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OBSERVATIONS ON THE USE OF THE HEN AND RABBIT ISOLATED AURICLES FOR THE DETERMINATION OF INOTROPIC POTENCY

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The electrically driven, isolated auricles from the rabbit and the hen have been used to compare the inotropic potency of a bufodiene aglycone with ouabain. The results from the hen auricle permit conventional statistical treatment and show a good degree of reproducibility. Results of inotropic potencies and toxicity determinations on the two drugs are discussed.

In 1961 several crystalline bufodienolide aglycones were obtained from *Bersama abyssinica* Fres., sub-species *abyssinica* (Lock, 1962). The major component, designated Material R, had a lethal dose on intravenous infusion into cats of 122 ± 12 μg/kg (mean and standard error), which is similar to the value of 116 μg/kg obtained by Chen, Henderson & Anderson (1951) for ouabain. Before proceeding to a clinical trial it was decided to compare the inotropic potencies of the two drugs.

Two preparations frequently used for this purpose are the dog heart-lung preparation (Farah & Maresh, 1948) and the cat isolated papillary muscle (Cattell & Gold, 1938). With both these methods statistical evaluation of results presents some difficulties. It was therefore decided to investigate the inotropic effects of these drugs on isolated auricles.

Bhatt & MacDonald (1960) have used the rabbit spontaneously beating isolated auricles for digitalis assays. The object of the present work, however, was to estimate the inotropic potency of different drugs and it was therefore desirable to eliminate the possibility of interference from effects on conducting tissue or on the pacemaker. Accordingly, isolated, electrically driven auricles have been used. With the hen isolated auricle, inotropic potencies could be estimated and the results permitted conventional statistical treatment.

METHODS

Rabbits, guinea-pigs and rats were killed by a blow on the back of the neck, hens by wringing the neck. The rabbits weighed from 1.0 to 1.3 kg, the hens were a Light Sussex/Rhode Island Red cross and weighed about 2 kg. After killing the animal the heart was removed as quickly as possible. A thread was tied on to the base of the outer wall of the left auricle, and a fan-shaped piece of tissue was cut out of the auricle with the thread at the apex. The base of the fan was attached to a three-pronged silver electrode (Fig. 1), and

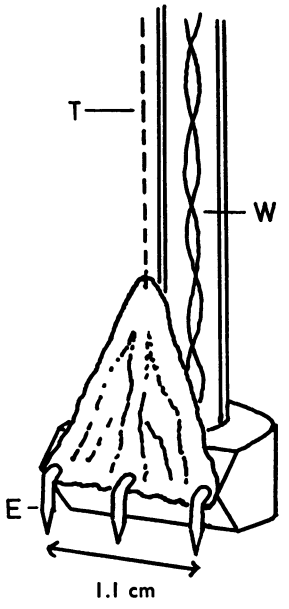

Fig. 1. Diagram of electrode, showing auricle wall in position. T: thread to recorder; E: silver prongs; the two outer prongs are electrically connected; W: wires to stimulator.

the apical thread attached to the recording device. The preparation was suspended in a 30 ml. organ-bath containing Locke solution of the following composition: NaCl 0.9% ; KCl 0.042% ; $NaHCO_3$ 0.3% ; $CaCl_2$ 0.024% ; and glucose 0.1% ; deionized water was added to make 100%. The bath was vigorously oxygenated The temperature was maintained at $30 \pm 0.1°$ C.

This " inverted " method of attaching the auricle was used because fat at the top of the heart made excision of a suitable piece of tissue difficult. The thread, tied to a fat-free portion of the heart just above the atrio-ventricular septum, facilitated rapid excision of a suitably shaped piece of tissue.

Speed in setting up the preparation greatly increased its longevity. The procedure could be completed within 3 min from the time of killing the animal.

Drugs were dissolved in aqueous ethanol, the concentration of ethanol being adequate to retain the drug in solution while not depressing the response of the tissue to stimulation.

