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Temperature modulation of α- and β-adrenoceptors in the isolated frog heart

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Summary

1. The effects of adrenaline on the isolated frog's heart at 27° C are not antagonized by phentolamine ($1{\cdot}5 \times 10^{-6}$M) but are abolished at 7° C.

2. At 27° C isoprenaline was more potent than noradrenaline, but at 7° C noradrenaline was more potent than isoprenaline.

3. Phenoxybenzamine ($1{\cdot}5 \times 10^{-5}$M) or dibenamine ($1{\cdot}5 \times 10^{-5}$M) at 7° C abolished the work output induced by adrenaline. When the temperature was raised to 24° C, adrenaline caused an increase in work output.

4. It is concluded that in the isolated frog heart there are at least two pools of adrenoceptors, the availability of which can be governed by temperature.

Introduction

Ahlquist (1948) classified the cardiac adrenoceptors as β-receptors, but subsequent workers have reported the existence of both α- and β-receptors in the hearts of mammals (Govier, Mosal, Whittington, & Broom, 1966; Wenzel & Su, 1966; Kunos & Szentivanyi, 1968; Berger & Mokler, 1969) and of frogs (Kunos & Szentivanyi, 1968). Kunos & Szentivanyi (1968), using isolated frog and rat hearts, have shown that alteration of the metabolic rate, by changing the temperature, resulted in a change in the type of adrenoceptor. Thus at higher temperatures the response to the physiological transmitter, adrenaline or noradrenaline, is blocked only by β-antagonists, whereas at lower temperatures the response is blocked by α-antagonists. The experiments described here were designed (i) to investigate the validity of the concept of temperature influenced receptors in the isolated frog heart, using α- and β-antagonists and agonists, and (ii) by the use of irreversible antagonists, to determine whether the effects observed are due to changes in a single receptor system, or to the existence of two separate receptor systems whose availability or effectiveness is governed by cell metabolism or temperature.

Methods

Frogs were maintained at 5° C with a low level of illumination for at least a week before use. The isolated frog hearts were perfused by a modified Symes (1918)

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technique, using as the perfusion cannula a J-shaped glass tube, with the heart attached to the shorter arm. The cannulated heart was suspended in a 70 ml bath of glucose-free, frog Ringer's solution so that the perfusion head was 15 cm. The bathing solution was continuously mixed by a stream of oxygen. The bath was maintained at the desired temperature by a water jacket in a vacuum flask. Spontaneous contractions of the hearts were recorded on a smoked drum kymograph using a spring lever with a 20 × magnification.

Kunos & Szentivanyi (1968) used dinitrophenol in conjunction with low temperature to reduce the metabolic rate. In the present experiments it was considered desirable to have only one variable, and so dinitrophenol was not used.

The drugs used were: adrenaline bitartrate, noradrenaline bitartrate, isoprenaline hydrochloride, phentolamine mesylate, propranolol hydrochloride, phenoxybenzamine hydrochloride, dibenamine hydrochloride. Solutions of phenoxybenzamine and dibenamine were freshly prepared in propane-2-diol. Phentolamine and propranolol were administered 10 min before and phenoxybenzamine and dibenamine 90 min before the addition of agonists.

Results

Responses to the catecholamines were obtained on a cumulative dose basis because this gave consistency of temperature during exposure to drugs. The time course of the responses to the drugs varied with the temperature of the experiment. The peak effect of the agonists was reached within 2 min at the higher temperatures, but only after 5 min at the low temperatures. The response of the hearts to catecholamines was measured as change in work output, which was computed as a function of force (F), represented by the height of contraction, and rate (R). The use of $F \times R$ gave more reproducible dose response curves than either F or R separately.

Phentolamine and propranolol

At a temperature of 7° C, adrenaline ($1{\cdot}6 \times 10^{-7}$ to $9{\cdot}1 \times 10^{-6}$M) caused an increased work output which was blocked by phentolamine ($1{\cdot}5 \times 10^{-6}$M). In the range of temperature 18°–22° C, phentolamine ($1{\cdot}5 \times 10^{-6}$M) only caused a partial block of the work output elicited by adrenaline, and at temperatures higher than 24° C phentolamine had no effect. The effectiveness of propranolol was found to be the reverse of that observed with phentolamine. At 7° C, propranolol ($1{\cdot}5 \times 10^{-6}$M) had no effect; at 18°–22° C it caused a partial block, and at 24° C completely blocked the effects of adrenaline. In all cases the effect of the antagonist was quickly abolished by washing with Ringer's solution.

Relative potencies of noradrenaline, adrenaline and isoprenaline

Figure 1 shows log dose-response curves for the effects of the three catecholamines at three different temperatures. The following changes in potency were observed:

8° C noradrenaline>adrenaline>isoprenaline
18° C adrenaline>isoprenaline>noradrenaline
27° C isoprenaline≥adrenaline>noradrenaline.

Phenoxybenzamine and dibenamine

In experiments with irreversible antagonists, the response to propranolol at 24° C and the absence of response at 7° C were first established. The hearts were then incubated at 7° C with phenoxybenzamine ($1{\cdot}5 \times 10^{-5}$M) or dibenamine ($1{\cdot}5 \times 10^{-5}$M) for 90 min. In two hearts the increased work output caused by adrenaline was not abolished by the antagonists. In five hearts the effect of adrenaline at 7° C was blocked, but at 24° C adrenaline still elicited a normal increase in work output. Antagonism of adrenaline at 7° C by phenoxybenzamine or dibenamine could not be reversed by prolonged washing at 7° C or 24° C.

