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THE EFFECT OF TEMPERATURE AND FREQUENCY ON THE CONTRACTILE FORCE AND INOTROPIC RESPONSE TO OUABAIN, CALCIUM AND SOME OTHER DRUGS OF THE HEN ISOLATED DRIVEN AURICLE

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In a previous paper (Lock, 1963) it was shown that the isolated auricles of the hen can be used for the determination of inotropic potencies.

Little general information occurs in the literature concerning avian auricles. Blinks & Koch-Weser (1961) mention the hen auricle in a list of species investigated, but give no details of behaviour. In view of the potential usefulness of this preparation in the investigation of cardiotonic substances, particularly because of the more rapid response to such drugs compared with mammalian preparations, a wider range of conditions has been investigated than was described in the previous communication. Many authors, since Weizacher (1913a, b), have shown the importance of both frequency and temperature in the inotropic responses of a heart preparation to a digitaloid, and the profound effect of temperature particularly is shown here to apply not only to digitaloids but also to calcium. Some general effects of temperature and frequency changes on the contractility of this preparation are also described.

METHODS

Auricle preparations. These were prepared in a manner similar to that described previously (Lock, 1963). Recordings of twitch tension were made with an R.C.A. 5437 transducer and a Mingograph 42 multichannel recorder.

Solutions. The bathing fluid was Locke solution containing (in mM): NaCl 154, KCl 5.6, $NaHCO_3$ 36, $CaCl_2$ 2.2 and glucose 5.5; de-ionized water was added to 100%. Where necessary, cardiotonic glycosides were dissolved in ethanol-water mixtures and doses were arranged so that not more than 0.05 ml. of ethanol was added to a 30-ml. organ-bath, to avoid the depressive effect of ethanol on the tissue. Temperature was controlled to $\pm 0.1°$ C.

RESULTS

Staircase effect. Repeated experiment showed that many auricles would not respond regularly to any frequency of stimulus at temperatures below 27° C up to a measured potential of 12.5 V (MacLeod & Koch-Weser, 1948) in the bath, whereas the life of the preparation was short at above 40° C. Not all preparations would follow stimuli at more

than 2 shocks/sec at 35° C or below, whereas at 39° C the same preparation continued to follow stimuli at up to 4 or 5 shocks/sec; few would respond to 6 shocks/sec. The average temperature of the birds before killing was 41±1° C.

In Fig. 1,*a* is shown the effects of frequencies of 0.5, 1, 2 and 3 shocks/sec on the isometric twitch tension at temperatures of 29, 33, 36 and 40° C. The results are the means of four repeated observations on two auricles at each frequency at the given temperature. Both temperature and frequency were randomized. In Fig. 1,*b* the results are expressed as changes produced by temperature for each given frequency.