Drugs were delivered by means of automatic syringes (Lock, 1961), and all cyclic operations were performed by a suitable device based on the use of Post Office selector switches and relays (Boura, Mongar & Schild, 1954).

An electronic rectangular-wave stimulator was used to stimulate the preparation, with supramaximal shocks usually at a frequency of 1 shock/sec.

Recording

Method (a). The thread from the auricle was tied to a semi-isometric torsion lever capable of rotational adjustment so that known tension could be applied to the preparation. The writing point consisted of aluminium foil at the end of a thin balsa arm 15 cm long and writing on cleared, smoked, photographic film. This provided a method of recording of relatively low friction ; after the film had been fixed by varnishing in the usual way, it could be placed in a photographic enlarger to facilitate measurement of the tracing. The lever

weighed 50 mg and gave a magnification of fifty-fold. Usually a tension of 1 g was applied to the tissue.

Method (b). The thread from the apex of the auricle was tied to the stylus of a mechanico-electronic transducer (RCA 5437), and a resting tension of 1 g was applied. Recordings were made using an oscillograph and camera.

RESULTS

The inotropic effect of drugs on these preparations was expressed as a percentage increase in force of contraction at various times after administration of a drug, over the force of contraction during the period immediately before adding the drug.

Rats

A few experiments performed on rat auricles showed that the response to ouabain was rapid, maxima occurring in 15 min and recovery taking a similar time. This time of response conforms with the results of Masuoka & Saunders (1950) on rat ventricles and Purkinje fibres. However, even in bicarbonate-free solutions (White & Salter, 1946) irregularities supervened when responses were more than 10 or 15%. The use of this auricle was not, therefore, pursued.

Rabbits

Auricles in Locke solution beat strongly when stimulated at 12 V and 5 msec duration and at a frequency of 1 shock/sec, but showed only slight responses to concentrations up to 20 μg/ml. of ouabain ; with higher concentrations irregularities and subsequent failure of the preparation occurred.

When placed in bicarbonate-free Locke solution the preparation rapidly became unresponsive and recovery with ouabain was not observed. In view of the complementary action of calcium and ouabain on heart muscle (Loewi, 1917), the effect of reducing the calcium concentration of the Locke solution to 50 and 30% of normal was investigated. The twitch tension rapidly diminished to a value dependent on the calcium concentration. Further, the responsiveness to ouabain increased as the calcium content was reduced. The maximal increase of twitch tension, plotted against the log of the dose, is expressed graphically in Fig. 2.

The force of contraction of the auricles in Locke solution with 30% of its normal calcium content was approximately half that of auricles in Locke solution with 50% of its calcium content and the response to ouabain was increased about three-fold, the response curve becoming manifestly steeper.

The sensitivity of the preparation was increased by reducing the calcium content still further, but its life was considerably shortened. In Locke solution with 25% of its normal calcium content the preparation rarely lived more than 8 hr, with 12.5% 6 hr or less. A calcium content of 30% of normal was, therefore, used in the following experiments with rabbit auricles.

Comparison of potency of ouabain and Material R. The previous experiment showed that the time for the development of maximal effect for ouabain was about 25 min. An attempt to compare Material R with ouabain was then made, with an approximately 1 hr cycle, allowing 30 min before recording and 30 min for

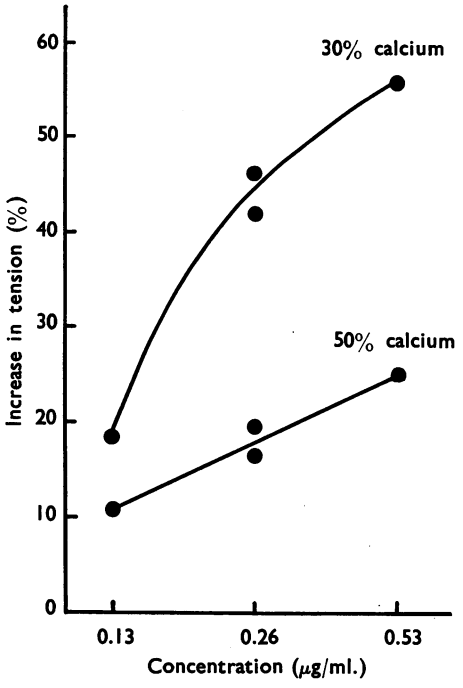

Fig. 2. The effect of 30 and 50% of the normal calcium concentration in Locke solution on the response of the rabbit auricle to ouabain, on the same preparation. Ordinate: maximal percentage increase in twitch tension. Abscissa: concentration (μg/ml.) of ouabain, log scale.

