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THE EFFECTS OF ENDOGENOUS CATECHOLAMINE AND PURE α- AND β-ADRENOCEPTOR STIMULATION ON MYOCARDIAL GLYCOGENOLYSIS, LACTATE PRODUCTION AND DISTRIBUTION

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Abstract—The stimulation of glycogenolysis and lactate production and distribution by endogenous catecholamines released from the perfused rat heart by high K^+ solutions in the presence and absence of an α- or β-adrenergic blocker has been assessed. In addition, the effects of pure α- (noradrenaline + atenolol) and pure β- (isoprenaline + thymoxamine) adrenoceptor stimulation have been examined. The results suggest that while glycogenolysis is under the control of the β-adrenoceptor, lactate accumulation in the tissue and perfusate is probably under both α- and β-adrenoceptor control. The distribution of lactate between intracellular and extracellular compartments appear to be governed by β-adrenoceptors in the coronary vessels or possibly by β-receptor induced activation of a permease on the cell membrane of the myocardial cell. Of these two suggestions the former seems the most likely.

It has been demonstrated by Carpenter and Nash [1] and by Boullin [2] that catecholamines are released from nerve-endings by solutions containing high levels of potassium ions. Potassium ions are rapidly released by heart tissue when subjected to ischaemic stress [3], and catecholamines have also been shown to be released under these conditions [4, 5]. The dramatic metabolic consequences of localised catecholamine release under ischaemic conditions are well documented [4, 6, 7]. The most rapidly observable metabolic changes are the rapid onset of glycogenolysis and the rapid production of lactate and its accumulation within the heart muscle cell [3, 7].

The catecholamine in highest concentration in cardiac nerve-endings is noradrenaline, an adrenoceptor agonist with both α- and β-stimulant properties.

The existence of α-adrenoceptors in addition to β-adrenoceptors in myocardial tissue is established. Positive inotropic effects of methoxamine in cat papillary muscle [8] have been observed. Additionally, α-adrenoceptor blocking drugs have been reported to inhibit contractility increases produced by phenylephrine in rabbit [9] and guinea-pig atria [10] and electrically-stimulated rat ventricle [11]. Imai *et al.* [12], using a reflectance fluorometer, have observed that the addition of noradrenaline to isolated perfused hearts causes an immediate transient reduction in the intracellular concentrations of reduced pyridine nucleotides followed by an increase in reduced pyridine nucleotide levels. This transient reduction was abolished by phentolamine while isoprenaline failed to demonstrate the transient effect. These authors conclude that this effect on metabolism is mediated by stimulation of α-adrenergic receptors in the myocardium. This work suggests that, although some metabolic effects are mediated by catecholamines, not all may be mediated by stimulation of β-receptors alone. Mayer and co-workers [13, 14] have demonstrated that mechanisms other than β-adrenoceptor stimulation, such as calcium influx, may be responsible for stimulation of glycogenolysis.

Williamson [15] and Regan *et al.* [16] have demonstrated that stimulation by catecholamines evokes lactate production by the myocardium both in intact animals [16] and isolated heart preparations [15].

The experiments described in this report endeavour to examine the effects of endogenous catecholamines released by the presence of a high potassium concentration as well as pure α- and pure β-adrenoceptor stimulation on glycogenolysis and lactate production by the isolated perfused rat heart. Hearts were perfused with a buffer containing a high potassium ion concentration to prevent contractility or heart rate changes caused by the presence of catecholamines from complicating the metabolic responses of the tissues to these drugs.

EXPERIMENTAL

All reagents used were of AnalaR grade wherever possible. Glucose oxidase (type II), Horseradish peroxidase (type II), *O*-dianisidine, *dl*-isoprenaline sulphate and l-noradrenaline (free base) were obtained from Sigma Chemical Co. (London). Atenolol ('Tenormin')* as the free base was a product of ICI Limited, Pharmaceuticals Division, Alderley Park, Cheshire. Thymoxamine was a product of the chemical laboratories of I.C.I.

*'Tenormin' is a Trademark, the property of Imperial Chemical Industries Ltd.