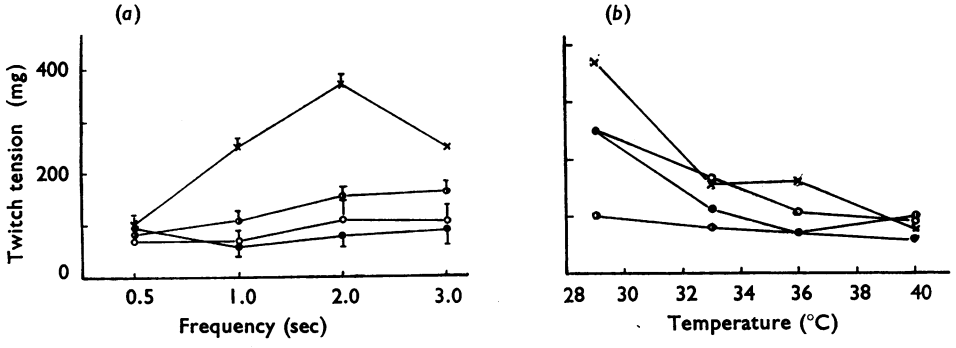

Fig. 1. The effect of temperature and frequency on the isometric twitch tension of the isolated hen auricle. (*a*) Ordinate: twitch tension (mg); abscissa: frequency (per sec). ×——× at 29° C; ◑——◑ at 33° C; ○——○ at 36° C; ●——● at 40° C. The vertical lines represent standard errors: only half these are shown for the sake of clarity. (*b*) Ordinate: twitch tension (mg); abscissa: temperature (°C). ◑——◑ 0.5 beat/sec; ●——● 1 beat/sec; ×——× 2 beats/sec; ○——○ 3 beats/sec. Standard errors are not shown but are identical to those in (*a*) at the appropriate position. The means are derived from four observations on two auricles at each frequency at the given temperature.

It will be seen that, for this range of frequencies, the staircase effect is in general positive, except at 29° C where there is a peak at 2 shocks/sec and between 2 and 3 shocks/sec where it becomes negative. At higher temperatures this effect is not observed; in general, at a given frequency an increase in temperature reduces the twitch tension to an almost steady value. The effect of temperature becomes less at the higher frequencies.

Alternate beating. This phenomenon, reported previously with the hen auricle (Lock, 1963), occurred with the majority of preparations at 29° C, and became particularly marked after an increase in frequency. This is shown in Fig. 2. Above this temperature it diminished and did not occur so frequently at 35° C except when the frequency was changed, and rarely at 39° C. Occasionally a change from regular to alternate beating occurred spontaneously, and where this apparently spontaneous change took place, either from alternate to uniform beating or *vice versa*, the sums of two pairs of twitch tensions, before and after the change, were equal.

The effect of temperature on the response to ouabain. Two doses of ouabain giving concentrations of 1.66 and 3.33 μg/ml. were added alternately to auricles stimulated at a frequency of 1 shock/sec at 29, 35 and 39° C. The ouabain was left in contact with the tissue until the maximal response was obtained—15 min. At least 30 min was allowed

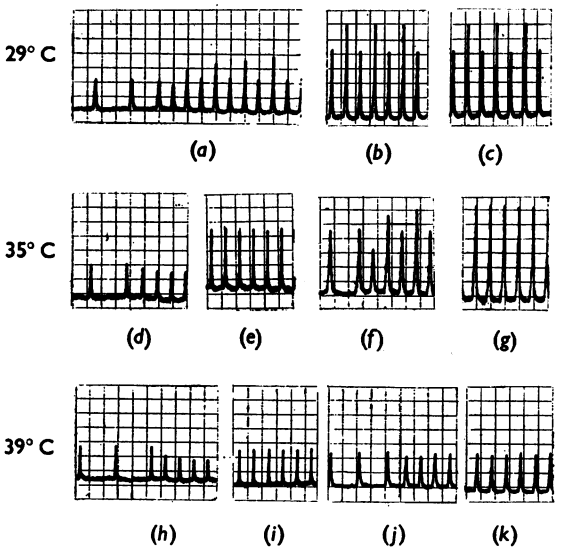

Fig. 2. The effect of frequency changes at various temperatures on the occurrence of " alternate " beating in the hen auricle. 5-msec-duration shocks were used at each frequency. 29° C: (*a*), alternations following a frequency change from 0.5 to 1 shock/sec; (*b*), 10 min later; (*c*), 15 min later. 35° C: (*d*), 0.5 to 1 shock/sec; (*e*), 10 min later; (*f*) 1 to 2 shocks/sec; (*g*), 10 min later. 39° C: (*h*), 0.5 to 1 shock/sec; (*i*), 10 min later; (*j*) 1 to 2 shocks/sec; (*k*) 10 min later.

between the temperature changes before dosing was restarted at the new temperature. Results are shown in Fig. 3.

It will be seen that the temperature effect depends on the dose, the greater the latter the greater the influence of temperature.

Lanatoside C and digitoxin. It was of interest to determine whether other glycosides were similar in their response to temperature. It has been found that on the hen preparation lanatoside C is of similar potency to ouabain, and produces a maximal response in the same time. On the other hand, digitoxin shows a potency of about three times that of ouabain, but takes twice as long to produce the maximal response. Table 1 shows effects of temperature on these two drugs compared with ouabain in approximately equipotent doses. The temperature effect on responses to both drugs is closely similar to that for ouabain.

