its effectiveness with temperature has been attributed to the former (Benfey, 1975). However, several observations strongly support the interpretation that inhibition of inotropic responses to sympathomimetics by Pbz at low temperatures is due to specific block of α -adrenoceptors: (1) The change in the effectiveness of Pbz with temperature is in the wrong direction to be due to nonspecific alkylation of tissue constituents. Formation of the intermediate aziridinium ion and, particularly, its subsequent, rate-limiting reaction with nucleophilic groups increases rapidly with temperature (Harvey & Nickerson, 1953; Rosen & Ehrenpreis, 1972). As expected from this change in chemical reactivity, we have observed that the nonspecific binding of [³H]-Pbz to frog heart homogenates increases progressively with incubation temperature, more than 10-fold over the range of 4 to 24°C (Kunos & Nickerson, unpublished results). In contrast, the block of inotropic responses of rat atria was greater at low temperatures. (2) The change in block with temperature was essentially the same for the competitive antagonist phentolamine as it was for Pbz. (3) The presence of phentolamine during incubation with Pbz at 17°C prevented block of responses to both NA and phenylephrine, but did not alter the potentiation of responses to NA at 31°C. Prevention of the block by phentolamine strongly supports the involvement of specific α -adrenoceptors because the same concentration of this competitive α -adrenoceptor antagonist had no effect on the block of responses to 5-HT by Pbz. (4) GD-131, a haloalkylamine that has been shown to lack significant α -adrenoceptor blocking activity in other preparations (Nickerson & Gump, 1949; Kalsner & Nickerson, 1969), but which has a nonspecific reactivity comparable to Pbz (Harvey & Nickerson, 1954),

potentiated rather than blocked responses to NA at 17°C. (5) Pbz effectively inhibited responses to NA at 17°C but had a minimal effect on those to CaCl₂. This indicated that the block was not due to the inhibition of calcium fluxes that can be produced by high concentrations of a haloalkylamine (see Triggle, 1972). (6) Block by Pbz was largely eliminated by pretreatment with 6-OHDA. It is difficult to see how exposure to this drug one and two weeks prior to the experiment could alter nonspecific alkylation of the myocardium.

Observations on the tissue retention of [³H]-Pbz also indicate an increase in the number of α -adrenoceptors at low temperatures. In contrast to our previous observations on frog hearts (Kunos & Nickerson, 1976), the total retention of label was not increased at low temperatures. However, effective protection of α -adrenoceptors by phentolamine significantly reduced the binding of [³H]-Pbz at 17°C, whereas phentolamine had little effect on either binding of or potentiation by [³H]-Pbz at 31°C. This indicated that, as in the frog heart, the binding of Pbz to α -adrenoceptors was increased at the low temperature to an identifiable fraction of the label. It is probable that failure to demonstrate a significant difference in the total radioactivity retained by rat atria at 31 and 17°C was due to greater nonspecific binding at the higher temperature. The reason for this difference in nonspecific binding in the two preparations is not clear, but it could be related to the fact that the experimental conditions (route of access of drugs, temperature) were more physiological for frog hearts than for rat atria. The presence of cut surfaces in rat left atria might also promote the type of temperature-dependent, nonspecific binding of Pbz that we have observed in frog heart homogenates (see above).

The results with antagonists provided the most clearcut evidence of changes in adrenoceptor characteristics. The effectiveness of both a competitive (phentolamine) and a nonequilibrium (Pbz) α -adrenoceptor antagonist increased as the temperature was lowered, and there was a reciprocal change in the effect of the β -adrenoceptor antagonist propranolol. α -Adrenoceptor blockade did not reduce responses to NA and had only a small effect on those to phenylephrine at 31°C, as would be expected from the many observations which indicate that inotropic responses of the mammalian myocardium at physiological temperatures are mediated predominantly by β -adrenoceptors (see Furchgott, 1970). As in many other studies of α -adrenoceptor blockade in the heart (Wenzel & Su, 1966; Govier, 1968; Nakashima, Maeda, Sekiya & Hagino, 1971; Schümann, Endoh & Wagner, 1974; Mugelli, Ledda & Mantelli, 1976), we used somewhat higher (micromolar) concentrations of antagonists than are needed in most smooth muscle preparations. However, even among smooth muscle structures, α -