Heart perfusions

Rats (male, Alderley Park strain, 300 g b.w.) were injected with 250 units of heparin intraperitoneally 20 min before induction of anaesthesia with Nembutal (100 mg kg^{-1} i.p.). After deep anaesthesia had been achieved hearts were removed by cutting along the line of the pericardium and dropped into ice-cold saline. Each heart was quickly cannulated and perfused via the aorta with Krebs–Henseleit bicarbonate buffer at 37° from a reservoir which exerted a hydrostatic pressure equivalent to 60 mm Hg on the heart. Perfusion in this way was continued for 5 min to ensure that the coronary vessels were cleared of blood. The buffer which passed through the heart during this period was discarded. At the end of this preliminary period the perfusate was switched to one which contained 20 mM potassium ion and various adrenergic agonists or antagonists, either alone or in combination. Perfusion pressure was maintained at 60 mm Hg. The normal Krebs–Henseleit buffer was composed of the following: 142.20 m-moles Na^+, 5.87 m-moles K^+, 1.17 m-moles Mg^{2+}, 3.02 m-moles Ca^{2+}, 128.14 m-moles Cl^-, 1.17 m-moles $H_2PO_4^-$, 1.17 m-moles $SO_4^=$, 24.80 m-moles HCo_3^-, 0.50 m-moles EDTA, 11.00 m-moles glucose/l, and the solution was kept continuously equilibrated with a 95% oxygen/5% carbon dioxide mixture. The high potassium buffer contained identical concentrations of all ions and glucose except for the following: 128.07 m-moles Na^+ and 20.00 m-moles K^+ per litre.

Recirculating perfusate contained either 10 μM thymoxamine, 2 μM atenolol, 0.5 μM noradrenaline or 0.5 μM isoprenaline either alone or in combination.

Perfusion with high potassium buffer was continued for 15 min and the buffer was recirculated. The volume of the recirculating buffer was 80 ml. Eight hearts constituted each treatment group. At the end of the perfusion period tissues were rapidly frozen between blocks of aluminium pre-cooled in liquid nitrogen. Samples of recirculated perfusate were also taken.

Tissue samples were powdered in a Braun Mikrodismembrator at the temperature of liquid nitrogen and weighed aliquots of these powders were assayed for glycogen. Other aliquots of powder were extracted with 0.6 M perchloric acid as described by Hohorst [17] and these extracts and samples of perfusate were assayed for lactate fluorometrically by the method of this same author. A portion of each powdered heart was weighed and then placed in an oven at 100° for 24 hr to obtain the wet/dry ratio, which was used to correct all tissue analyses for tissue water content.

Glycogen estimation

A weighed portion of tissue powder (*ca* 200 mg) was hydrolysed for 1 hr at 100° with 1 ml of 30% KOH (w/v). After cooling to room temperature, 0.1 ml of Na_2SO_4 solution (2% w/v) and 0.5 ml absolute ethanol were added and the solution mixed. The mixture was left standing overnight at 4° and glycogen harvested by centrifugation. The tubes were drained by inversion of filter paper and the pellet washed by two 0.5 ml aliquots of 65% (v/v) ethanol. The crude glycogen thus obtained was hydrolysed by heating in 2 ml of 2 N H_2SO_4 for 3 hr at 100°. After cooling to room temperature the hydrolysate was neutralized by the addition of 2 N NaOH and 4 ml of 1 M phosphate buffer pH 7.0. The pH was adjusted in this way to 7.0 and the final vols made up to 10 ml by the addition of water. The glucose solutions thus prepared were assayed using a glucose oxidase procedure: 0.5 ml aliquots of glycogen hydrolysate were added to 3 ml of the enzyme–dye reagent. The resulting mixture was incubated for 1 hr at 37°, mixed and read at 420 nm. The enzyme–dye reagent was prepared as follows: 12.5 mg of glucose oxidase and 5 mg of Horseradish peroxidase were dissolved in 100 ml of 0.1 M phophate buffer pH 7.0 and 0.5 ml of *O*-dianisidine solution (10 mg/ml methanol) added. A calibration curve from 0 to 2 μmoles ml^{-1} glucose was constructed. Absorbance was linearly related to glucose content over this range of concentration.

Cyclic AMP extimation

Cyclic AMP was extracted from weighed tissue powder by boiling for 30 min with 0.5 ml of 50 mM HCl. Tissue residue was removed by centrifugation and 0.3 ml of 200 mM Tris was added. The solution was clarified by centrifugation and the supernatant taken for assay. Cyclic AMP was assayed by a competitive binding technique purchased in kit form from The Radiochemical Centre (Amersham, Bucks.).