TABLE 1

THE EFFECT OF TEMPERATURE ON THE INOTROPIC RESPONSE OF THE HEN AURICLE TO OUABAIN, LANATOSIDE C AND DIGITOXIN

The auricle was stimulated at 1 shock/sec. Values are means and standard errors

	Maximal inotropic response (%) after		
Temperature (°C)	Ouabain 3·3 μg/ml.	Lanatoside C 3·3 μg/ml.	Digitoxin 1·0 μg/ml.
28	98± 4	105± 6	82± 4
33	197±12	230±14	170±12
38	570±35	603±43	500±20
40	720±48	—	612±35

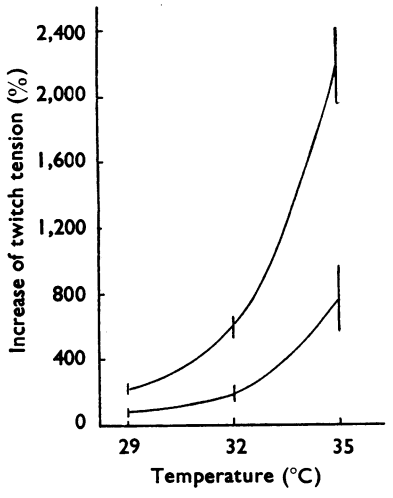

Fig. 3.

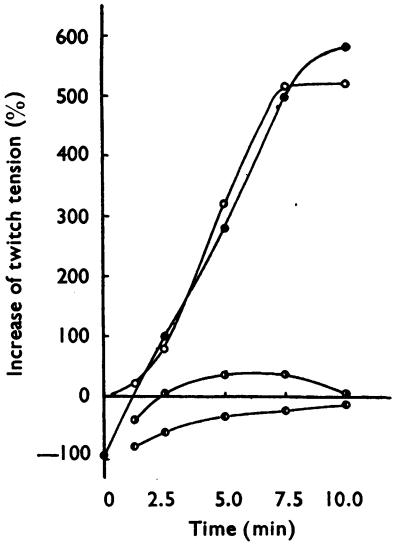

Fig. 4.

Fig. 3. The effect of temperature on the inotropic response of the hen auricle to ouabain. Ordinate: increase of twitch tension (%); abscissa: temperature (°C). Frequency, 1 shock/sec; duration, 5 msec. The responses are the means of three results on each of two preparations. Standard errors are shown by the vertical lines. The upper curve shows responses to 3.33 μg/ml., and the lower to 1.66 μg/ml. of ouabain.

Fig. 4. The dependence on beating of the hen auricle for the inotropic effect of ouabain. Ordinate: increase of twitch tension (%); abscissa: time (min). Temperature, 35° C; frequency, 2 shocks/sec. ○——○, The normal development of the inotropic response to 3.3 μg/ml. of ouabain; ●——●, 3.3 μg/ml. of ouabain added to the bath after switching the stimulator off. After 10 min the stimulator was restarted. The lowest point, showing −99%, was recorded immediately. ◑——◑, 3.3 μg/ml. of ouabain was added to the bath with the stimulator off. The preparation was allowed to rest for 10 min. The drug was then removed by washing the bath twice, and the stimulator restarted. ◐——◐, This shows the recovery after stopping the stimulator for 10 min in the absence of drug.

Calcium. Added quantities of calcium, as calcium chloride, also showed a high dose-dependent temperature effect, and, in order to determine whether the effects were similar, four-point comparisons of inotropic potency were conducted between the two drugs on the same auricle preparation at temperatures of 30 and 35° C, and at 35 and 39° C. It has previously been shown for a variety of species (for example, Niedergerke, 1957) that the inotropic effect of calcium ions is much faster than that of ouabain, and this was found to be so for the hen, maxima occurring in about one-third of the time required for ouabain.

