Effect of Epinephrine on Contractile Tension and Phosphorylase Activation in Rat and Dog Hearts.* (29566)

GEORGE I. DRUMMOND, J. R. E. VALADARES AND LOVERNE DUNCAN
(Introduced by J. G. Foulks)
Department of Pharmacology, School of Medicine, University of British Columbia, Vancouver, Canada

It is well known that epinephrine, in addition to its ability to increase cardiac contractile force, can cause *in vivo* conversion of cardiac phosphorylase *b* to the active enzyme, phosphorylase *a*. The possibility has been considered by a number of investigators that these two actions may be associated. Haugaard and his associates(1,2,3) have reported a correlation between increased contractile force and phosphorylase *b* to *a* conversion induced by epinephrine in isolated perfused rat hearts. This correlation was reported to hold even for small doses of the amine which caused only minimal increases in contractile tension(3). Haugaard(4) has thus suggested that a direct relationship exists between phosphorylase activity and some stage of the contraction cycle of the heart. In contrast to this, Mayer and co-workers(5), using open-chest dog heart preparations, have shown that small doses of epinephrine can significantly increase ventricular contractile force without altering phosphorylase *a* levels. These latter authors have concluded that activation of phosphorylase is not associated with the positive inotropic action of the drug in the heart *in situ*.

The present studies are concerned with the effects of epinephrine on inotropic action and phosphorylase activation both in perfused rat hearts and in dog hearts *in situ*. Particular attention has been paid to the effects of low doses of the drug in an effort to clarify whether increased contractile tension is always accompanied by increases in phosphorylase *a* levels.

Methods. Rat hearts were perfused by the Langendorf technique, using Tyrode's solution at a flow rate of 5 ml per minute. Contractions were measured isometrically with a Grass Force Transducer, the arm of which was sutured to the apex of the ventricle. Contractile activity was recorded with a Grass Polygraph. A diastolic tension of 5 g was imposed on each heart at the beginning of an experiment. After a 5-minute control period, epinephrine was administered in a volume of 0.2 ml of 0.9% saline over a period of 12 seconds. Delivery of the dose was through a thin polyethylene catheter inserted in the cannula immediately above the heart. In control experiments, 0.2 ml of 0.9% saline was injected. Following epinephrine administration, when contractile response reached a maximum, the tissue was rapidly frozen. Two methods of freezing were employed. In one series (Series A), the suture attaching the heart to the transducer arm was cut, and a beaker containing dichlorodifluoromethane (freon-12) immersed in liquid N_2, was quickly placed into position so as to immerse the heart completely. The frozen heart was then transferred to liquid N_2 and a portion of the ventricle at the apex (200-250 mg) was chiseled off and stored in liquid N_2 until assayed (1 or 2 days). In another series (Series B), a portion of the left ventricle was frozen by crushing with a modified Wollenberger clamp (6) pre-chilled in liquid N_2. That portion of the tissue (30-60 mg) frozen between the jaws of the clamp was rapidly cut from the heart with strabismus scissors and placed in freon-12 at —150°. Removal of the tissue sample in this way could easily be accomplished in less than 2 seconds. Tissue was then chipped from the clamp under liquid N_2 and stored until assayed.

Frozen tissue was rapidly weighed in a cold room and homogenized in 100 volumes of 20 mM NaF containing 2 mM EDTA, pH 6.7 in a Potter-Elvehjem homogenizer.

* This investigation was supported by grants from the Life Insurance Medical Research Fund, Philadelphia, and the B. C. Heart Foundation, Vancouver, to George I. Drummond. We are deeply indebted to Mr. G. Kent for technical assistance.

TABLE I. Effect of Epinephrine on Isometric Tension and Phosphorylase *a* Activity in Perfused Rat Hearts.

