Effects of Epinephrine and 5-Hydroxytryptamine on Strips of Frog and Turtle Ventricle.* (27192)

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This study is a contribution to the comparative cardiac pharmacology of epinephrine and 5-hydroxytryptamine (5-HT). Epinephrine when applied to the hearts of most mammals(1,2) has positive inotropic and chronotropic effects. However, certain fish and molluscan hearts(3,4) do not so respond. Similarly 5-HT has positive inotropic and chronotropic effects in many classes ranging from mammalia (rabbits, dogs and cats 5,6,18) to mollusca (clams, 7,8) and arthropoda (crustacea, 9).

Amphibian and reptilian hearts have been favorite preparations for testing cardiac drugs. The effects of epinephrine and 5-HT on stimulus threshold, conduction velocity, Q-T interval (refractory period), and force of contraction were recorded from strips of frog and turtle ventricle. In addition ouabain and procainamide were applied in order to check with data available in the literature and thereby establish the adequacy of our technic. Epinephrine and 5-HT were also injected into turtles intravenously. In addition both substances were applied at acid and alkaline pH's to strips of frog ventricle.

Materials and methods. Hearts from painted turtles (*Pseudonyms elegans*) and from frogs (*Rana pipiens*) were excised and dropped into phosphate buffered Ringer's solution. A v-shaped strip extending from the heart's base to apex and back to the base on the opposite side was cut from the lateral edges of the ventricle. The strip was supported in air and stimulated at a constant frequency by means of an American Electronics Laboratories model 104A stimulator and a model 112 stimulus isolation unit. The stimulus rate was 6/min. during electrical recording and 12/min. during mechanical recording. Stimulus duration was constant at 1 msec. for all experiments. Twice threshold voltage was used for stimulus intensity.

Stimulating and recording electrodes were arranged as shown in Fig. 1 and 3. When the waves of depolarization and repolarization passed the proximal pickup electrode (P), the potential difference between P and the indifferent electrode (I) was recorded on a 2-channel Sanborn recorder. A similar recording was taken at the distal pickup electrode D. A moistened piece of filter paper touched the tissue and supported the indifferent electrode; it formed a conducting pathway between P and I and between D and I. Test periods were one to 2 minutes apart when the agent was first applied. Later, the periods between measurements were lengthened progressively. Four or 5 successive electrograms were recorded at each test period. From the electrograms (Fig. 2) we

Figure 1

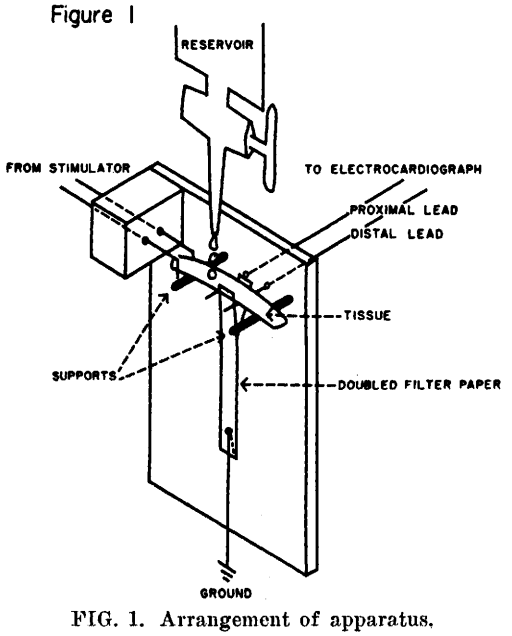

FIG. 1. Arrangement of apparatus.

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FIGURE 2

DIAGRAM OF ELECTROGRAMS

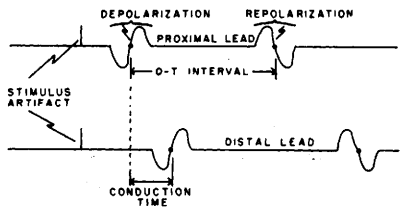

FIGURE 3

DIAGRAM OF PREPARATION

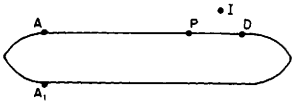

FIG. 2. Electrograms of ventricular strips obtained from proximal (upper) and distal (lower) monopolar leads.

