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THE INTERACTION OF SOME STIMULANT AND DEPRESSANT DRUGS ON THE FROG HEART

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The activity of frog isolated hearts was depressed by altering the perfusing Ringer solution in five different ways: by reducing the calcium content, by increasing the potassium content, and by adding ether, thiopentone or acetylcholine. Depressed hearts were perfused with Ringer solution containing the following stimulant drugs: paullinia tannin, tannic acid, hydrogen peroxide, sodium oleate, sodium caprylate and ouabain. All these stimulant drugs had similar actions on hearts depressed by calcium lack, ether and thiopentone; hearts depressed by acetylcholine were, however, only weakly stimulated. Hearts depressed by potassium were readily stimulated by oleate, caprylate and paullinia tannin; ouabain and hydrogen peroxide had weak stimulant actions on hearts depressed by potassium, and tannic acid had a negative inotropic action. The differing actions on hearts depressed by potassium are probably related to differences in the degrees of fixation of the stimulant drugs. The mode of action of ouabain and the functional lesion in hearts depressed by narcotics are discussed.

Frog hearts depressed by perfusion with low-calcium Ringer solution lose calcium from a superficial site in the heart where calcium is required for the onward propagation of the excitatory process (Niedergerke, 1957). Hydrogen peroxide, tannic acid, paullinia tannin, sodium oleate and sodium caprylate stimulate these hearts only when there is calcium in the perfusing fluid (Broadbent, 1962b). Cardiac glycosides, on the other hand, mobilize bound calcium inside the heart (Lüllmann & Holland, 1962; Klaus & Kuschinsky, 1962). If this mobilization is the basis of the stimulant action of ouabain then all the drugs stimulate by a common mechanism, and one would expect close similarities in the abilities of the drugs to stimulate hearts depressed in different ways. A comparison was therefore made of the abilities of the drugs to stimulate hearts depressed in five different ways: by perfusion with low-calcium or high-potassium Ringer solutions, or by the addition of ether, thiopentone or acetylcholine to the Ringer solution.

METHODS

Frog hearts were dissected and perfused as previously described (Broadbent, 1962a). The perfusion assembly maintained constant venous and arterial pressures and permitted simultaneous recordings of heart movements and cardiac output (in drops per given time interval). The heart rate was counted. Experiments were performed at room temperature and the frogs (*Rana temporaria*) were stored at 3° C and allowed to warm up 24 hr before use. The Ringer solution had the following composition: NaCl 6.5 g (112.0 mM), $CaCl_2$ 0.12 g (1.08 mM), KCl 0.14 g (1.88 mM), $NaHCO_3$ 0.5 g (5.95 mM) and distilled water to make 1 l. In

low-calcium Ringer solution the calcium chloride was reduced to 0.03 g/l. (0.27 mM). In experiments where the heart was depressed by potassium the potassium chloride concentration was raised to 0.56 g/l. (7.52 mM). The other depressant drugs were ether anaesthetic B.P. (5×10^{-3}), thiopentone sodium (5×10^{-5}) and acetylcholine chloride (1×10^{-8}). All solutions were freshly prepared.

Hearts were perfused with Ringer solution until heart rate, ventricular movements and cardiac output were steady. They were then perfused either with low-calcium Ringer solution or with Ringer solution containing the depressant drug for 5 min; next with the previous solution with addition of the cardiotonic drug, usually for 30 min, and finally with Ringer solution. The cardiotonic drugs and their concentrations (w/v) were ouabain U.S.P., 2×10^{-6}; paullinia tannin, prepared from *Paullinia pinnata* L., as described by Bowden (1962), 1×10^{-4}; tannic acid B.P., 1×10^{-4}; hydrogen peroxide, 1×10^{-5}; sodium oleate, 1×10^{-4}; and sodium caprylate, 1.6×10^{-4}.

For each heart the cardiac stroke output, in drops per heart beat, was calculated for a preliminary "control" period; for the period of depression, at the time of maximum depression; and for the period of stimulation (if any), at the time of maximal stimulation. Each stimulant drug was tested on two depressed hearts. Usually the degrees of stimulation in the two experiments were similar. If a large difference occurred further experiments were performed and the results from two typical experiments were recorded. Where unexpected results were obtained, such as the negative inotropic action of tannic acid upon hearts depressed by potassium, additional confirmatory experiments were performed. Since six stimulant drugs were studied and the effect of each was recorded on two hearts, the heart rates and cardiac stroke outputs in Table 1 are the means of twelve readings.