Dose (μg)	No. of hearts	Increase in isometric tension ± S.E.M. (%)	Phosphorylase *a* ± S.E.M. (%)
Series A			
Control	36	—	12.7 ± .51
.025	25	23.2 ± 1.6	13.4 ± .9
.04	24	44.1 ± 3.4	10.3 ± .7
.05	33	58.0 ± 3.2	15.9 ± .95
.10	18	81.1 ± 4.3	37.1 ± 3.0
.20	9	104.4 ± 8.4	64.5 ± 1.6
1.0	12	112.4 ± 9.3	75.1 ± 2.1
Series B			
Control	12	—	10.8 ± .58
.025	11	26.3 ± 4.8	8.0 ± .43
.04	11	45.3 ± 4.7	9.9 ± .75
.05	13	62.0 ± 6.1	12.1 ± 1.01

After centrifugation, phosphorylase determinations were conducted in triplicate in the manner described by Mayer and co-workers (5). Activity of phosphorylase *a* was calculated in per cent of total phosphorylase activity.

In open-chest dog heart studies, contractile force was recorded by means of a strain gauge arch, according to the procedure of Mayer and co-workers(5). Epinephrine was injected intravenously and at the height of the contractile response a sample of right ventricular tissue was removed, using blunt forceps pre-chilled in liquid N_2. The time required to remove a sample was less than 4 seconds and the tissue was immediately placed in freon-12 at —150°. Usually 8 to 10 biopsy samples (25-50 mg) were removed from each dog heart. Samples were stored in liquid N_2 and were homogenized and assayed for phosphorylase as described for rat hearts.

Results. The effect of epinephrine on contractile tension and phosphorylase *a* levels in perfused rat hearts is shown in Table I. In Series A (hearts frozen by immersion in freon-12) it is apparent that small doses of epinephrine (0.025 and 0.04 μg) caused significant increases in contractile tension but did not increase phosphorylase *a* levels. At a dose of 0.05 μg when there was a 58% increase in contractile force, phosphorylase *a* activity began to rise above the controls. At doses above this which produced greatly increased contractile force, phosphorylase *a* levels rose sharply. When tissue was removed from perfused hearts by freezing with a modified Wollenberger clamp (Series B), control phosphorylase *a* levels were slightly lower than those obtained by the immersion method. This may be due to more rapid freezing. Here again, epinephrine in doses of 0.025 and 0.04 μg failed to increase phosphorylase *a* levels but caused significant increases in contractile tension. Even at a dose of 0.05 μg which caused a 60% increase in contractile force, phosphorylase *a* activity was not significantly higher than the control.

Contractile activity in dog hearts *in situ* was also significantly increased by small doses of epinephrine which did not affect phosphorylase *a* levels (Table II). A dose of 0.4 μg/kg was required to produce a significant increase in phosphorylase *a* activity and this dose produced a 60% increase in contractile force. Doses of 0.1 and 0.2 μg/kg did not affect phosphorylase *a* levels, but caused an 18.7 and 34.6% increase in contractile force respectively.

Discussion. Our results with perfused rat hearts do not agree with those described by Hess, Shanfeld and Haugaard(3), who reported a significant rise in enzyme activity after a dose of epinephrine which caused only a 10% increase in contractile tension. In the present experiments at least a 45% increase in contractile tension occurred before phosphorylase *a* levels rose. Relatively large numbers of hearts have been used to augment the significance of the values obtained. The data indicate that significant inotropic action can occur without changes in phosphorylase *a*

TABLE II. Effect of Epinephrine on Contractile Force and Phosphorylase *a* Activity in Dog Hearts *in situ*.