RESULTS AND DISCUSSION

Carpenter and Nash have shown that high concentrations of potassium ion in the perfusion medium of heart muscle cause a release of catecholamines from nerve-endings. For this reason hearts blocked with both atenolol and thymoxamine were used as control preparations.

Effect of α- or β-stimulation on myocardial glycogen stores

The results obtained are shown in Fig. 1. Isoprenaline alone caused a significant reduction in myocardial glycogen during the 15 min perfusion period (51.43 ± 7.48 μmoles/g dry tissue vs 109.41 ± 7.95 μmoles hexose/g dry tissue, $P < 0.001$). This

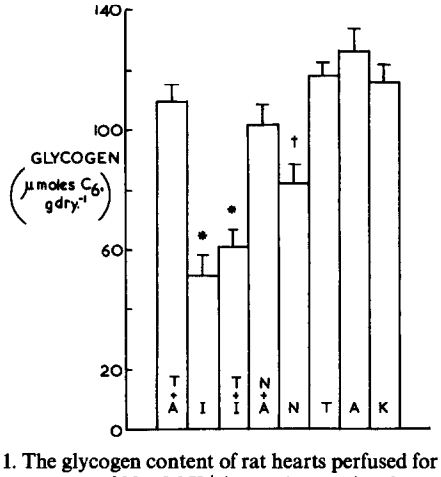

Fig. 1. The glycogen content of rat hearts perfused for 15 min in the presence of 20 mM K^+ ion under varying drug regimes. T: thymoxamine, A: atenolol, I: isoprenaline, N: noradrenaline, K; 20 mM potassium ion only. Bars show mean and S.E.M. of 8 hearts.*: $P < 0.001$,†: $P < 0.05$ vs T + A.

effect was not effectively modified by the addition of thymoxamine illustrating the absence of α-stimulant activity in isoprenaline. Noradrenaline alone stimulated a significant, but smaller reduction of intramyocardial stores (82.27 ± 9.0 μmoles hexose/g dry tissue, $P < 0.05$). This stimulation was prevented by atenolol. In the presence of atenolol and noradrenaline, a condition in which only α-adrenergic stimulation is present, tissue glycogen levels were not significantly different from those of control tissues, illustrating an apparent lack of stimulation of glycogenolysis by α-receptor stimulation. However, no significant difference was observed between myocardial glycogen levels in hearts treated with noradrenaline alone and noradrenaline and atenolol combined. Such an observation may possibly indicate that other weak effects could be involved in stimulation of glycogenolysis. Mayer and colleagues [14] have demonstrated that glycogenolysis can be stimulated by factors other than catecholamine stimulation. Hartley and McNeill [18] have presented evidence to suggest that calcium can directly activate phosphorylase; however, in a recent paper Keely *et al.* [19] have concluded that glycogenolysis is predominantly under the control of the β-adrenoceptor.

Endogenous catecholamines released by the presence of high circulating potassium concentrations appeared to be without effect on myocardial glycogen stores. The observation of Keeley *et al.* [19] that phentolamine, an α-blocker, potentiates phenylephrine-stimulated glycogenolysis, suggests that α-stimulation may ameliorate the effects of β-stimulation and may explain the nil effect reported here if endogenous catecholamine concentrations are low.

Effect of α- and β-stimulation of tissue cAMP *concentrations*

The effects of stimulation on tissue-cAMP levels are shown in Fig. 2. Isoprenaline stimulated cAMP production by the heart (1.689 ± 0.155 n moles/g dry vs 0.573 ± 0.053 n moles/g dry, $P < 0.001$). Thymoxamine was unable to overcome this effect. Significant potentiation by thymoxamine was not observed as has been previously reported for α-blockers [19]. Noradrenaline significantly increased cyclic AMP production by myocardial tissue (1.177 ± 0.133 n moles/g dry, $P < 0.001$) under conditions of potassium arrest and this effect was reversed by atenolol (0.8868 ± 0.1064 n moles, value of P vs noradrenaline < 0.05). There was good negative correlation between cAMP level and glycogen content of tissues ($r = 0.83$, $P < 0.01$)

