On the positive inotropic action of glucagon in the isolated atria of the guinea-pig

Glucagon is known to exert a positive inotropic action in laboratory animals and in man (Klein, Morch & Mahon, 1968; Linhart, Barold & others, 1968; Lucchesi, 1968; Parmley, Matloff & Sonnenblick, 1969). However, the nature of the positive inotropic effect remains virtually unknown. It is probably different from that of cardiac glycosides (Parmley & others, 1969) and glucagon is not a sympathomimetic agent (Glick, Parmley & others, 1968; Mayer, Namm & Rice, 1970). Glucagon activates cardiac adenyl cyclase (Entman, Levey & Epstein, 1969; Mayer & others, 1970) but there is no conclusive evidence for a correlation between biochemical and positive inotropic effects. We therefore attempted to investigate the mechanism of action more closely.

Electrically stimulated, isolated atria (frequency 180/min) obtained from guinea-pigs, 280–420 g, of either sex, were suspended in oxygenated Muralt-Tyrode solution at 30°. Mechanical activity was recorded by means of a strain gauge and Helcoscriptor HE 86 t device. After 2 h of equilibration glucagon was added to the bath. The maximum increase in contractile force was approximately 20% of the initial value at a final glucagon concentration of 10^{-7}M. The effect was dose-dependent. The maximum effect was much lower than that of cardiac glycosides or sympathomimetic agents. Glucagon showed the same effect on the atria of animals pretreated with reserpine (3 mg/kg intraperitoneally for 2 days).

Pretreatment of normal atria with 10^{-7}M propranolol did not diminish the positive inotropic effect of glucagon (cf. Glick & others, 1966; Mayer & others, 1970). If glucose in the medium was replaced by sodium pyruvate (10 mM) the atria did not show a decrease in contractile force or frequency when compared with atria incubated in glucose-containing Tyrode solution. The tissue glycogen will have disappeared largely after incubation in a glucose-free medium (cf. Willebrands and van der Veen, 1967). The effect of glucagon (10^{-7}M) was the same in both media. Hence, an increased glucose concentration cannot explain the inotropic action. *N*-Isopropylmethoxamine (IMA) blocks the noradrenaline-induced rise in cyclic 3:5′-AMP (Shanfeld, Frazer & Hess, 1969). IMA (10^{-5} g/ml) added to the medium did not diminish the positive inotropic action of 10^{-7}M glucagon. Thus, a rise in 3,5′-AMP cannot be held responsible for the effect of glucagon.

Exposure of guinea-pig atria to 10^{-7}M glucagon (for 60 min) did not affect the sodium and potassium contents of the muscle preparations, nor were the membrane potential and the shape and size of the action potential changed. Obviously, glucagon does not give rise to changes of the cell membrane's properties which might explain the inotropic effect.

In separate studies we established the influence of glucagon (10^{-7}M) on the exchange of extracellular $^{45}Ca^{2+}$ against tissue Ca^{2+} in isolated atria (cf. Lahrtz, Lüllmann & van Zwieten, 1967).

Since the total calcium content was not affected by the presence of 10^{-7}M glucagon, the value shown for the total calcium concentration in Fig. 1 represents the mean value (± s.e.) for both control organs and for atria treated with glucagon. Since neither $^{45}Ca^{2+}$-uptake nor the total calcium content were affected (Fig. 1), the exchangeable calcium fraction was not influenced either. The effect of 10^{-7}M glucagon on calcium metabolism was also studied in isolated atria that had been rendered failing upon equilibration in Tyrode solution for 12 h. The contractile force of these organs was much reduced in comparison with that of atria, equilibrated for only 2 h. The $^{45}Ca^{2+}$ content of the failing organs after either 15 or 60 min of incubation was not affected by 10^{-7}M glucagon in the medium. Accordingly, changes in Ca^{2+}

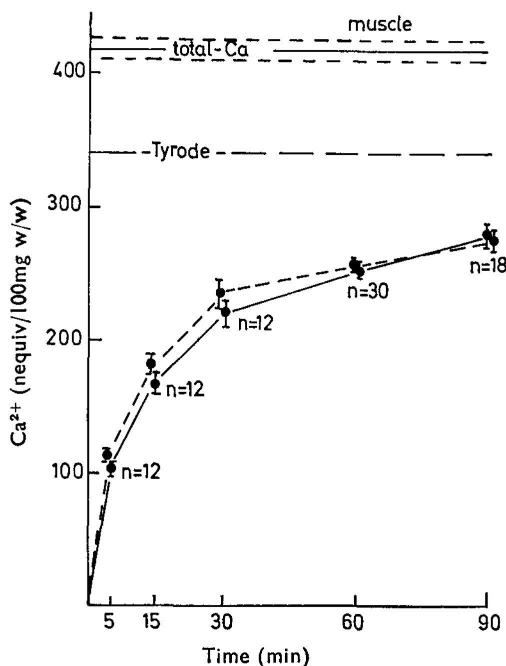

FIG. 1. Influence of glucagon (10^{-7}M) on calcium exchange in guinea pig isolated atria. The exchange, determined by means of $^{45}Ca^{2+}$, was expressed as nequiv Ca^{2+} per 100 mg wet weight. Each point on the curves represents the mean value ± s.e. for 12–30 atria. The total calcium content for both series of atria (glucagon and controls) is shown.

fluxes cannot be held responsible for the drug's inotropic effect.

Our results are in disagreement with those recently reported by Nayler, McInnes & others, (1970), who observed an increased $^{45}Ca^{2+}$ uptake by dog papillary muscle preparations upon treatment with glucagon. Since in these studies total calcium was not determined, the increased $^{45}Ca^{2+}$ uptake might as well be explained by means of changes in total calcium.