FIG. 3. Electrode placement along ventricular strip. A_1A_1 are stimulating electrodes. P is proximal, D distal and I the indifferent recording electrode (farther away than shown here).

measured conduction time and interval between depolarization and repolarization, which is labelled "Q-T interval". Stimulus threshold was determined during each test period by reducing the stimulus intensity until the tissue just failed to respond.

The force of isometric contraction was measured in a series of experiments on turtle and frog strips and on turtle hearts *in situ*. The strips were stimulated as described above and arranged to pull vertically on a Statham strain gauge. The responses were recorded on a Sanborn recorder.

The ventricular strips were superfused with a phosphate buffered turtle Ringer's solution at pH 7.0-7.2 (no glucose). The superfusion solution was dropped directly onto the tissue at approximately 30 drops per minute. This procedure kept the entire preparation moist. The drip was turned off one minute before taking electrical recordings. When mechanical contraction was recorded, the superfusion was continued throughout the experiment.

Drug solutions were prepared just before use from pure dry salts dissolved in turtle Ringer's solution, except for ouabain which was prepared by diluting a commercially available solution (Eli Lilly and Co.) The following drugs were used: procainamide hydrochloride (USP), l-epinephrine bitartrate (USP), l-arterenol bitartrate (USP), 5-hydroxytryptamine creatinine sulfate (Mann Research Laboratories, Inc.), dl-n-isopropyl-arterenol hydrochloride (NNR), and ouabain.

Two other procedures were used in a few experiments. In one procedure epinephrine or 5-HT was injected intravenously into turtles. The frenulum of the exposed heart was attached to the strain gauge and the force of contraction was measured. In the other variation frog strips were subjected to 5-HT at different pH values. The superfusing fluid was buffered with imidazol (20 mM) and histidine (20 mM). Hydrochloric acid (0.1 N) was added to lower the pH to the desired value before superfusion began.

Results. Effects following the administration of ouabain and procainamide to strips of turtle ventricle are summarized in Table I. Values shown are averages obtained from 4 or 5 electrograms obtained during each of several consecutive test periods. Each parameter is expressed as a percent change from control value. Asterisks mark the results that are significantly different from this control at or above the 95% level of significance (T test).

Procainamide at concentrations of 0.5 μg/ml and 5 μg/ml did not alter significantly the Q-T interval. However, at the end of one hour conduction time was increased more than 800% by the 5 μg/ml concentration and 83% by the 0.5 μg/ml solution. The stimulus threshold also rose at each of these concentrations. Ouabain reduced significantly

TABLE I. Procainamide and Ouabain on Turtle Strips.

	Duration of exposure, hr	Change expressed as % of control: Q-T interval	Conduction time	Threshold
Procainamide, 5 μg/ml	1	+ 6	+836*	+139*
Procainamide, .5 μg/ml	1	+ 2	+ 83*	+218*
Ouabain, 2.5 μg/ml	¾	- 18*	+250*	0
Ouabain, 1 μg/ml	1½	- 51*	+ 26	+ 21

* Values significantly different from zero ($p < .05$).

TABLE II. Epinephrine and Isoproterenol on Turtle Strips.

	Change in % from control		
	Q-T interval	Conduction time	Threshold
Epinephrine			
.5 μg/ml	+ 2.1	−4.1	+16.8
1 "	+12.7	− .4	+12.2
5 "	+ 5.1	+1.8	+17.7
5 "	+ 1.8	−5.4	− 3.8
Isoproterenol			
1 μg/ml	+ 3.2	+2.5	+ 3.1

No values are significantly different from zero ($p < .05$).

the Q-T interval and increased conduction time without altering significantly the threshold when it was applied in concentrations of 1 μg/ml and 2.5 μg/ml.

Table II shows the results when epinephrine was applied to turtle strips. Concentrations of 0.5 μg/ml, 1 μg/ml, and 5 μg/ml had no significant effect on Q-T interval, conduction velocity, or stimulus threshold. Three out of 4 experiments showed increases in stimulus threshold, but these increases were not significant at the 95% level. Isoproterenol in a concentration of 1 μg/ml was applied without significant effect on the parameters under consideration.