During the comparisons, whilst 1.66 and 3.33 μg/ml. of ouabain and 1.13 and 0.565 mg/ml. of calcium were satisfactory at 30 and 35° C, the higher sensitivity and increased slope of the dose/response line necessitated a reduction to concentrations of 0.0116 and 0.0083 μg/ml. of ouabain and 0.396 and 0.283 mg/ml. of calcium, to avoid toxic irregularities and for the responses to remain on the flat portion of the dose/response curve. Results of typical experiments are shown in Table 2. It will be seen that the potencies of the two drugs are

TABLE 2

RESULTS FROM FOUR-POINT ASSAYS BETWEEN OUABAIN AND CALCIUM CHLORIDE WITH THE HEN AURICLE

The auricles were stimulated at 2 shocks/sec. Doses of drug were given in random order. The assays at 30 and 35° C were done on the same auricle. The calcium equivalent is in moles of calcium equivalent to 1 mole of ouabain. Combined slopes are means and standard errors, on a $\log_e$ basis

Temperature (°C)	Calcium equivalent	95% fiducial limits (%)	Slopes for calcium and ouabain: Combined	Slopes for calcium and ouabain: Difference (*P* value)	No. of results at each dose level	Time of dosing cycle (min)
39	$1{\cdot}16\times10^8$	110 & 87	436±31	0·2	6	30
35	$1{\cdot}09\times10^8$	110 & 91	225±22	0·2	6	30
30	$1{\cdot}12\times10^8$	107 & 93	96± 4	0·1	5	20

almost constant at the three temperatures and also the log dose/response slopes of both drugs are not significantly different at any temperature used, notwithstanding the changes of mean slopes with temperature. It has been repeatedly found that at 39° C the potency of calcium is slightly less than at the two lower temperatures. (It became apparent that difficulties in recording the high percentage changes at 39° C were introducing external errors into the results of assays, which were probably responsible for reducing the overall increase of accuracy which was expected from the steeper dose/response curve.)

The effect of temperature on the negative inotropic effect of potassium chloride and carbachol. Potassium chloride (190 μg/ml.) and carbachol (0.06 μg/ml.) were added alternately to auricles driven by 2 shocks/sec and 5 msec duration at 29, 35 and 39° C. The results are shown in Table 3. Both drugs are significantly less potent at the highest temperature than the lowest—the temperature coefficient, in contrast to ouabain and calcium, is negative.

TABLE 3

THE EFFECT OF TEMPERATURE ON THE NEGATIVE INOTROPIC RESPONSE OF THE HEN AURICLE TO POTASSIUM CHLORIDE AND CARBACHOL

The results are the means of three observations on each of two auricles, frequency 2 beats/sec, shock duration 5 msec. Values are means and standard errors

Temperature (°C)	Negative inotropic response (%) after: Potassium chloride 190 μg/ml.	Negative inotropic response (%) after: Carbachol 0·059 μg/ml.
29	50·5±3·7	48·6±2·5
34·5	24·5±2·6	41·0±9·1
39	24·5±1·4	40·0±3·0

The effect of frequency changes. Table 4 shows the maximal responses to ouabain, 3.3 and 1.6 μg/ml., at frequencies of 0.5, 1 and 2 shocks/sec at temperatures of 30 and 35° C. The maxima shown are mean results of six observations on two auricles at each combination of conditions. It was found necessary to allow 30 min between changes of frequency or temperature to ensure a steady state. It is seen that at 29° C the response to the larger dose falls nearly 50% from 0.5 to 2 shocks/sec. The response to the smaller dose is less affected. In consequence, the slope of the dose/response curve becomes less at the higher frequency. At 35° C there is little change in the slope, and smaller responses occur at 1 shock/sec than at the other two frequencies. The figures in parentheses indicate the approximate time taken to achieve maximal response. The rate of attainment of maximal response increases

TABLE 4

THE PERCENTAGE INOTROPIC RESPONSE OF THE HEN AURICLE TO OUABAIN AT 29 AND 35° C, AND AT FREQUENCIES OF 0·5, 1·0 AND 2·0 BEATS/SEC