Glucagon influences calcium transport in isolated *subcellular* particles (Entman & others, 1969). However, it seems doubtful whether such observations will allow conclusions on the nature of the drug's effects in intact organs.

The present studies would suggest that none of the so-far known mechanisms underlying the positive inotropic action of drugs like cardiac glycosides, sympathomimetic agents, methylxanthines or Ca^{2+}-ions may explain the cardiac effect of glucagon. The nature of its positive inotropic action, therefore, requires further investigations.

This work was supported by the Deutsche Forschungsgemeinschaft. The skilful technical assistance of Mrs. M. Dorn is gratefully acknowledged.

Department of Pharmacology,
Christian-Albrechts-University,
Kiel, W.-Germany.

W. MIELKE
P. A. VAN ZWIETEN

December 3, 1970

REFERENCES

ENTMAN, M. L., LEVEY, G. S. & EPSTEIN, S. E. (1969). *Circulation Res.*, **25**, 429–438.
GLICK, G., PARMLEY, W. W., WECHSLER, A. S. & SONNENBLICK, E. M. (1968). *Ibid.*, **22**, 789–800.
KLEIN, S. W., MORCH, J. E. & MAHON, W. A. (1968). *Can. Med. Ass. J.*, **98**, 1161–1164.

LAHRTZ, H., LÜLLMANN, H. & VAN ZWIETEN, P. A. (1967). *Biochim. Biophys. Acta*, **135**, 701–709.
LINHART, J. W., BAROLD, S. S., COHEN, L. S., MILDNER, F. J. & SAMET, P. (1968). *Am. J. Cardiol.*, **22**, 706–710.
LUCCHESI, B. R. (1968). *Circulation Res.*, **22**, 777–788.
MAYER, S. E., NAMM, D. H. & RICE, L. (1970). *Ibid.*, **26**, 225–234.
NAYLER, W. G., MCINNES, I., CHIPPERFIELD, D., CARSON, V. & DAILE, (1970). *J. Pharmac. exp. Ther.*, **17**, 265–275.
PARMLEY, W. W., MATLOFF, J. M. & SONNENBLICK, E. M. (1969). *Circulation Res.*, **24**, Suppl. I, 163–167.
SHANFIELD, J., FRAZER, A. & HESS, M. E. (1969). *J. Pharmac. exp. Ther.*, **169**, 315–320.
WILLEBRANDS, A. F. & VAN DER VEEN, K. J. (1967). *Am. J. Physiol.*, **212**, (6), 1529–1535.

In vivo electrically evoked release of [^{3}H]noradrenaline from cat brain

A neurotransmitter role has been postulated for brain noradrenaline and dopamine. Before a substance can be considered to be a transmitter it must be shown to be released from nerve endings upon depolarizing stimulation. Baldessarini & Kopin (1966) demonstrated that electrical stimulation of brain slices caused the efflux of [^{3}H]noradrenaline, presumably from depolarized nerve endings. Attempts have also been made to detect endogenous dopamine release *in vivo* after electrical stimulation of various brain regions (McLennan, 1964), but the minute amounts of dopamine released were at the limits of the spectrophotofluorometric measurement. Philippu, Heyd & Burger (1970) reported that after the intraventricular injection of [^{14}C] noradrenaline, stimulation of the hypothalamus increased the concentration of ^{14}C (noradrenaline and metabolites) in ventricular perfusates. We now report an increased outflow of [^{3}H]noradrenaline into the ventricular system after electrical stimulation of the caudate nucleus.

Cats (2–3 kg) had their spinal cords sectioned and prepared for cerebroventricular perfusion (Carr & Moore, 1970). Five μCi of [^{3}H]noradrenaline (8·76 Ci/mmol, New England Nuclear Corp.) were injected in an effective volume of 10 μl through a cannula in a lateral ventricle at 16·5 A, 3·5 L (left or right) and + 8·0 D (Snider & Niemer, 1961). After 1 h the ventricular system was perfused with artificial cerebrospinal fluid (Pappenheimer, Heisey & others, 1962) at a rate of 0·1 ml/min. After washout for 110 min, the perfusion rate was increased to 0·5 ml/min and the collection of 1 ml samples of perfusate every 2 min was begun. During one or two of the collection periods, constant current square waves of 1 ms duration, 350 μA intensity and various frequencies were applied to the caudate nucleus by an electrode pair (anode at 13·0 A, 4·0 L and +5·0 D, cathode at 18·0 A, 4·0 L and +5·0 D). [^{3}H]Noradrenaline and metabolites in the perfusates were separated by alumina absorption and ion-exchange chromatography and quantified by liquid scintillation spectrometry (Carr & Moore, 1970). Throughout the course of the experiments blood pressure was recorded from the femoral artery and the rectal temperature monitored and maintained at 37·5° with a heating pad. All cannula and electrode placements were verified by gross dissection of the cat brain after formalin fixation.

In four experiments the mean ($\pm$ s.e.) concentrations of [^{3}H]noradrenaline and [^{3}H]normetanephrine in the perfusate samples before stimulation were 4·6 $\pm$ 1·1 and 2·6 $\pm$ 0·7 nCi/ml respectively. Electrical stimulation for 2 min significantly increased ($P < 0·05$) the perfusate concentrations in the periods during and immediately after the stimulation period (7·4 $\pm$ 1·1); stimulation did not alter the perfusate concentration of [^{3}H]normetanephrine. The other metabolites, deaminated catechols and deaminated *O*-methylated products, were present in the perfusates (10 and 15% of total radioactivity respectively), but they did not increase in concentration during or after stimulation.