Tracings demonstrating the effects of epinephrine and norepinephrine on the force of contraction are shown in Fig. 4. Epinephrine in concentrations up to 10 μg/ml (not shown in the figure) had slight positive inotropic effects on turtle ventricular strips.

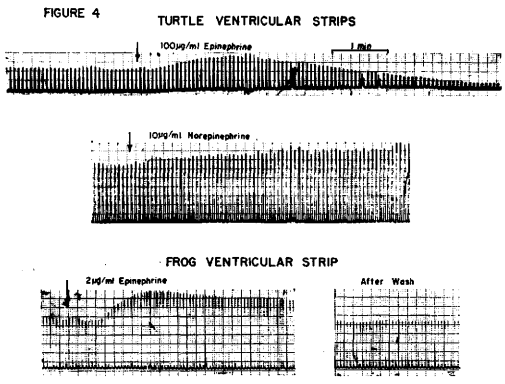

FIG. 4. To show effects of epinephrine and norepinephrine on force of contraction (isometric recording) of turtle and frog ventricular strips. Note occasional premature systoles.

Norepinephrine in a 10 μg/ml concentration increased the force of contraction as shown. This positive inotropic action of the catecholamines was demonstrated in all preparations of turtle ventricle tested in this way. At 100 μg/ml epinephrine first increased contractile force and then depressed it. A 1000 μg/ml solution of epinephrine was found to be only depressant. Both the enhancing and depressing effects caused by epinephrine could be reversed by returning to the control superfusion fluid. A 2 μg/ml solution of epinephrine produced a marked increase in the force of contraction of a frog ventricular strip (Fig. 4).

TABLE III. 5-Hydroxytryptamine (5-HT) on Turtle Strips.

5-HT conc.	Q-T interval	Conduction time	Threshold
1 μg/ml	− 1.6	− 2.1	+ 4.5
10 "	+ 3.9	− 8.3	+14.1
" "	+ .1	− .7	+ 2.0
" "	− 9.9	+ 5.0	15.8*
" "	+15.7	−32.2	+47.1*
" "	−25.6	−14.4	−10.3
" "	+ 2.4	− 7.2	− 3.8

* Values significantly different from zero ($p < .05$).

Table III summarizes the 3 electrical properties in 7 experiments where 5-HT was applied in frog Ringer's at pH 7. The results are variable. Only 2 figures are significantly different from zero, and no consistent trends appear. The force of mechanical contraction was not altered by 5-HT in concentrations from 0.1 μg/ml to 100 μg/ml in 14 experiments on turtle ventricular strips and 3 experiments on frog strips. At 1000 μg/ml 5-HT was depressant.

In 15 experiments on frog strips where the pH was adjusted to values ranging from 8.2 to 6.0, no change in the force of contraction was measured when 5-HT was applied in concentrations of 1 μg/ml and 10 μg/ml. In one experiment a strip showed an increased amplitude after 1 μg/ml 5-HT at pH 7.8 and at 7.5 but not at pH 8.2. Premature systoles and short periods of autorhythmicity were present during these periods of enhanced contractile force, but the same strip, when it responded exclusively to the driving stimulus,

did not show an increase in force associated with 5-HT. Epinephrine as well as 5-HT occasionally produced premature systoles and periods of autorhythmicity.

Discussion. Some of the basic studies on the heart actions of digitalis and quinidine have been done on isolated strips of turtle ventricle(10,11). The adequacy of our preparation has been tested by use of ouabain and procainamide and our results agree with earlier findings. In accordance with the procedure suggested by Blair, Wedd, and Young(12) the term Q-T interval is used to describe the period between depolarization and repolarization, *i.e.*, the refractory period.

Wedd and others have reported that epinephrine in concentrations up to 100 μg/ml has little or no effect on contraction, refractory period or fiber conduction in ventricular strips(1,13,14). Our results on the force of contraction differ. We find that in lesser concentrations epinephrine and norepinephrine stimulate contractile force and in higher concentrations depress it. These differences of observations may be due to differences in technic, *i.e.*, superfusion rather than submersion and measurement of isometric rather than isotonic contractions. Although we have no specific data concerning the mechanism of action of epinephrine, we have demonstrated that it does act on a strip of turtle ventricle, perhaps directly on muscle cells but perhaps on specialized tissue or even on sympathetic nerve fibers both of which are present(15-17). Strips of ventricular tissue from frogs are sensitive to even lower concentrations of epinephrine. It is probable that the active agent penetrates the thinner frog strip more readily. Deficient diffusion throughout the extracellular spaces of the turtle strip may be the reason for the lack of response to epinephrine reported by Wedd(1).