adrenoceptors can vary significantly (Sheys & Green, 1972), and a considerable difference between smooth and cardiac muscle (Schümann *et al.*, 1974) is not surprising. Although their contributions are difficult to assess, other characteristics of both the drugs and the tissue could also contribute to the apparent relative resistance to blockade. The significant β -adrenoceptor activity still present at 17°C could be important in 'masking' the degree of block produced by α -adrenoceptor antagonists. Pronethalol has been reported to reveal an α -adrenoceptor component of the inotropic action of adrenaline and NA, but not of isoprenaline, at 32°C (Govier, 1968), and the combination of pindolol and phentolamine produces a greater than additive block of responses to phenylephrine (Schümann *et al.*, 1974). It is of interest that the concentration-response relation for propranolol is flatter at 17 than at 31°C in control atria (Table 1), but is much steeper at 17°C in atria from 6-OHDA pretreated rats (Table 3) in which the response has little or no α -adrenoceptor component. Similarly, the slope for phentolamine block of responses to phenylephrine at 37°C was very low, but was converted to that expected of a competitive antagonist by a low concentration of pindolol (Schümann *et al.*, 1974).

Occult potentiation could also reduce the apparent block of responses to NA by Pbz at 17°C; protection of α -adrenoceptors by phentolamine revealed a comparable potentiation at all temperatures. The interaction of blockade and potentiation is also apparent in the observation that the significant shift to the left of the NA concentration-response curve produced by Pbz at 31°C was abolished in guinea-pig atria incubated with Pbz at 17°C and subsequently tested at the higher temperature (Benfey, 1975, Table 2). This observation also indicates that the block produced by Pbz at a low temperature persisted when the temperature was raised, as previously reported for frog hearts (Kunos *et al.*, 1973b; Kunos & Nickerson, 1976).

Irrespective of the mechanisms responsible for differences in the apparent sensitivity of different tissues to adrenoceptor blockade, dose or concentration *per se* does not establish or exclude the interaction of a drug with specific receptors. This must be assessed on the basis of the relative inhibition of responses to specific and nonspecific agonists, and in the case of nonequilibrium antagonists, particularly, by 'receptor protection' experiments (Furchgott, 1954).

Although changes in the effects of sympathomimetic amines observed in the present study are compatible with the changes in adrenoceptor characteristics deduced from the effects of antagonists, some details are difficult to explain. The receptor specificity of adrenoceptor agonists is lower than that of antagonists, but the potency ratio of an

agonist with predominantly β - and one with relatively less β - and more α -adrenoceptor activity would be expected to decrease as the temperature was lowered if there is a shift from β towards α in receptor characteristics. The potency ratios of isoprenaline and phenylephrine for both inotropic and chronotropic responses did decrease considerably with temperature when the drugs were tested on spontaneously-beating preparations, but there was no change in the ratio in electrically-driven rat atria. A similar failure to demonstrate temperature-induced changes in agonist ratios in electrically-driven frog ventricle strips was recently reported (Benfey, 1975), but these results are difficult to compare with others because the preparations were much less sensitive to adrenaline and isoprenaline than are spontaneously-beating frog hearts (Erlj, Cetrangolo & Valadez, 1965; Kunos & Nickerson, 1976).

Both low temperature and electrical stimulation can influence the potency of agonists by mechanisms other than changes in receptor characteristics, and a coincidence of these effects could mask a change in receptor properties. Changes in the balance of α - and β -adrenoceptors are readily demonstrated by agonist ratios when only one of these factors is involved, i.e. temperature in spontaneously-beating preparations (Buckley & Jordan, 1970; Broadley, 1972; Harri, 1973; Tirri *et al.*, 1974; Amer & Byrne, 1975), or electrical driving at a high temperature in atria from rats with altered thyroid state (Nakashima *et al.*, 1971; Kunos *et al.*, 1974).

Factors other than changes in receptor characteristics that could affect agonist potency ratios include the release of endogenous NA by electrical stimulation (Jewell & Blinks, 1968), and the effects of released transmitter could be augmented by reduced reuptake (Kirpekar & Wakade, 1968) and decreased catechol-*O*-methyltransferase (COMT) activity (Opperman, Ryan & Haavik, 1972; Munoz-Ramirez, Ryan & Buckner, 1975) at lower temperatures. Any change in COMT activity could also have a direct effect on the isoprenaline-phenylephrine potency ratio. Some involvement of released endogenous NA is suggested by our observation that reserpine pretreatment increased the apparent α -adrenoceptor characteristics of electrically-driven rat atria, but a full explanation of differences between the responses of electrically-driven and spontaneously-beating preparations to agonists must await further studies.