Effect of α- and β-stimulation on myocardial lactate production

Hearts perfused with buffer containing both atenolol and thymoxamine exhibited significantly lower tissue lactate than did hearts perfused without any drugs added to the buffer (2.804 ± 0.116 μmoles/g dry vs 4.597 ± 0.445 μmoles/g dry, $P < 0.02$) (Fig. 3). The endogenous catecholamines released by potassium ions alone (predominantly noradrenaline) may be expected to exhibit both α- and β-stimulatory effects upon the tissues. The data presented show that blockade of both receptors will reduce lactate accumulation by the tissue. Additionally, the presence of thymoxamine alone is sufficient to cause a reduction in the lactate accumulation in myocardial tissue caused by release of endogenous catecholamines (3.048 ± 0.135 μ moles/g dry vs 4.597 ± 0.445 μ moles/g dry, $P < 0.01$). Atenolol was without effect.

The presence of added noradrenaline caused a significant increase in lactate accumulation (7.387 ± 1.377 μmoles/g dry, $P < 0.001$). This effect is attenuated by the action of atenolol (3.767 ± 0.385 μmoles/g dry, value of P vs noradrenaline < 0.05). The effect evoked by noradrenaline and atenolol together (α-stimulation alone) is still higher ($P < 0.05$) than that observed in hearts treated with both atenolol and thymoxamine. These data suggest that lactate accumulation by the tissue results from α-as well as β-adrenoceptor stimulation.

Isoprenaline alone produced significantly increased lactate accumulation in the tissue (5.075 ± 0.282 μ moles/g dry, $P < 0.001$). This effect was not reversed or attenuated by thymoxamine illustrating the predominantly β-stimulant nature of isoprenaline. Tissue lactate concentrations did not correlate with tissue

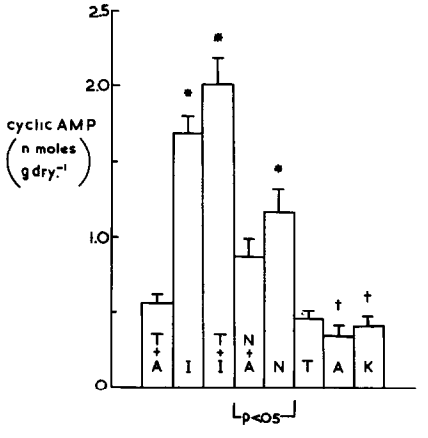

Fig. 2. The cAMP content of rat hearts perfused for 15 min in the presence of 20 mM K^+ ion under varying drug regimes. T: thymoxamine, A: atenolol, I: isoprenaline, N: noradrenaline, K: 20 mM potassium only. Bars show mean and S.E.M. of 8 hearts. *: $P < 0.001$, †: $P < 0.05$ vs T + A.

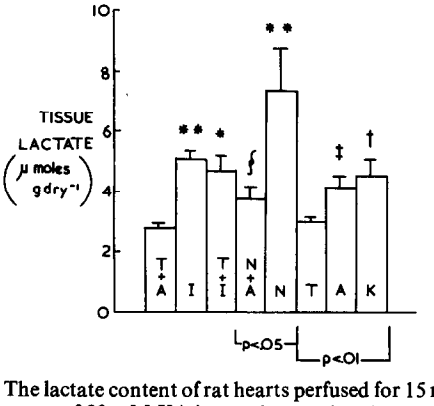

Fig. 3. The lactate content of rat hearts perfused for 15 min in the presence of 20 mM K^+ ion under varying drug regimes. T: thymoxamine, A: atenolol, I: isoprenaline, N: noradrenaline, K: 20 mM potassium ion only. Bars show mean and S.E.M. of 8 hearts. **: $P < 0.001$, *: $P < 0.01$ †: $P < 0.02$, §: $P < 0.05$ vs T + A.

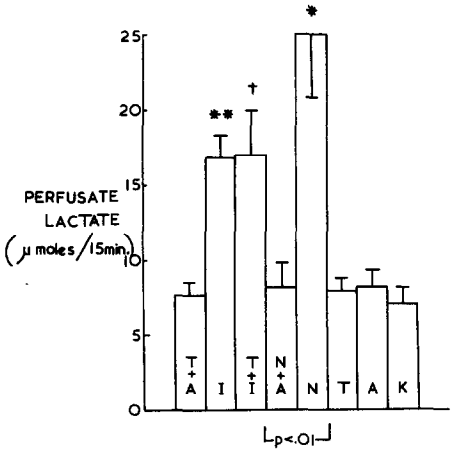

Fig. 4. The lactate content of perfusate of hearts perfused for 15 min in the presence of 20 mM potassium ions under varying drug regimes. T: thymoxamine, A: atenolol, I: isoprenaline, N: noradrenaline, K: 20 mM potassium ion only. Bars show mean and S.E.M. for 8 hearts. **: $P < 0.001$, *: $P < 0.01$ vs T + A.