recovery. The preparation was washed three times during the recovery period. In Fig. 3 is shown the plot of the maximal responses obtained in two such experiments against the log of the dose.

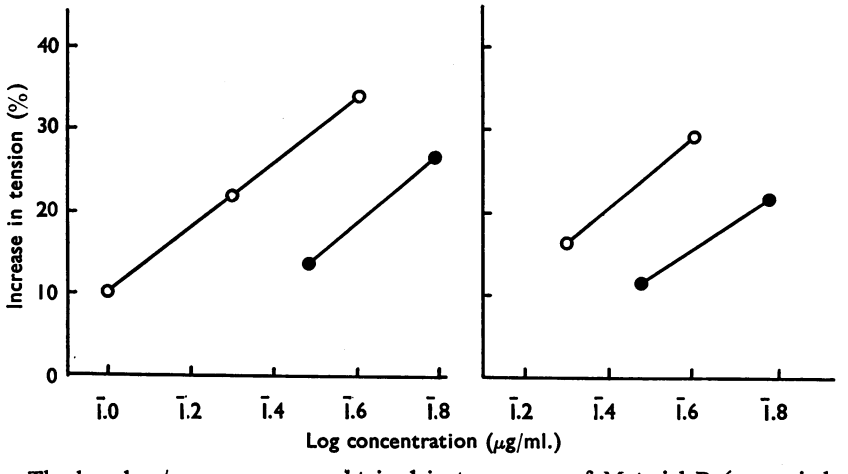

Fig. 3. The log dose/response curves obtained in two assays of Material R (open circles) and ouabain (filled circles) on the rabbit auricle. Ordinate: maximal percentage increase in twitch tension. Abscissa: log concentration (μg/ml.).

The rates of development of maxima for the two drugs were similar. From four such experiments (thirty-five observations), Material R was estimated to be 2.17-times (standard error, ±0.11) more potent than ouabain.

The method is obviously limited by the restricted responsive life of the preparation, and by the slow rate of development of, and recovery from, the effect of the drug.

The guinea-pig auricle

When placed in calcium deficient Locke solution this preparation behaved very similarly to that of the rabbit. The time to achieve maximal effect in response to ouabain was again about 30 min. There appeared to be no advantage in using auricles from guinea-pigs.

The hen isolated auricle

This preparation showed striking differences from those described above. In Locke solution containing the usual amount of calcium the preparation responded to ouabain and to Material R, the maximal effect occurring in about half the time required for the rabbit auricle. The time for recovery was also shorter. The preparation usually lasted longer than that of the rabbit; in most experiments it continued to respond for 24 to 40 hr. With the electrode system used for the rabbit, the hen auricle responded with alternate strong and weak beats. This phenomenon was observed in most experiments, and varied during the course of a single experiment. It was not an artefact due to a defect in the stimulator. The alternations of beat could be influenced by variation of the stimulus, and by the addition of extra calcium or of atropine. Furthermore, the weaker beats were more sensitive to inotropic substances.

The effects of the addition of extra calcium and of atropine are shown in Fig. 4. These effects and those of drugs other than digitaloids will be reported in greater detail elsewhere.