The degree of stimulation was expressed by a system of scoring for each heart: −=negative inotropic action; 1=any degree of stimulation up to 50% of the difference between depression and control; 2=stimulation between 50 and 100% of the difference between depression and control; and 3=stimulation equal to, or above, the control level. So in Table 2, stimulation of two hearts to the control level or above is shown by a score of 6, and scores of 5, 4, 3, 2, and 1 represent progressively lesser degrees of stimulation.

RESULTS

All the depressant agents reduced the mean heart rate, but the reduction was much greater and highly significant with potassium (Table 1). Hearts depressed by ether or thiopentone, especially the latter, frequently developed bradycardia when subsequently perfused with tannic acid or ouabain. Because of these changes in heart rate, cardiac output was expressed as stroke volume. Each of the depressant agents significantly reduced stroke volume (Table 1).

TABLE 1

EFFECTS OF DEPRESSANT AGENTS ON RATE AND STROKE OUTPUT OF THE FROG HEART

The concentration of calcium refers to calcium chloride; that of potassium to potassium chloride. Values are means±s.e. each of twelve observations. * Difference not significant

		Heart rate (beats/min)			Stroke output (drops/beat)		
Depressant agent	Concentration	Control period	Depression	*P*	Control period	Depression	*P*
Low calcium	3×10^{-5}	48·0±2·24	45·8±2·15	*	1·29±0·14	0·18±0·07	<0·001
Ether	5×10^{-3}	51·0±1·96	47·5±2·39	*	1·23±0·12	0·54±0·10	<0·001
Thiopentone sodium	5×10^{-5}	56·1±2·95	49·7±2·74	*	1·56±0·15	0·71±0·12	<0·001
Acetylcholine	1×10^{-8}	46·3±2·64	41·3±2·29	*	1·37±0·21	0·38±0·10	<0·001
Potassium	$5·6\times10^{-4}$	51·7±3·04	31·9±3·53	<0·001	2·10±0·23	0·64±0·09	<0·001

