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Received September 19, 1966. P.S.E.B.M., 1967, v124.

Action of Isoproterenol on Heart Cells in Tissue Culture.* (31680)

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For many years it has been possible to grow dispersed heart cells in tissue culture, and rhythmic contraction of these cells has been noted(1). The rate of contraction of these heart cells has been measured by direct observation and electrical depolarization has been recorded by micropuncture techniques (2). The effects of cardioactive drugs, including epinephrine(3), quinidine(4), and digitalis(5), have been determined on these cells. Our studies were designed to assess the effects of drugs which stimulate or block specific adrenergic receptors in the heart(6). These experiments allow studies in an isolated system of cardiovascular tissue devoid of all neural connections.

Method. Beating hearts are removed from 9- to 11-day-old chick embryos. They are chopped into fragments and incubated for 20 minutes at 37°C in 0.125% trypsin in phosphate buffered saline containig glucose. The dissociating fluid is decanted and the settled cells resuspended in a modified Puck's medium. Dispersion of the cells is accomplished by gentle up and down pipetting. The larger tissue fragments are removed by filtering the cell suspension through a sterile mesh gauze. The cells are collected by centrifugation at 1500 RPM for four minutes and then resuspended in Puck's medium containing 20% horse serum and antibiotics. Approximately 5×10^5 cells in 2 ml of culture media are placed into 6 × 150 mm plastic plates. The cells are grown at 37°C in an incubator in a 5% carbon dioxide atmosphere. Cells attach to the bottom of the plates and rhythmic contraction starts in some cells within 24 hours. Medium changes are made every 2 days while the cells are developing. Many cells develop regular contractions at rates between 12 and 150 per minute. All studies are carried out at 3 to 6 days after plating the cells, although beating may continue to 9 or 10 days in some cell clusters.

The rate of contractions of cells was observed with a Zeiss Plankton microscope with inverted phase contrast optics. The observer records the contractions which occur in 2 one-minute periods. Increases and decreases in contraction rate were defined as changes greater than ± 3/minute. Cells were considered to have stopped when no spontaneous activity was noted for 2 minutes. All studies are carried out at 25° and all reagents are preincubated to that temperature. Drugs were added by medium change after they have been mixed with Puck's medium and all doses are reported as the base compound.

Results. The morphology of developing cells is illustrated in Fig. 1 and 2. No attempt to distinguish myocytes from other cell types was made except on the basis of their contractile properties. Myofibrillar structure was not noted in these cultures. As the cells aged, highly refractile inclusions are noted within their cytoplasm (Fig. 2).

Isoproterenol, a specific stimulator of beta adrenergic receptors(7) produces increases in the rate of contraction of cells (Table I). The threshold dose appears to be between 0.2 μg

* Supported in part by Grants HE 09058-03, AI-05629-04, and Training Grant 1 T1 HE 5709-01.

FIG. 1. Chick heart cells in culture at 6 days after plating.