TABLE 1

THE RATE OF DEVELOPMENT AND RECOVERY OF TWITCH TENSION OF THE HEN AURICLE IN RESPONSE TO MATERIAL R AND OUABAIN

Each drug was given in a concentration of 0·8 μg/ml. The preparation was washed at 15, 20 and 25 min

Material R			Ouabain		
Time (min)	Response (mm × 5)	Increase (%)	Time (min)	Response (mm × 5)	Increase (%)
0	8·5	0	0	7·0	0
2	14·0	67·5	2	8·5	12·0
4	22·0	159·0	4	14·0	100·0
6	26·0	206·0	6	20·0	157·0
8	29·0	241·0	8	21·0	186·0
10	30·0	253·0	10	21·5	207·0
12	30·0	253·0	12	23·0	229·0
14	31·0	265·0	14	23·0	229·0
20	12·0	41·0	20	12·5	79·0
25	9·7	15·0	25	7·5	7·0
30	8·4	0·0	30	6·9	0·0

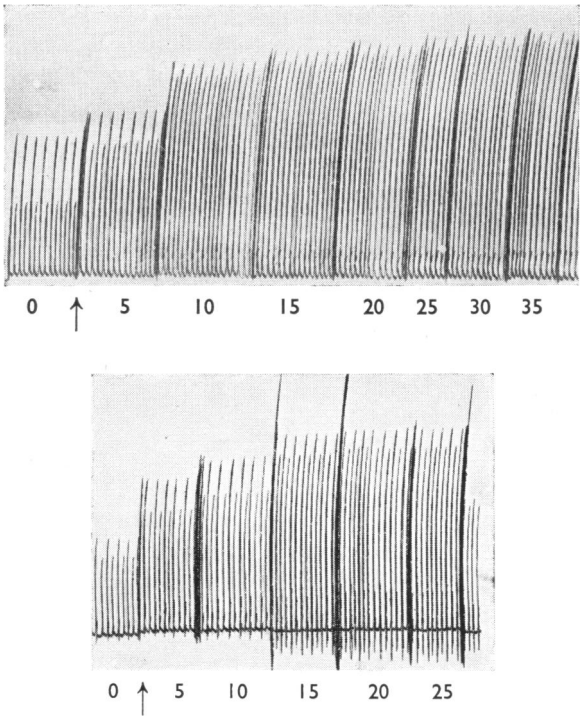

Fig. 4. The effects of extra calcium and of atropine on the twitch tension of the chick auricle. Upper tracing: at the arrow, the calcium content of the Locke solution was increased to 170% of normal. Recordings were then taken at 5 min intervals. Lower tracing: at the arrow atropine sulphate (3 μg/ml.) was added. Recordings at 5 min intervals. Well marked alternate beating is seen in both preparations. Numbers indicate times (in minutes) from adding drugs.

Table 1 shows protocols of the rate of increase of twitch tension of the hen auricle in response to Material R and to ouabain, and also of recovery following washing. Maximal tension occurred at about 15 min, and recovery was complete in a further 15 min. Accordingly, trial assays were made using a 30 min cycle, with recording at 10 and 15 min, followed by washing which was repeated at 20 and 25 min.

Reference has been made to the occurrence of alternate " beating " with this preparation, and this is well seen in Fig. 5. Both strong and weak beats were sensitive to inotropic substances: the weaker beats were more so. In a trial assay of digitoxigenin and ouabain, increases of both the weak and the strong beats were measured. Fig. 5 shows log dose/response lines taken from these results. Calculated from the effects on the weak beats, digitoxigenin was estimated to be 0.43-times as potent as ouabain (fiducial limits, $P=0.01$, 95 and 107%).

The greater percentage responses from results from the weaker beats, and also the greater degree of parallelism, compared with results from the stronger, are