In similar experiments, three hearts were incubated with phenoxybenzamine at 24° C. In two cases the response to adrenaline at 24° C and 7° C remained unaltered and in the third case the sensitivity to adrenaline at 24° C was diminished but that at 7° C was unaffected.

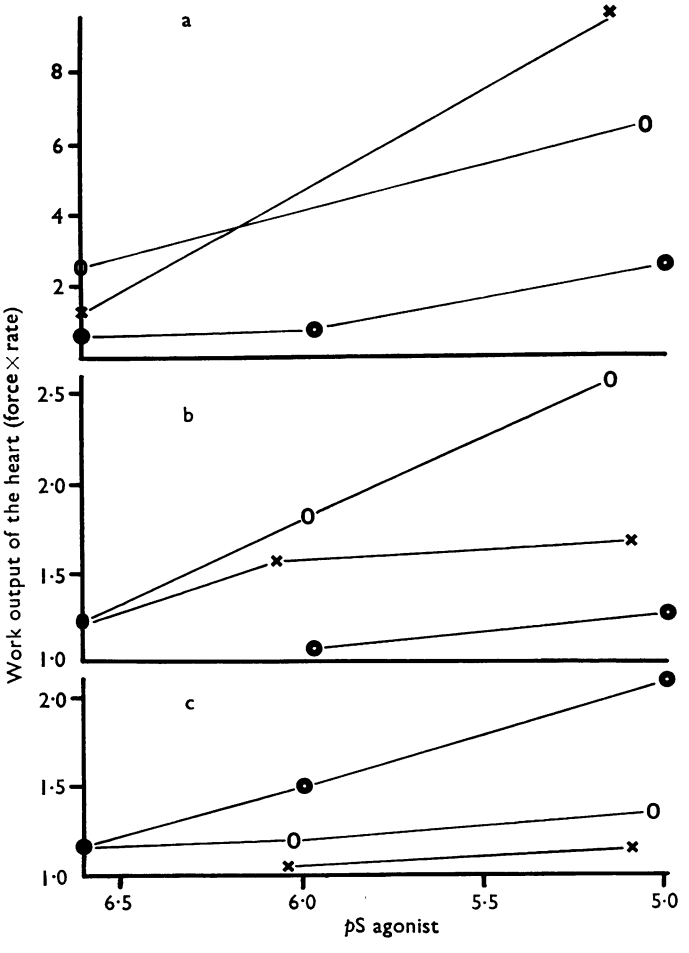

FIG. 1. Effect of catecholamines on work output of the isolated frog heart maintained at different temperatures. (a) 27° C; (b) 18° C; (c) 7° C. Catecholamines: (**O**) noradrenaline; (○) adrenaline; (×) isoprenaline.

Discussion

The results obtained using the α- and β-adrenoceptor antagonists phentolamine and propranolol support the observations of Kunos & Szentivanyi (1968) of a transition from β- to α-receptors on cooling the isolated frog heart. Further evidence is provided by the changes observed in relative potencies of noradrenaline, adrenaline and isoprenaline as the temperature of the heart is lowered. Price, Swann & Nayler (1967) also showed that β-receptors in the dog heart-lung bypass preparation became ineffective at 15° C, whereas α-receptors retained their effectiveness. It is important to note, however, the observations of Nayler & Wright (1963) on isolated toad hearts at 2° C. These authors could not show an effect of adrenaline at 2° C and offered the explanation that because of spontaneously released adrenaline, the hearts were already contracting maximally.

If a change in temperature produces a change only in metabolic rate, then the results presented here are consistent with the concept of a metabolic influence on receptor type. This concept, however, is not supported by results obtained with hyperthyroid and hypothyroid rats. Strubelt (1968) observed no differences in the sensitivities to noradrenaline and adrenaline of isolated atria from hypo- and hyperthyroid rats. The oxygen consumption of the hyperthyroid rats was 161% of control values, compared with hypothyroid rats with 78% of control values.

Kunos & Szentivanyi (1968) suggested that their results indicate the existence of a single receptor system which undergoes qualitative changes as metabolic rate is altered. They considered that the mid-point of this change occurs at 22°–24° C in the winter frog heart. The evidence provided by Kunos & Szentivanyi (1968) is that an equal and separate block is obtained at 22°–24° C with phentolamine or propranolol, but it is not clear whether the block obtained was complete or partial. Our observations showed that the mid-point of the change occurred at 18°–22° C, phentolamine and propranolol having only a partial blocking action within this temperature range. The decreased effectiveness of both antagonists at 18°–22° C is compatible with the concept of a single adrenoceptor.

If there is a single adrenoceptor system which is qualitatively altered by changing the temperature, then an irreversible blockade of the α-component at low temperature should render the receptors unavailable for conversion to β-receptors at higher temperature. The experiments with phenoxybenzamine and dibenamine show that alkylation of the receptors at 7° C has no effect on the response to adrenaline at 24° C. Furthermore, the lack of response of the hearts following recooling to 7° C showed that the receptors which became effective at 24° C, became unavailable or ineffective at the lower temperature. Thus the α- and β-components must be discrete entities and not alternative forms of a single adrenoceptor.

Incubation at 24° C with phenoxybenzamine had little if any effect on the response at 24° C and no effect on the response following recooling to 7° C. This is in contrast to the observations of Nickerson (1949) (who reported from unpublished results) and Graham (1962) that β-haloalkylamines always block the response to adrenaline in the amphibian heart.

We therefore conclude that in the isolated frog heart there are at least two pools of adrenoceptors, the effectiveness of which can be governed by temperature.

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