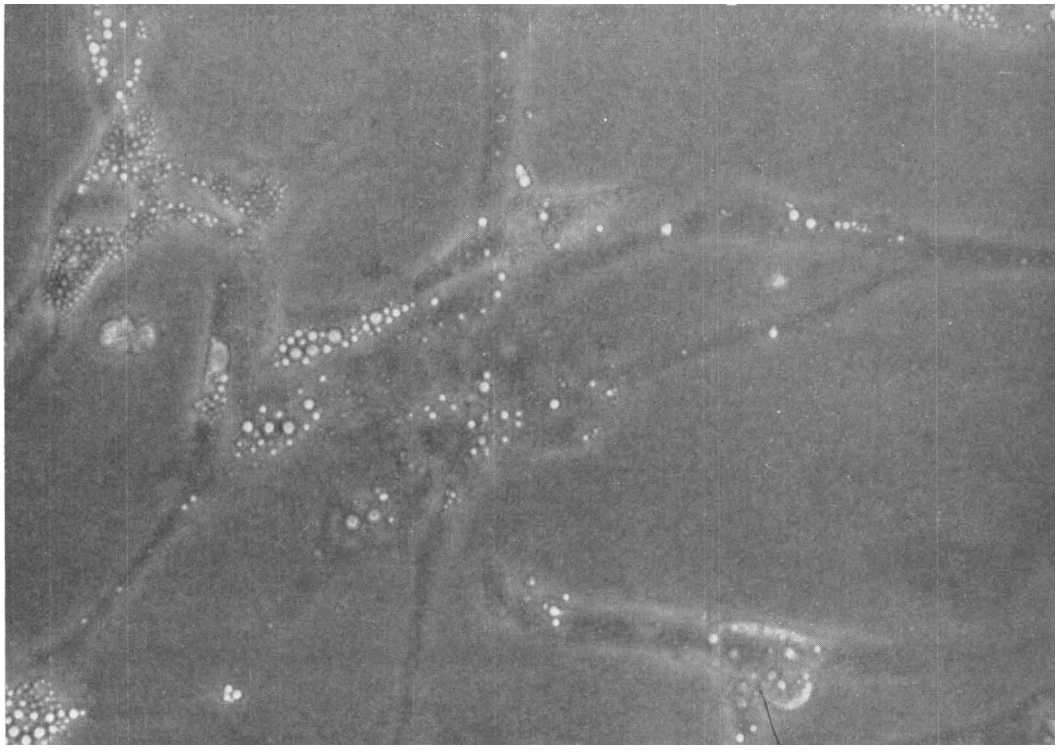

FIG. 2. Heart cells in tissue culture at 6 days. Note interconnecting processes from cells and refractile inclusions.

TABLE I. Response of Heart Cells to Isoproterenol.

Concentration ISO	No. of studies	Increase	Decrease	No change	Stop
0 (medium alone)	38	5	3	27	3
.2 μg/cc	6	1	1	4	0
1.0 "	20	14	1	4	1
10 "	16	12	0	2	2
100 "	10	8	0	0	2
1000 "	8	2	0	0	6

and 1.0 μg/cc of medium. Medium change alone without drug rarely results in increases and decreases in the contraction rate of cells, but in most instances produces no change in the rate (Table I). Dose response curves showing the average change in rate produced by medium change and isoproterenol are illustrated in Fig. 3. The standard errors of the mean at the highest dose and the media change are ± 7 beats/minute and ± 2 beats/minute, respectively. At higher concentrations, *viz.*, 1000 μg/cc isoproterenol, most cells transiently increase their rate but then develop periods of quivering movements prior to stopping (Table I). The pattern of contraction also changes after addition of isoproterenol, appearing more forceful.

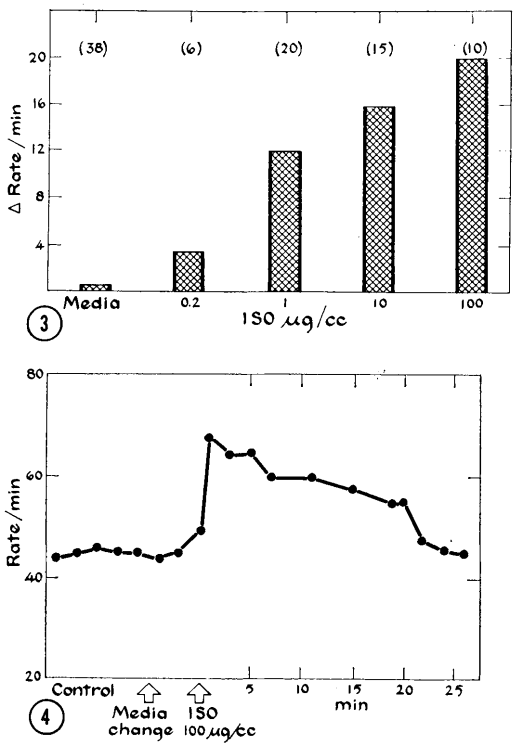

FIG. 3. Dose response curve for isoproterenol showing average change in contraction rate at various concentrations of isoproterenol (ISO μg/cc). Number of experiments in each group is shown above each column.