Many workers have reported on the effect of 5-HT on heart tissue(5-9,18). Mammalian and lamellibranch hearts increase their force of contraction when exposed to 5-HT. In our experiments on reptilian and amphibian strips, 5-HT in concentrations up to 10 μg/ml produced no effect on the 3 electrical parameters under study. Concentrations up to 100 μg/ml did not affect the contractile force of turtle or frog strips. We assume that the test agent was able to enter the extracellular space, especially in the thin strips of frog ventricle, but we have no conclusive proof that 5-HT reached a site where it might act. However, the lack of effect following intravenous injections gives us confidence that 5-HT does not have a significant action on turtle ventricular muscle in the doses used except for occasional production of premature beats.

Summary and conclusions. Ventricular strips from turtles and frogs were isolated, suspended in air, superfused with buffered Ringer's solution and driven at a constant frequency. The conduction velocity, stimulus threshold, Q-T interval (refractory period), and force of isometric contraction were measured. 1. Ouabain and procainamide elicited their previously documented effects on these isolated ventricular strips. 2. Contrary to a previous report, epinephrine increased the force of contraction of an electrically driven strip of turtle ventricle, but did not affect the other parameters. 3. 5-HT did not alter significantly conduction time, stimulus threshold, Q-T interval or force of contraction of either ventricular strips or of turtle hearts *in situ*, when applied in neutral, acid, or alkaline solutions. 4. Epinephrine and 5-HT occasionally produced premature systoles and periods of autorhythmicity.

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Effects of Acetylsalicylic Acid on Excretion of Endogenous Metabolites By Man.* (27193)

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Much of the current information on the biochemistry of salicylate has recently been reviewed by Smith(1). Despopoulos(2) has suggested the possibility that the salicylate inhibition of "active accumulation" of p-amino hippuric acid (PAH) may be due to disruption of mitochondrial metabolism. Yu and Gutman(3) have observed a paradoxical effect of varying doses of salicylate on excretion of uric acid by man. On the basis of these types of studies it seemed important to determine whether salicylate would affect the excretion of endogenous metabolites in man since many of these metabolites are "actively transported". The effect of varying amounts of acetylsalicylic acid on excretion of creatinine, urea, uric acid, amino nitrogen, inorganic phosphate, sodium, potassium and chloride was studied.

Procedure and methods. A group of 25 college and medical students (males) served as experimental subjects. Urine samples were collected by voiding after hydration with water until urine flow ranged between 4-20 ml/min. Base line studies were obtained on all subjects as previously described by Wu and Elliott(4). Analytical methods used include alpha amino nitrogen by Yemm and Cocking(5), amino acid chromatography by Hardy(6), flame photometric determination of sodium and potassium(7) and uric acid analysis by the methods of Caraway(8) and Liddle(9).

A series of 4 control samples of urine was collected from each subject and 0.6 or 1.8 g of acetylsalicylic acid (aspirin) were given by mouth. Urine samples were then collected at 15-20-minute intervals for 4 hours.

Results. Fifty-four experiments with varying oral doses of acetylsalicylic acid were performed. The findings are as follows: *sodium, potassium and chloride.* The drug caused a decrease in sodium and chloride excretion in every subject studied except one, following both the 0.6 and the 1.8 g amounts of the drug. The data in Table I show that both amounts had essentially the same effect. Excretion rate data in meq/min for a typical experiment are plotted in Fig. 1. Potassium excretion was not affected in these experiments. *Amino acids.* Aminoaciduria was found in every subject following administration of 1.8 g of the drug. Average increase in excretion rate was 202%. Data from one of these experiments are shown in Fig. 2 and Table I. *Uric acid.* Varying degrees of uricosuria resulted from administration of 1.8 g of drug in all but 3 subjects. Small doses (0.6 g) caused varying retention of uric acid in all subjects so that ex-

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