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EFFECTS OF SYMPATHOMIMETIC AMINES ON ^{45}Ca EFFLUX FROM LIVER SLICES

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The efflux of ^{45}Ca from slices of guinea-pig and rabbit liver is greatly increased by α-adrenoceptor agonists. Isoprenaline is much less effective. The effects of these agents on the efflux of ^{45}Ca mirror their actions on ^{42}K loss and suggest that the two may be related. Glucose release from both rabbit and guinea-pig liver slices is increased to a similar extent by either α- or β-receptor agonists. The possible relationship between Ca and K movements and the production of glucose is discussed.

Introduction Previous work (Haylett & Jenkinson, 1972a, b) has shown that activation of an α-like receptor causes a rise in the potassium permeability of guinea-pig liver cells. This is reflected by an increase in the rate coefficient for ^{42}K efflux, a net loss of cell potassium, increased membrane conductance and hyperpolarization of the cells. The same study also rather unexpectedly showed that α- as well as β-adrenoceptor agonists increased glucose release from guinea-pig liver. The β-mediated response almost certainly involves an increase in the activity of adenylate cyclase. However, α--receptors are not thought to activate this enzyme. In keeping with this, recent work in this laboratory (Osborn, 1975) has shown that α-agonists (in contrast to β-agonists) do not cause a significant rise in cyclic adenosine-3′,5′-monophosphate content of guinea-pig liver slices. An alternative possibility is that the α-mediated increase in glucose release results from changes in intracellular ion concentrations. It has been shown in skeletal muscle that phosphorylase b kinase, an important enzyme in the glycogenolytic pathway, can be activated by low concentrations of Ca (Heilmeyer, Meyer, Haschke & Fisher, 1970) and it seemed possible that α-receptor activation might increase the breakdown of hepatic glycogen in a similar way. It thus seemed worthwhile to study the actions of sympathomimetic amines on calcium movements in this tissue.

Methods The experiments were performed on slices cut from the liver of either male guinea-pigs or rabbits. Details of the slicing procedure and information on the condition of the slices may be found in Haylett & Jenkinson (1972a). The slices were incubated at 38°C in a medium containing (mM): NaCl 125, KCl 6, $CaCl_2$ 1, $MgSO_4$ 1.2, NaH_2PO_4 1, $NaHCO_3$ 15, Na pyruvate 2 (pH 7.4 when gassed with 5% CO_2 in O_2) and were loaded with ^{42}K and ^{45}Ca for the period from 90 to 180 min after their preparation. Efflux of the isotopes and release of glucose were measured by passing the slices through a series of test-tubes containing 5 ml of non-radioactive solution at 2 min intervals. ^{42}K released from the slices was measured by Cerenkov counting of 2 ml aliquots of the washout solutions, and ^{45}Ca in the same samples was counted by adding scintillation fluid after ^{42}K had decayed. The slices were weighed and sonicated in incubation medium at the end of the experiment and samples of the suspension taken for counting. Glucose in the washout fluid was analysed by the simple colorimetric method, based on the reducing property of glucose, described previously (Haylett & Jenkinson, 1972b).

Results Figure 1 shows the effects of (−)-noradrenaline (1 μM), (−)-amidephrine (10 μM) and (−)-isoprenaline (1 μM) on ^{45}Ca, ^{42}K and glucose release from rabbit liver slices. Amidephrine is a selective α-adrenoceptor agonist (Dungan, Stanton & Lish, 1965) and isoprenaline at this concentration will have little effect on α-receptors. It can be seen that noradrenaline and amidephrine greatly increase the loss of ^{42}K, ^{45}Ca and glucose whereas isoprenaline produces a substantial change in glucose release alone. The effect of isoprenaline on ^{42}K and ^{45}Ca loss was rather variable, and sometimes undetectable. Taken together, the results suggest that α-receptors can elicit all three responses and that β-receptors, whilst consistently causing release of glucose, have a variable and generally small effect on ion movement.

Similar experiments with guinea-pig liver slices showed that (−)-amidephrine (10 μM) again caused a substantial increase (300–400%) in the rate of ^{45}Ca efflux whereas (−)-isoprenaline (50 nM, maximal for glucose release from guinea-pig liver) had a very small effect (increased by about 15%).

Examination of the ^{45}Ca efflux curves for guinea-pig liver slices indicated that at the time of drug application (35 min after removal from the ^{45}Ca solution) a substantial part of the accumulated ^{45}Ca has already been lost, mainly from a rapidly exchanging fraction which probably includes tracer in the extracellular space and perhaps also some bound to damaged tissue at the surface of the slices. The