FIG. 4. Rate of response of typical heart cells to media change and isoproterenol (ISO μg/cc).

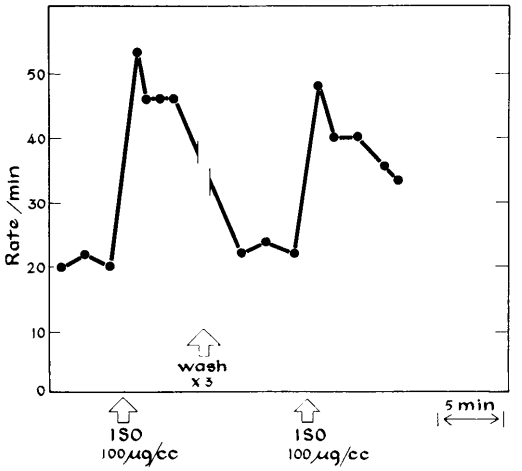

FIG. 5. A typical response of cells to isoproterenol, 100 μg/cc before and after washing 3 times with Puck's medium.

After addition of 100 μg/cc isoproterenol, approximately 25 to 30 minutes are required for a gradual return to control rate (Fig. 4). In some instances the effects of the drug can be removed by washing several times with fresh medium and increases produced again by addition of the same dose of isoproterenol (Fig. 5).

Since the cells appear to be stimulated by the highly specific beta receptor agonist, isoproterenol, further studies to determine the effects of propranolol, a new highly specific beta receptor blocking agent(8), were performed. The addition of propranolol produces marked slowing of the contraction rate (Fig. 6), and in large concentrations, 100 μg/cc, most clones stopped contracting. The addition of isoproterenol only partially restores the contraction rate to control levels. When

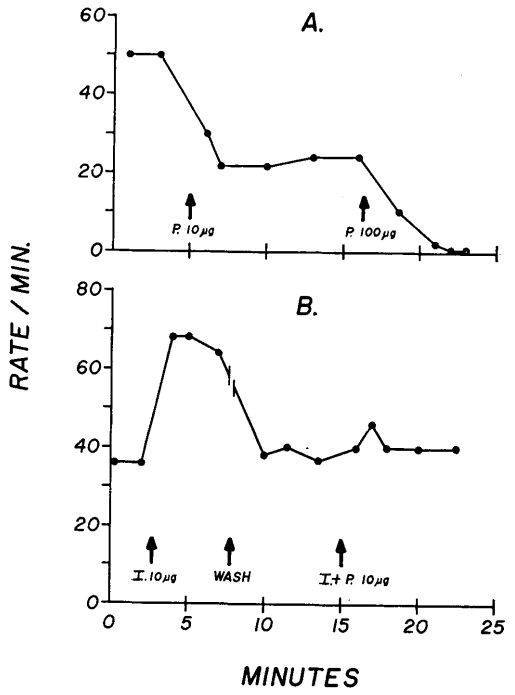

FIG. 6A. A typical rate response of cells to propranolol (P) 10 μg/cc and 100 μg/cc.

FIG. 6B. A typical rate response of cells to isoproterenol (I) 10 μg/cc followed by a wash with Puck's medium and second challenge with isoproterenol (I) and propranolol (P) administered simultaneously.

isoproterenol and propranolol are added simultaneously to stimulate the cells, only minimal increases in the rate of contraction are noted (Fig. 6).

Discussion. Isoproterenol is active in stimulating the rate of heart cells grown in tissue culture which lack sympathetic nerves. Although the threshold dosage of isoproterenol is much higher than *in vivo* studies, progressively larger doses produce greater increments in the rate of contraction. The large concentrations of isoproterenol required to stimulate these cells *in vivo* may be a result of the lower temperature of 25°C at which the drug studies were performed. At extremely large doses, the quivering movements we have observed may represent fibrillatory activity prior to total arrest of the cells. These findings with isoproterenol correlate with *in vivo* studies which have shown a greater rate of spontaneous electrical depolarization of ventricular tissue in patients with heart block when treated with isoproterenol(9).