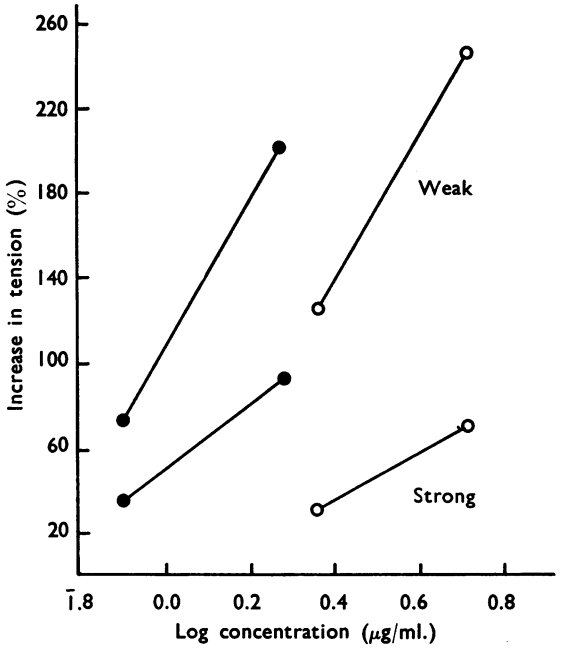

Fig. 5. The log dose/response curves obtained in an assay of ouabain (filled circles) versus digitoxigenin (open circles), using percentage increases of twitch tension calculated from weak (upper lines) and strong (lower lines) beats. Abscissa: percentage increase in twitch tension. Ordinate: log concentration (μg/ml.).

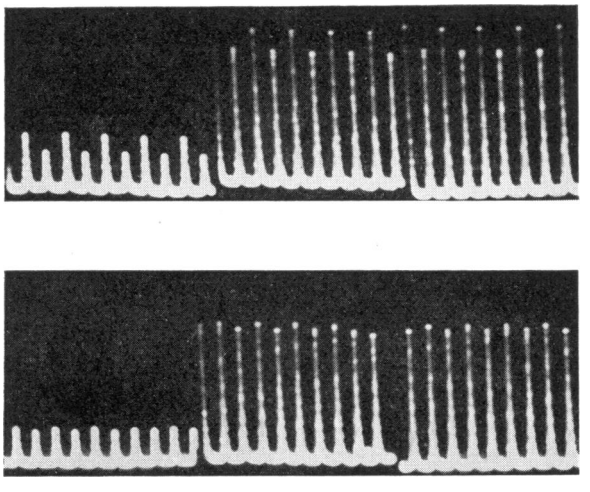

Fig. 6. Contractions of hen auricle, recorded electrically. Upper record: the effect of 2.6 μg/ml. of ouabain at 1 hr. Lower record: the same dose at 5 hr on the same preparation. Alternations are pronounced in the upper record and slight in the lower.

immediately apparent. Experience showed that the occurrence of these alternating responses was not consistent. While they occurred in most preparations, a few showed none during many hours. In a few others they varied during the course of an experiment. Thus Fig. 6 shows that, during the course of an experiment with ouabain, at 1 hr the alternations are well marked and at 5 hr they are much less well defined. Consistent results could be obtained with measurements of only the weaker beats.

The relative inotropic potency of Material R and ouabain was determined on the hen isolated auricles. The results (fiducial limits in parentheses) from the assays were 1.88 (85 and 144%), 1.75 (80 and 117%) and 2.10 (77 and 148%).

In view of the apparently different relation of responsiveness of the auricles of hen and rabbit to digitaloids and calcium, serum calcium determinations were made (Clark & Collip, 1926). The mean and standard error of ten determinations were 12.0 ± 0.3 mequiv/l.

DISCUSSION

The hen auricle provides a means of assay of inotropic potency to which conventional statistical methods may be applied, and from which a good degree of reproducibility may be expected. It is of interest that the results of comparisons of inotropic potency of ouabain and of Material R on the avian and mammalian auricles closely agree, namely that Material R shows an activity twice that of ouabain.

That the hen auricle is responsive to digitaloids in Locke solution of normal mammalian composition, containing 4.3 mequiv/l. of calcium, is apparently due to the fact that the hens used in these experiments were killed a little after the peak of their laying period, when the calcium content of the serum is raised (Dukes, 1955) compared with that during the non-laying period. Thus the serum calcium of the hens used was 12.5 mequiv/l.; the calcium content of the Locke solution would be about 30% of that of normal hen's serum, disregarding the possibility of bound calcium.

It is clear that comparisons between further digitaloid materials must be made before the full value of this method, and its bearing on structure/activity relationships, can be properly assessed.

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