The results are means and standard errors of three responses from two auricles. Figures in parentheses are the approximate times taken to reach the maximal response

Temperature (°C)	Concentration of ouabain (μg/ml.)	Inotropic responses (%) at frequency (per sec)		
		0·5	1·0	2·0
29	3·33	374±18 (19)	310±60 (12)	154±18 (8)
	1·66	114± 8	108± 8	69± 8
35	3·33	839±27 (24)	618±42 (18)	832±56 (12)
	1·66	328±28	204±18	334±25

with increasing frequency but is independent of temperature, except in so far as a higher percentage inotropic response produced either by a higher dose at a given temperature, or the same dose at a higher temperature, requires somewhat longer.

The dependence of inotropic response on beating. Moran (1963) has shown that development of an inotropic effect in the rabbit depends on the beating of the preparation, and this is confirmed for the hen. Fig. 4 shows a comparison of the rate of growth of inotropic effect when the stimulator is on during and after the addition of ouabain, with the rate of growth when the stimulator is switched off just before the addition of the drug. It will be seen that only after restarting the stimulator does the growth of the inotropic effect take place and that the rate of growth is similar to that of the control. When the drug was washed out before the stimulator was restarted only a slight inotropic effect occurred.

DISCUSSION

Blinks & Koch-Weser (1961) have emphasized the generality of behaviour of the isolated myocardium from various species when made to beat at various rates, but have noted that over various frequency and temperature ranges species differences may be considerable. The investigation here, of more limited application, shows that, over the range of frequencies 0.1 to 3 per sec, the hen differs from the cat (Koch-Weser & Blinks, 1963) and rabbit (Kruta, 1937) in having a less strongly positive staircase effect. Further observations showed that up to 5 beats/sec no increase in contractile force occurred, but rather it tended to diminish. The hen preparation is relatively thin, but it cannot be said with certainty that oxygen deficiency does not influence the contractility at these higher frequencies. It is improbable that the fall in contractility between 2 and 3 beats/sec at 29° C is associated with deficient oxygenation, for at 3 beats/sec the auricle will maintain an increase of twitch tension several hundred per cent greater, and there is not a corresponding fall from 2 to 3 beats/sec at higher temperatures. It appears then, that for the hen over this limited range the sign and degree of the staircase effect has little effect on the ability of the tissue to respond to digitaloids (Farah & Witt, 1963).

The occurrence of marked alternate beating, which does not appear to have been recorded in the literature except for rat preparations under the influence of quinidine (Benforado, 1958), is in the hen preparation a function of temperature (Fig. 2). It was seen during many experiments at 29° C that there is considerable variability in the occur-

rence of this phenomenon; some preparations showed it at frequencies as low as 0.1 beats/sec; in others, for example Fig. 2,*a*, it did not occur at 0.5 beats/sec but was consistently present at 1 beat/sec and higher frequencies. At 35° C only a change of frequency temporarily provoked its appearance but again, at this temperature, in a few preparations it was constantly present but less in degree than at 29° C. At 39° C it occasionally occurred to a slight extent under the influence of strong enhancement by ouabain.

The influence of temperature on the response to ouabain is shown here to be profound, and is shown to be a property common to digitaloids. Fig. 3 shows that over a range of only 6° C the inotropic response changes from approximately 200 to 2,200%—an elevenfold difference. Many authors have shown that in general an increase in temperature reduces the size of the twitch tension of a heart preparation in the absence of drug, and it has been postulated that this decrease of twitch tension may influence the percentage increase possible by a given dose of a digitaloid. This is shown to be at most only a minor factor, for it will be seen from Fig. 1,*b* that at a frequency of 2 beats/sec, between 36 and 40° C, the reduction of size of basal twitch tension is negligible, whereas over this same range the percentage inotropic response increased sixfold. Saunders & Sanyal (1958) using relatively much smaller doses of ouabain showed this positive temperature effect on the response of rabbit auricle, although to a less dramatic degree because of the smaller doses used. These authors postulated that a physical rather than a chemical explanation should be sought because of the high temperature coefficient. This view is well borne out by the effect of the more extended range of doses used in this present work. It is known (Repke, 1963) that the cardiac glycosides influence various enzymes such as adenosine triphosphatase, but since the TQ_{10} of enzyme promoted reactions is normally 2 to 3° C it is improbable that this effect relates to a chemical activity, unless chain reactions are involved. Waser (1963) has recently shown that the thixotrophy of actomyosin is greatly affected by cardioactive and not by inactive glycosides. It would be of interest to determine whether such effects are influenced by temperature in the same way as the inotropic response.