It seems likely that the stimulation produced by isoproterenol results from activation of a beta receptor mechanism. Furthermore, it appears that this mechanism does not depend on the presence of sympathetic nerve endings because they are not present in tissue culture. We expected these results for Fingi *et al* (10) had shown that 16- to 21-day embryonic chick hearts *in situ* are responsive to the action of sympathomimetic agents at a time prior to the growth of sympathetic nerve fibers into the heart. Investigations utilizing intracellular electrodes to study electrical depolarization of dispersed heart cells in tissue culture from embryonic chick heart at 5-7 days have shown that epinephrine did not alter the rate of depolarization(11). In addition, these workers failed to demonstrate any effect of autonomic agents on heart cells taken from 6-day-old chickens, although Barry(12) has clearly demonstrated an action of epinephrine on the intact heart of the 3-day-old chick. These discrepancies may be due to an alteration of excitation-contraction coupling produced by the micropuncture techniques or to other differences in design of these experiments.

It is not possible to ascertain whether the loss of the isoproterenol effect is due to enzymatic degradation of the drug in this system, although the enzymes that degrade catecholamines have been demonstrated in denervated tissue(13).

The effects of the beta blocking agent, propranolol, in slowing the contraction rate of these cells may result from its non-specific effects similar to that of pronethalol(14) on cardiac tissue. This non-specific action is similar to that noted for quinidine in the intact dog heart(15) and in tissue culture(4). When added simultaneously with isoproterenol, propranolol blocked the increase in contraction rate which was produced by the isoproterenol in the control period. Although this would support the concept that propranolol is a competitive receptor blocking agent, its non-specific slowing effects could produce these findings. The dose of propranolol used in these studies was large when compared to *in vivo* studies where this

drug has been utilized. The precise mechanism of the agonist-antagonist action of isoproterenol and propranolol for the beta receptor is under further investigation.

Summary. Trypsin dispersed chick embryo heart cells have been grown in tissue culture to produce rhythmic contraction cell clusters. Isoproterenol, a specific beta adrenergic stimulating drug, has been shown to increase the rate of contraction of these cells. This effect persists for 20-30 minutes and can be removed by washing the cells with fresh control media. Propranolol, a specific beta adrenergic blocking agent, blocks the stimulating action of isoproterenol. It appears likely that stimulation of beta adrenergic receptors of the Ahlquist classification can be used to explain these findings. These receptors apparently develop in the absence of adrenergic nerve endings.

ADDENDUM. At a recent symposium on the structure and function of heart muscle, Wollenberger described his unpublished observations(16) on the effects of adrenergic stimulating and blocking drugs on heart cells in tissue culture which are in general agreement with this report.

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Received September 19, 1966. P.S.E.B.M., 1967, v124.

Effects of Acutely Induced Ischemic Heart Failure on Myocardial High Energy Phosphate Stores. (31681)

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When severe myocardial ischemia occurs, a deterioration of cardiac contractile function takes place. It is generally assumed that the primary metabolic consequence of a severe impairment of oxygen delivery consists of a shift from aerobic to anaerobic metabolism (1-3), with an attendant reduction in the production of high energy phosphate compounds(3). This reduction is thought to be the ultimate cause of the breakdown of cardiac function(3-7). It was the purpose of the present investigation to test this hypothesis and specifically to determine whether there is a relation between the store of high energy phosphate compounds available to the myocardium and the depression of myocardial function produced by acute ischemia. Severe myocardial ischemia was produced by abruptly reducing coronary arterial flow in anesthetized dogs. Biopsies of the left ventricle were obtained before and during the development of heart failure, and correlations were made between the sequential changes in left ventricular function and the high energy phosphate stores.

Methods. Preparation. Twelve mongrel dogs weighing 15.0 to 26.4 kg were anesthetized with an average dose of sodium pento-