Dose (μg/kg)	No. of biopsies	Increase in contractile force ± S.E.M. (%)	Phosphorylase *a* ± S.E.M. (%)
Control	29	—	3.6 ± .17
.1	12	18.7 ± 2.3	3.76 ± .55
.2	17	34.6 ± 3.0	3.37 ± .40
.4	17	60.6 ± 2.0	5.10 ± .42
.7	12	97.4 ± 10.0	11.8 ± 1.18
1.0	15	98.9 ± 8.1	20.1 ± 2.7
3.0	9	112.6 ± 14.3	36.8 ± 3.3

activity. Our data obtained with biopsy samples from open-chest dog heart preparations are in agreement with those of Mayer and co-workers(5). It should be mentioned that control phosphorylase *a* levels found in perfused rat hearts are considerably higher than those found in dog hearts. The control values we have obtained, however, are much below those previously reported(2,3). We have evidence that this is due to rapid freezing of the tissue and to high dilution of the extract before assay.

Our results would suggest that phosphorylase activation may not be associated with epinephrine-induced cardiac contractile force. Small doses of epinephrine significantly increase contractile force both in perfused rat hearts and in dog hearts *in situ* without increasing phosphorylase *a* levels. It must be emphasized that at higher doses, phosphorylase *a* levels rise sharply. Further work is clearly necessary to establish the true role of phosphorylase in epinephrine-induced cardiac contractility.

Summary. The effects of epinephrine on inotropic action and on phosphorylase activation have been examined in perfused rat hearts and in dog hearts *in situ*. Small doses of epinephrine caused significant increases in contractile force without augmenting phosphorylase *a* levels. It is concluded that activation of phosphorylase does not correlate with the inotropic action of the drug, but occurs only after cardiac activity is significantly increased.

1. Hess, M. E., Haugaard, N., *J. Pharmacol. Exp. Therap.*, 1958, v122, 169.

2. Kukovetz, W. R., Hess, M. E., Shanfeld, J., Haugaard, N., *J. Pharmacol. Exp. Therap.*, 1959, v127, 122.

3. Hess, M. E., Shanfeld, J., Haugaard, N., *Biochem. Pharmacol.*, 1962, v11, 1031.

4. Haugaard, N., *Nature*, 1963, v197, 1072.

5. Mayer, S. E., Cotten, M. de V., Moran, N. C., *J. Pharmacol. Exp. Therap.*, 1963, v139, 275.

6. Wollenberger, A., Ristau, O., Schoffa, G., *Pflüg. Arch. ges. Physiol.*, 1960, v270, 399.

Received June 25, 1964. P.S.E.B.M., 1964, v117.

In vitro Stimulation of Pituitary Follicle-Stimulating-Hormone Release by Hypothalamic Extract.*† (29567)

JAMES C. MITTLER AND JOSEPH MEITES
Michigan State University, Department of Physiology, East Lansing

Campbell *et al*(1) demonstrated that direct infusion of median eminence extracts into the pituitary of rabbits was effective in inducing ovulation, and Nikitovitch-Winer (2) similarly induced ovulation by infusion of median eminence extract into the pituitary of pentobarbital-blocked proestrus rats. McCann *et al*(3) and McCann(4) first demonstrated the presence of a hypothalamic luteinizing-hormone-releasing factor in acid extracts of stalk-median-eminence tissue of rats, using the ovarian ascorbic acid depletion method of Parlow(5). These results have been confirmed by Courrier *et al*(6) and Guillemin *et al*(7). Courrier *et al*(6) and Johnson(8) also observed that the luteinizing-hormone-releasing factor in hypothalamic extracts could induce ovulation in androgen-sterilized female rats. Schiavi *et al*(9) used acid extracts of sheep hypothalamus to produce ovulation in rats rendered anovulatory by hypothalamic lesions.

Kobayashi *et al*(10) reported that crude saline extracts of hypothalamus induced an increase in "total gonadotropin" release from monolayer cultures of rat anterior pituitary tissue. These workers used the immature mouse uterine weight assay method, which measures the combined effect of FSH and LH. Their results can be considered of doubt-

* This work was supported in part by NIH grant AM 4784-04

† Published with the approval of Mich. Agri. Exp. Station as journal article no. 3413.