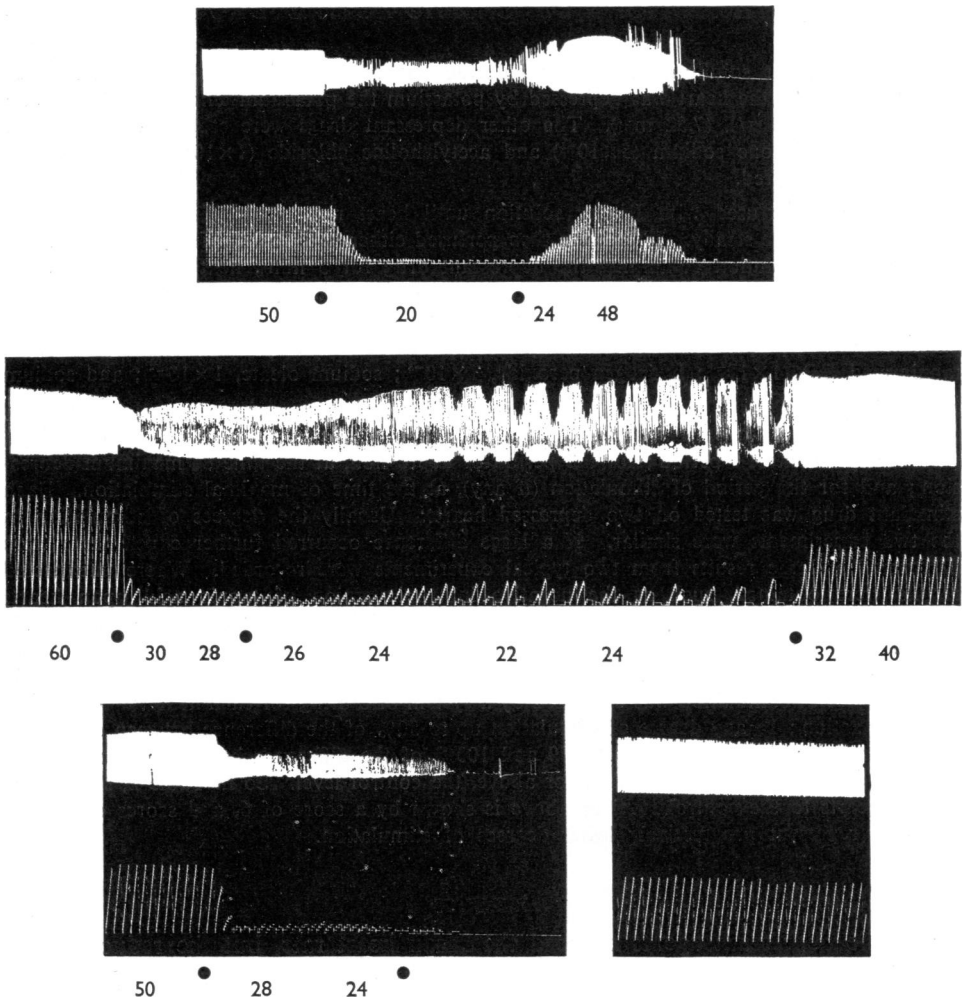

Fig. 1. The effects of some cardiotonic drugs on hearts depressed by 7.52 mM-KCl.

Top record: A frog isolated heart perfused with Ringer solution. Upper tracing, ventricular movements (systole upwards); lower tracing, cardiac output over periods of 10 sec; numerals give heart rates (beats/min). At the first dot the perfusion fluid was changed to high-potassium Ringer solution. At the second dot perfusion with high-potassium Ringer solution containing sodium oleate (1×10^{-4}) was started. Sodium oleate produced its usual strong cardiac stimulation, which ended in arrest of the ventricle in systole.

Middle record: A similar heart perfused with Ringer solution. Tracings as above except that cardiac output is recorded for periods of 30 sec. At the first dot the perfusion fluid was changed to high-potassium Ringer solution. At the second dot perfusion with high-potassium Ringer solution containing ouabain (2×10^{-6}) was started. Slight stimulation interspersed with periods of depression occurred. At the third dot, 30 min later, perfusion with ordinary Ringer was resumed. The relatively normal cardiac function indicates reduced fixation of ouabain.

Bottom record: A similar heart perfused with Ringer solution. Tracings as in the middle record. At the first dot the perfusion fluid was changed to high-potassium Ringer solution. At the second dot perfusion with high-potassium Ringer solution containing tannic acid (1×10^{-4}) was started. This compound had a negative inotropic action which led to ventricular arrest. The second part of the record shows recovery of the heart 15 min after the return to perfusion with Ringer solution.

The effects of the stimulant drugs on the stroke volume of depressed hearts are summarized in Table 2. It can be seen that strong stimulants of hearts depressed by calcium lack, namely ouabain, paullinia tannin, hydrogen peroxide and sodium oleate, also stimulated strongly hearts depressed by the narcotics, ether and thiopentone sodium. Weaker stimulants of hearts depressed by calcium lack, namely, tannic acid and sodium caprylate, had weaker actions on the hearts depressed by narcotics.

Each of the stimulant drugs had only weak actions on hearts depressed by acetylcholine. However, oleate (1×10^{-4}), which usually only stimulated hearts depressed by acetylcholine weakly, stimulated a few hearts strongly.

In hearts depressed by potassium, ouabain frequently caused slight stimulation alternating with periods of depression, so producing a " wavy " appearance of the

TABLE 2

EFFECTS OF SOME CARDIAC STIMULANT DRUGS ON THE STROKE OUTPUT OF DEPRESSED FROG HEARTS

6, 5, 4, 3, 2 and 1 represent decreasing degrees of stimulation; — = negative inotropic effect. The figures are derived from two experiments for each combination of depressant and stimulant drug