The close association between digitalis and calcium has been a matter of interest since Loewi (1917) and recently Walker & Weatherall (1964) have presented further reasons for the hypothesis that digitaloids may enhance the concentration of calcium at the site of excitation contractions-coupling. The high temperature effect on the digitaloids is shown here to apply to calcium; indeed, it appears from the comparative assays shown in Table 2 that it is identical within highly significant limits of probability. That two chemically dissimilar substances should show such closely similar potency ratios, under conditions producing very different dose/response slopes, would appear to provide further strong indirect evidence of a close identity of action. Indeed, it is perhaps a more feasible hypothesis that temperature is acting on only one drug-tissue relationship, that of calcium. But, if this were so, why does not an increase of temperature invariably stimulate, instead of depressing, contractility of isolated myocardium, as is known to be the case ?

Further, does this temperature effect on " added " calcium indicate a difference between its role in myocardial contraction and the steady state of higher contractility achieved by a bathing fluid which has a greater than normal calcium ion content and in which the temperature effect is then absent. It would appear that there is a close connection between the effect of " added " calcium and the action of digitalis (Niedergerke, 1957) but none

between digitalis and calcium effects at hypothetical deeper levels which are apparently insensitive to temperature changes.

By contrast with temperature effects on the actions of calcium and ouabain, the coefficients for potassium and carbachol are negative. The relatively slight change in activity produced by temperature on these drugs may be illusory, if considered from the point of view of the range of ability of the heart on the one hand to increase contraction, and on the other to be depressed. It is not yet clear from the present work whether there is a connection between the negative effect on the latter drugs and the positive effect for digitalis-like drugs and calcium.

Whilst more than one author (Garbs & Penna, 1957; Koch-Weser & Blinks, 1962) have noted that the myocardium from the cat is influenced by digitaloids in the absence of active beating, that from the hen is shown to be similar to the rabbit and guinea-pig (Moran, 1963). It is clear that further work is desirable to throw light on this divergence.

SUMMARY

1. Isolated hen auricles were stimulated at frequencies of 0.5 to 5.0 shocks/sec with 5-msec-duration shocks in Locke solution containing 24 mg of calcium chloride per 100 ml.

2. The staircase effect at 29, 35 and 39° C is considerably less positive than for the rabbit and cat.

3. The phenomenon of alternate beating in this preparation is a function of temperature, occurring frequently at 29° C and infrequently at 39° C.

4. The inotropic effect of digitalis-like drugs is greatly increased by raising the temperature over the range 27 to 39° C.

5. Calcium added to the bath, in addition to the normal calcium content of the Locke solution, produces a rapid inotropic effect which is affected by temperature in a way closely similar to that due to digitalis-like drugs. The slopes of the dose/response curves for both ouabain and added calcium increase with temperature to the same extent.

6. The inotropic effect of ouabain in this species depends on beating for its development.

7. The temperature effects on digitaloids and calcium are greater than those to be expected from chemical reactions, and may be associated with physical changes of myocardial proteins, unless a consecutive chain of chemical reactions are involved.

I wish to express my thanks to Mr N. M. Casperd and Mrs Y. H. Lock for technical assistance; to Sandoz (Basel) for cardiac glycosides; to the Medical Research Council for financial help; and to the Wellcome Trust for a Mingograph multichannel recorder.

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