Short communications

Negative inotropic responses of the isolated heart of the rat to isoprenaline

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The isolated heart of the rat perfused at 38° C responded to isoprenaline with an initial positive inotropism followed by a biphasic negative inotropic effect. The latter was associated with the simultaneous biphasic rate increase. On reduction of the perfusion temperature to 20° C, isoprenaline no longer increased the rate but a negative inotropic response remained. Blockade of this by practolol suggested that it was mediated via β_1-adrenoceptors.

Typically, catecholamines exert positive inotropic effects on both intact and isolated hearts. However, negative inotropic responses have been reported (Claes, 1922; Serin, 1952) and have been attributed to the concomitant rate increase (Ando, Naito, Shiozu, Saito, Okumura & Iwata, 1959), which shortens the duration of individual cardiac contractions and reduces the total tension developed. At lowered temperatures, the catecholamine-induced tachycardia diminishes (Brown & Cotten, 1954) and finally disappears at 20° C in the rat isolated perfused heart. This paper describes a negative inotropic response occurring during hypothermia in the absence of any rate changes.

Methods.—Wistar rats of either sex and weight range 200–350 g received heparin (1,000 iu/kg) via the femoral vein under ether anaesthesia. This prevented subsequent occlusion of the coronary vessels by thrombi, to which the rat was found to be particularly susceptible. Ten minutes after regaining consciousness, the animals were killed by a blow on the head. Control experiments showed that there were no residual effects of the ether on cardiac responses. The hearts were rapidly excised and the cut aorta tied on to a glass cannula for retrograde perfusion by a modified method of Langendorff (Broadley, 1970). A constant rate (3–5 ml/min) of perfusion with oxygenated Locke Ringer solution (composition in g/l. distilled water: NaCl, 9; KCl, 0·42; $CaCl_2$, 0·24; $NaHCO_3$, 0·5; glucose, 1·0) was achieved with a Watson–Marlow flow inducer. Alterations in perfusion pressure arising from changes in coronary vascular resistance were recorded on a Devices M4 polygraph by means of a pressure transducer (Consolidated Electrodynamics, Type 4-327-L221). Isometric tension changes of the heart were recorded with a transducer (Ether, Type UF1, 57 g sensitivity range) attached via a pulley to a clip on the apex of the left ventricle. A measure of amplitude of contraction was obtained with an optical wedge transducer (Devices 2LDO1). The wedge was connected via a second pulley to a heart clip on the right ventricle and was returned after each contraction by a light spring. The heart rate was recorded with a Nielson instantaneous rate meter which was triggered by the tension record.

Experiments were also performed with hearts from guinea-pigs of either sex which were set up by the same technique except heparin pretreatment was omitted and Krebs-bicarbonate perfusion solution (Broadley, 1970) gassed with 5% CO_2 in oxygen was used.

Initially, all hearts were perfused and jacketed at 38° C. The temperature was lowered by means of a refrigerator coil (Grant Instruments (Cambridge) Ltd.) in an open water bath forming part of the warming system being driven by a Churchill circulator (CH/LTC/3). (−)-Isoprenaline sulphate (B.D.H. Ltd.) was injected (0·1 ml) into the perfusion solution and practolol (I.C.I. Ltd.) was introduced into the Ringer reservoir.

Results.—Typical responses to isoprenaline (10 ng) are shown in Figure 1A. The heart rate increased biphasically. The amplitude and tension changed triphasically; initially there was a small positive inotropism followed by a more prolonged biphasic negative inotropic component. Close inspection of many responses showed that the second increase in rate preceded the secondary fall in tension and amplitude. Isoprenaline caused a fall in perfusion pressure. After the perfusion temperature had been lowered to 20° C,

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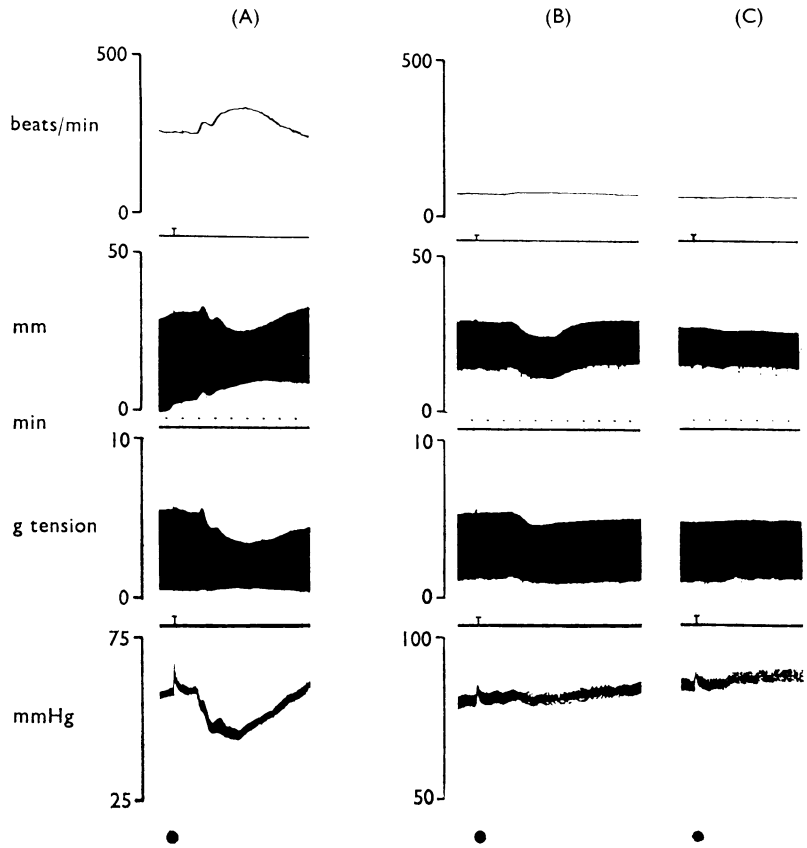