		Depressant agent				
Stimulant drug	Concentration	Ca^{++} lack	Ether	Thiopentone	Acetylcholine	K^{+} increase
Ouabain	2×10^{-6}	6	5	6	3	3
Paullinia tannin	1×10^{-4}	6	6	5	3	6
Hydrogen peroxide	1×10^{-5}	6	6	5	3	3
Tannic acid	1×10^{-4}	3	4	3	3	—
Sodium oleate	1×10^{-4}	6	6	6	2	6
Sodium caprylate	$1{\cdot}6 \times 10^{-4}$	2	3	4	1	3

record of heart movements (Fig. 1). After perfusion of these hearts for 30 min with solutions containing ouabain or hydrogen peroxide, and then a change to perfusion with Ringer solution, the signs of toxicity (irregularities of contraction) disappeared, and systolic arrest did not occur, which indicated reduced fixation of these cardiotonic drugs. Paullinia tannin, sodium oleate and sodium caprylate stimulated hearts depressed by potassium to the same extent as hearts depressed by other means, but the stimulant properties of ouabain and hydrogen peroxide were less in hearts depressed by potassium than in those depressed otherwise, and tannic acid had a negative inotropic action. These differing actions of the stimulant drugs on hearts depressed by potassium are illustrated in Fig. 1.

DISCUSSION

The six stimulant drugs have many features in common: they are weak stimulants of normal frog hearts; they stimulate hearts depressed by calcium lack and by the narcotic drugs, ether and thiopentone; and they all usually cause systolic arrest of the ventricle. Also, all the stimulants except tannic acid (Table 2) have weaker actions on hearts depressed by acetylcholine, which inhibits both the contractile process and the process maintaining the surface potentials (Clark,

Eggleton, Eggleton, Gaddie & Stewart, 1938). Similarly, Loewi (1949) reported that the augmentative effect of oleate was greatly diminished on hearts depressed by acetylcholine (but not by potassium).

However, the stimulant drugs differ in their actions on hearts depressed by potassium. These differentiations are probably due to differences in the degrees of fixation of the stimulant drugs, and can be explained as follows: high potassium concentrations prevent the fixation of ouabain in guinea-pig atria (Holland & Sekul, 1961) and Fig. 1 suggests that the same is true for the frog heart. Hydrogen peroxide and the two tannins are fixed at the same cardiac site as ouabain, but paullinia tannin has a greater affinity for the heart than tannic acid (Broadbent, 1962a, b). High concentrations of potassium do not prevent the fixation of paullinia tannin, which has its usual stimulant action, but fixation of ouabain and of hydrogen peroxide is reduced and they have weak actions. Fixation of tannic acid is prevented and the formation in the Ringer solution of tannin-calcium complexes reduces the Ca^{++} concentration of the Ringer solution. Hence tannic acid acts as a chelating agent for calcium, and the actions on the heart are similar to those of edetic acid in calcium-free Ringer solution (Broadbent, 1962b). Oleate and caprylate, which are fixed at a different cardiac site or sites, have their usual stimulant actions.

The general similarities in the stimulations produced by hydrogen peroxide, the two tannins, oleate and caprylate on the one hand, and ouabain on the other, suggest that a common mechanism may be involved. Since five of the drugs depend on the presence of calcium in the Ringer solution for their stimulant action and since ouabain mobilizes intracellular calcium (Lüllmann & Holland, 1962; Klaus & Kuschinsky, 1962), it is likely that this mobilization of calcium is the cause of the positive inotropic action of ouabain.

Similarities between hearts depressed by calcum lack and hearts depressed by narcotic drugs have been noticed previously (Clark *et al.*, 1938; Wollenberger, 1949). In the present experiments drugs which strongly stimulate hearts depressed by calcium lack, namely ouabain, paullinia tannin and oleate, were strong stimulants of hearts depressed by the two narcotics; and drugs which weakly stimulated hearts depressed by calcium lack, tannic acid and caprylate, were weaker stimulants of hearts depressed by narcotics. As hearts depressed by calcium lack lose calcium from a superficial site in the heart where calcium is required for the onward propagation of the excitatory process (Niedergerke, 1957), it may be inferred that the depressed condition of narcotized hearts is due to a similar depression in the propagation of the excitatory process (as opposed to the contractile process) and that this depression can be reversed by calcium. The observation of Berwick (1951), that concentrations of ether similar to those employed in the present experiments caused calcium loss from the frog gastrocnemius muscle, is consistent with this hypothesis. Moreover the depressant effect of pentobarbitone on cardiac contractility has been attributed to decreased mobilization of calcium at the surface membrane of the heart (Daniel, Johnson & Foulkes, 1962).

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