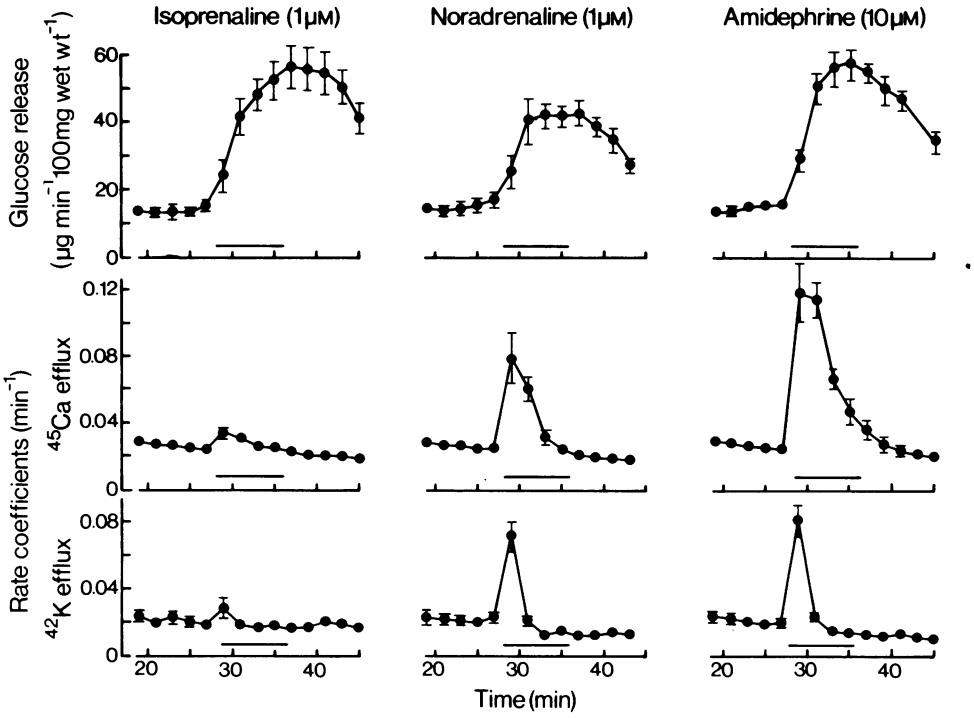

Figure 1 Effects of (−)-isoprenaline (1 μM), (−)-noradrenaline (1 μM) and (−)-amidephrine (10 μM) on the simultaneously measured release of glucose, ^{45}Ca and ^{42}K from rabbit liver slices. Drugs were applied for 4 successive 2 min periods as indicated by the horizontal bars. Each point is the mean of 3 or 4 observations and s.e. means are indicated where they exceed the size of the symbol.

remaining efflux appears to be coming from tissue compartments containing less than 1 mmol Ca/kg wet wt. as compared with a total Ca content of 2.6 ± 0.1 (s.e., $n = 22$) (K. Koller, personal communication). However, the fact that α-receptor activation can cause the rapid loss (within a few minutes) of more than 30% of this residual ^{45}Ca suggests that substantial changes in calcium distribution are occurring.

Discussion The main new finding is that α-receptor activation can greatly increase the efflux of ^{45}Ca from both rabbit and guinea-pig liver slices. The mechanism for this loss is as yet uncertain; active extrusion of calcium from the cells, release from internal binding sites (in particular mitochondria) and increased membrane permeability to Ca all being possible. The last two would probably lead a to a rise in the free concentration of Ca, $[Ca]_i$, within the cell. As already suggested this could lead to the activation of glycogenolytic enzymes. It is also possible that the effect on cell membrane potassium permeability may be the result of a rise in $[Ca]_i$. Experiments by Romero & Whittam (1971) suggested that the permeability of the erythrocyte membrane to potassium could be increased by raising $[Ca]_i$ and recent support for this has come from studies with the Ca-ionophore, A23187, which causes a Ca-dependent loss of K from red cells (Reed, 1973) and from salivary glands (Selinger, Eimerl & Schramm, 1974). Furthermore, Meech (1974) has shown that injection of Ca directly into snail ganglion cells leads to an increase in K permeability and hyperpolarization.

In conclusion it is tempting to propose that α-receptor activation in guinea-pig or rabbit liver leads to a rise in $[Ca]_i$ which then brings about the three actions examined in the present work; increased loss of ^{45}Ca, ^{42}K and glucose.

References

DUNGAN, K.W., STANTON, H.C. & LISH, P.M. (1965). Amidephrine I; Pharmacological characterization of a sympathomimetic alkylsulfonamidophenethanolamine. *Int. J. Neuropharmac.*, **4**, 219–234.

HAYLETT, D.G. & JENKINSON, D.H. (1972a). Effects of noradrenaline on potassium efflux, membrane potential and electrolyte levels in tissue slices prepared from guinea-pig liver. *J. Physiol., Lond.*, **225**, 721–750.

HAYLETT, D.G. & JENKINSON, D.H. (1972b). The receptors concerned in the actions of catecholamines on glucose release, membrane potential and ion movements in guinea-pig liver. *J. Physiol., Lond.*, **225**, 751–772.

HEILMEYER, L.M.G., MEYER, F., HASCHKE, R.H. & FISCHER, E.H. (1970). Control of phosphorylase activity in a muscle glycogen particle. II. Activation by calcium. *J. biol. Chem.*, **245**, 6649–6656.

MEECH, R.W. (1974). The sensitivity of *Helix aspersa* neurones to injected calcium ions. *J. Physiol., Lond.*, **237**, 259–277.

OSBORN, D. (1975). Comparison of the effects of selective α- and β-receptor agonists on intracellular cyclic AMP levels and glycogen phosphorylase activity in guinea-pig liver. *Br. J. Pharmac.*, **55**, 286P.

REED, P.W. (1973). Calcium-dependent potassium efflux from rat erythrocytes incubated with antibiotic A23187. *Fedn Proc.*, **32**, 635.

ROMERO, P.J. & WHITTAM, R. (1971). The control by internal calcium of membrane permeability to sodium and potassium. *J. Physiol., Lond.*, **214**, 481–507.

SELINGER, Z., EIMERL, S. & SCHRAMM, M. (1974). A calcium ionophore simulating the action of epinephrine on the α-adrenergic receptor. *Proc. Nat. Acad. Sci.*, **71**, 128–131.

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