The fact that shifts in receptor characteristics are more readily revealed by antagonists than by agonists could be due to some action of the former that would 'direct' unstable, transitional phase receptors into a specific configuration. There is no direct evidence for this mechanism at present, but it might provide an explanation for the unexpected reduction by phentolamine of maximal responses to phenylephrine. This was a consistent finding, but it cannot be

explained on the basis of current receptor theory. Such an action of antagonists might also be the basis for the observations that very low concentrations of propranolol, present during incubation with Pbz and subsequently washed out, greatly increase the block produced by a standard exposure to Pbz in frog hearts at low temperature (Kunos & Nickerson, unpublished observations), and decrease the threshold blocking concentration of Pbz in atria from hypothyroid rats (Kunos, 1977).

Our experiments on atria from 6-OHDA pretreated rats revealed an unexpected effect of sympathetic innervation on receptor characteristics. In addition to supersensitivity to the inotropic effect of NA, atria from pretreated rats showed almost pure β -adrenoceptor characteristics under all conditions tested. Pbz did not inhibit responses to NA at 17°C, and propranolol was even more effective at this temperature than at 31°C. In addition, block of responses to phenylephrine by Pbz was markedly reduced at both temperatures, which indicated that even the small α -adrenoceptor component of responses at 31°C was decreased by denervation. Similarly, denervation did not alter the binding of [³H]-Pbz at 31°C, but significantly less label was retained by denervated than by control atria at 17°C. These results all indicate that the α -adrenoceptor component of inotropic responses requires intact sympathetic innervation and that denervation 'stabilizes' adrenoceptors in the β configuration. A similar decreased effectiveness of α -adrenoceptor antagonists on chronically denervated cat nictitating membranes has been observed, and has been attributed to qualitative changes in the α -adrenoceptors (Varma, 1966) or to other postsynaptic changes (Langer & Trendelenburg, 1968).

Depletion of the NA content of rat atria by reserpine pretreatment produced moderate supersensitivity, but did not prevent the changes in adrenoceptor properties induced by temperature. Indeed, Pbz blocked responses to NA at 17°C even more effectively than it did in control atria. These observations suggest that adrenergic innervation may affect adrenoceptor characteristics by a mechanism independent of the presence of neurotransmitter. The nature of this effect is unknown, but passage of neuroplasmic constituents other than acetylcholine across the myoneural junction has been shown in skeletal muscle (Korr, Wilkinson & Chornock, 1967), and the possibility that such substances are involved in various 'neurotrophic' phenomena has been considered (Guth, 1968).

The shift in the properties of myocardial adrenoceptors from β towards α after thyroidectomy was also prevented by 6-OHDA but not by reserpine pretreatment (Kunos & Mucci, 1975). This further illustrates the role of sympathetic innervation in determining adrenoceptor characteristics and the

similarity of temperature- and hormone-induced adrenoceptor interconversions.

Few details of metabolic changes that may accompany denervation are known, but it appears not unreasonable to suggest that denervation supersensitivity may be associated with an increase in some component of tissue metabolism. If this is so, the observed effects of chemical denervation would fit into the general pattern relating adrenoceptor properties to tissue metabolism that is emerging from other studies. In addition to low temperature, metabolic inhibitors (Nickerson & Nomaguchi, 1950; Kunos & Szentiványi, 1968; Matheny & Ahlquist, 1975), thyroid deficiency (Nakashima *et al.*, 1971; Kunos *et al.*, 1974) and a reduced rate of contraction of

ventricular muscle (Endoh & Schümann, 1975; Mugelli *et al.*, 1976) appear to promote α -adrenoceptor properties and, conversely, muscle activity augments β -adrenoceptor characteristics (Szentiványi, Kunos & Juhász-Nagy, 1970). β -Adrenoceptors are generally more sensitive than α to noradrenaline (β_1) and adrenaline, and this interconversion may serve to adapt the mechanical response of a tissue to its metabolic state.

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