physiological significance than are the *in vitro* effects.

The data reported herein support the hypothesis that the hypoglycemic action of L-tryptophan and of some of its metabolic derivatives is related to a decrease in the rate of destruction of endogenous insulin. The precise mechanism involved remains unknown.

*Conclusions.* Metabolic derivatives of L-tryptophan which produce a hypoglycemic response in rats are effective also as inhibitors of the destruction of insulin by fresh extracts of rat livers and by intact mice.

1. Mirsky, I. A., Perisutti, G., and Jinks, R., *Endocrinology*, 1957, v60, 318.

2. Mirsky, I. A., Perisutti, G., and Dixon, F. J., *J Biol. Chem.*, 1955, v214, 397.

3. Sörensen, S. P. L., *Ergebn. Physiol.*, 1912, v12, 393.

4. Mirsky, I. A., Perisutti, G., and Jinks, R., *Endocrinology*, 1955, v56, 484.

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## Action of Ouabain upon Normal and Hypodynamic Myocardium.* (23151)

PULAK N. SANYAL AND PAUL R. SAUNDERS

*Department of Pharmacology, School of Medicine, University of Southern California, Los Angeles.*

The positive inotropic action of cardiac glycosides upon hypodynamic isolated mammalian cardiac muscle has been demonstrated many times. The papillary muscle of the cat has been used most frequently, and the usual experimental procedure has been to stimulate the muscle, suspended in a phosphate medium containing glucose, over a period of hours until it becomes hypodynamic and to then add the drug(1,2). Isolated papillary muscles become hypodynamic within a few hours in phosphate media, in contrast to their stability in bicarbonate media(2). It has been claimed that the typical increase in force of contraction upon addition of non-toxic concentrations of cardiac glycosides does not occur in myocardium which is not hypodynamic, *e.g.*, in bicarbonate medium(2) or in phosphate medium when the drug is added prior to the development of the hypodynamic state(3). We have investigated this matter in guinea pig myocardium.

*Methods.* Isolated, electrically stimulated strips, prepared from the right ventricular wall of the guinea pig (150-200 g males) heart in a manner similar to that described previously for the rat(4), were used. The basic medium employed had the following composition (m*M*): NaCl 154, KCl 5.6, and $CaCl_2$ 3.2. The bicarbonate medium contained in addition 5 m*M* $NaHCO_3$ and was gassed with 1% $CO_2$-99% $O_2$ mixture (final pH 7.4). The phosphate medium contained 1 m*M* sodium phosphate buffer in place of bicarbonate and was gassed with 100% $O_2$ (final pH 7.4). Both media contained 0.1% glucose. The strips were stimulated at 37°C at a rate of 100/minute at a constant resting tension of 750 mg. The absolute values of the "initial force" were of the same order of magnitude in the two media (approximately 100 mg).

*Results. Phosphate medium.* Fig. 1 shows the results of typical experiments in which ouabain was added to a hypodynamic preparation (A) and to one in which the force of contraction had not fallen appreciably from the initial value (B). Magnitude and duration of the positive inotropic actions were similar by comparison with the control values.

*Bicarbonate medium.* Fig. 2 shows the effect of the same concentration of ouabain upon a strip in bicarbonate medium. A positive inotropic action occurred in this typical experiment which persisted above the control value for many hours. The great stability of the muscle in the bicarbonate medium, in contrast to its behavior in phosphate medium, is evident in this figure.

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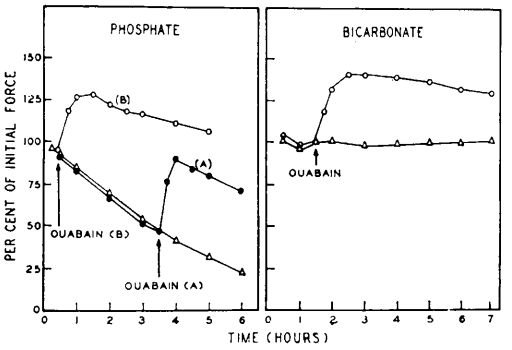


FIG. 1 (left). Effect of ouabain (1:6,000,000) upon force of contraction of ventricular strips in phosphate-buffered medium. -●-●- drug added to hypodynamic strip (A); -○-○- drug added soon after setting up strip (B); -△-△- control strip (no drug added). "Initial force" defined as force extrapolated to zero time.

FIG. 2 (right). Effect of ouabain (1:6,000,000) upon force of contraction of ventricular strips in bicarbonate-buffered medium. -○-○- drug added as indicated; -△-△- control strip (no drug added). "Initial force" defined as force after equilibration period of approximately 1.5 hr.

The concentration of ouabain used above (1:6,000,000) was considerably less than that required to produce a toxic effect upon the muscle. A concentration of 1:1,000,000 led consistently to the appearance of spontaneous contractions, the rate of which was independent of the frequency of the stimulator, and to a marked increase in resting tension. Such toxic effects of high concentrations of cardiac glycosides upon papillary muscles have been noted previously(1). A concentration of 1:10,000,000 produced a positive inotropic action in bicarbonate medium of approximately one-half that shown in Fig. 2.

*Discussion.* The view that a hypodynamic state must be produced in isolated mammalian myocardium in order to demonstrate a positive inotropic action of cardiac glycosides has resulted in the use of unphysiological (*e.g.*, phosphate-buffered) media in order to induce this state(1,2). The unphysiological nature of phosphate in comparison with bicarbonate media is evident from the steady decrease in developed tension which occurs in the former and from the failure of glucose to restore the force of contraction normally after prolonged contraction in substrate-free phosphate medium(4). Our finding that the use of such media is not necessary in order to demonstrate the typical action of ouabain implies that the system(s) in guinea pig myocardium affected by the cardiac glycosides with resultant increased force of contraction are present and affected as readily in "normal" as in hypodynamic isolated myocardium. It might be noted that a positive inotropic response to cardiac glycosides occurred also in dog heart *in situ* in the absence of evidence of failure (5).

*Summary.* The action of ouabain upon force of contraction of guinea pig ventricular strips in phosphate and bicarbonate media was studied. A positive inotropic response occurred in both "fresh" and hypodynamic strips in phosphate medium, and also in bicarbonate medium.

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1. Cattell, McK., and Gold, H., *J. Pharmacol. and Exp. Therap.*, 1938, v62, 116.

2. White, W. F., and Salter, W. T., *ibid.*, 1946, v88, 1.

3. Sciarini, L. J., Ackerman, E. M., and Salter, W. T., *ibid.*, 1948, v92, 432.

4. Berman, D. A., and Saunders, P. R., *Circulation Res.*, 1955, v3, 559.

5. Walton, R. P., Leary, J. S., and Jones, H. P., *J. Pharmacol. and Exp. Therap.*, 1950, v98, 346.

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