FIG. 1. The effects of isoprenaline, 10 ng (●) on a rat isolated heart perfused at 38° C in (A) and at 20° C in (B) and (C). The records (from the top down) are: heart rate; amplitude of contractions; force of contractions; and coronary perfusion pressure. Between (B) and (C) practolol (0·4 μg/ml) was introduced to the perfusion solution.

isoprenaline administration was repeated (Fig. 1B). The fall in perfusion pressure and the rate response were no longer evident and the positive inotropic component was absent. However in 23 of the 26 hearts examined, a monophasic negative inotropic response remained, and this was abolished by the inclusion of practolol (0·4 ug/ml) in the perfusion solution (Fig. 1C).

In more than 35 experiments with guinea-pig hearts, neither isoprenaline nor adrenaline have been observed to produce negative inotropic responses at any of the temperatures examined. These were 38° C, 35° C, 30° C and 25° C, below which the hearts normally stopped.

Discussion.—Negative inotropic responses are well known with antagonists at β-adrenoceptors (Blinks, 1967) but have been rarely demonstrated by the agonists. One example is the noradrenaline-induced reduction of cardiac contractions in the intact hypothermic dog following digitalization (Cotten & Cooper, 1962). Booker (1960) also produced negative inotropism and bradycardia with large concentrations of noradrenaline after introducing several increasing supramaximal doses to a guinea-pig isolated heart. The responses seen by Booker (1960) may well be equivalent to those of β-adrenoceptor antagonism due to saturation of the receptors. In the present investigation the negative inotropic response to isoprenaline at 20° C would appear to be an agonistic β_1-adrenoceptor effect since it was abolished by practolol, a selective β_1-blocking agent.

Even at normal temperatures (38° C) isoprenaline produced a multiphasic inotropic response, the predominant portion of which was a reduction of tension and

amplitude of contractions. This negative inotropism has been accounted for by the accompanying rate increases (Ando *et al.*, 1959). In their review, Koch-Weser & Blinks (1963) show that a tachycardia shortens the duration of the active state and in the rat heart causes the accumulation of a 'negative inotropic effect of activation'. The nature of the latter is not clear but may be related to intracellular Ca^{++} levels. In the hearts of guinea-pigs a 'positive inotropic effect of activation' predominates, and since this species exhibited no negative inotropism it would appear that the negative inotropism of the rat is due to the negative inotropic effect of activation rather than the reduction of the active state. The fact that both the positive chronotropic response and negative inotropic responses at 38° C were biphasic and that the tension and amplitude changes occurred slightly after the rate changes would support the view that this negative inotropism is related to the rate changes. At 20° C there were no rate changes and the negative inotropic response to isoprenaline could not be related to the interval-strength phenomena.

The normal catecholamine-induced increases in cardiac contractions have been shown to be inhibited by hypothermia (Price, Swann & Nayler, 1967). Any residual positive inotropism has been attributed to the predominance of α-adrenoceptors at low temperatures (Kunos & Szentivanyi, 1968; Buckley & Jordan, 1970). Govier (1968) also has identified positive inotropic α-adrenoceptor effects at normal and low temperatures and Wenzel & Su (1966) have demonstrated both positive and negative inotropic effects mediated via α-adrenoceptors. The present finding that β_1-adrenoceptors are still present in the rat heart at reduced temperatures but responsible for an opposite effect to that at physiological temperatures confuses the situation even further. The question arises as to whether the β_1-adrenoceptors are the same at all temperatures and cooling merely reverses the type of response or whether there are both β_1-positive inotropic and β_1-negative inotropic adrenoceptors, the temperature controlling which should predominate.

Finally, the coronary vasodilator response to isoprenaline has been linked in a previous paper (Broadley, 1970) to the increased metabolic activity of the heart and this explains its absence on cooling.

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