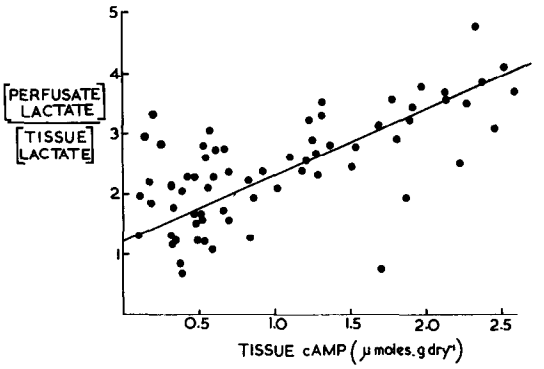

Fig. 5. The relation between [perfusate lactate]/[Tissue lactate] and heart tissue cAMP.

cyclic AMP unlike the relationship between tissue glycogen and cyclic AMP. Williamson [15] has demonstrated an increase in glucose uptake and utilization by perfused heart tissue consequent upon adrenergic stimulation. The data obtained would support this idea and further suggest that to some extent it may be under α-adrenergic control while the supply of glycosyl residues from glycogen are predominantly under β-adrenergic control. Such a suggestion has been made in part by Keely *et al.* [19].

The observation of Imai *et al.* [12] that intracellular NADH levels transiently decrease just after noradrenaline stimulation may be explained by an enhancement of glycolysis leading to a modest accumulation of pyruvate due to only partial activity of pyruvate dehydrogenase [20]. Some of this accumulated pyruvate is converted to lactate with concomitant oxidation of NADH.

Lactate accumulated in the perfusate during the 15 min perfusion period was assayed and the results obtained are depicted in Fig. 4. Isoprenaline alone and in combination with thymoxamine raised the lactate level of the perfusate significantly (16.70 ± 1.47 μmoles, $P < 0.001$; 16.87 ± 2.86 μmoles, $P < 0.01$). Similar behaviour was observed for noradrenaline although this effect was abolished by atenolol (24 ± 4.26 μmoles, $P < 0.002$; 8.17 ± 1.58 μmoles, NS: value of P for noradrenaline vs noradrenaline + atenolol < 0.01). These results apparently show some β-adrenergic rather than α-adrenergic dependence of lactate production, although there was no significant correlation between tissue cAMP levels and perfusate lactate concentration.

The distribution ratio ([Perfusate lactate]/[Tissue lactate]) showed a positive correlation ($r = 0.985$, $P < 0.001$) with tissue cyclic AMP levels (Fig. 5), perhaps illustrating the importance of β-adrenergic stimulation to this phenomenon. This effect may be caused principally by an increase in coronary dilation occasioned by stimulation of the β-adrenoceptors in the coronary vessel, although the observation of Mowbray and Ottaway [21], that lactate does not equilibrate between intra- and extracellular compartments by diffusion alone, suggests that β-stimulation may perhaps facilitate transport of this metabolite across the cell membrane by some additional mechanism perhaps by activation of a permease [20]. Because of this apparent β-adrenoceptor stimulation of lactate transport, perfusate lactate accumulation may be a less sensitive indicator of α- and β-stimulation than is tissue lactate accumulation.

In conclusion, although these results are not definitive they do suggest that in the potassium arrested heart, glycogenolysis is controlled very largely by β-adrenoceptor stimulation and that glucosyl units thus liberated are converted into lactate. Simultaneous α- and β-adrenoceptor stimulation shows similar effects but when the β-adrenergic effects are blocked by the use of a highly specific β-receptor antagonist glycogenolysis is effectively prevented, but an acceleration of glycolysis, as evidenced by a small but significant production of lactate, is attributable to α-stimulation although some effect of β-receptor stimulation on glycolysis cannot be discounted. Distribution of lactate between intra- and extracellular compartments appears to be dependent upon β-adrenoceptor stimulation and this effect could be due to increased coronary perfusion or to activation of a lactate permease, or both. Further studies to clarify this point seem justified.

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