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ACTION OF OUABAIN ON THE GUINEA-PIG ISOLATED UTERUS

BY

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Uterine activity *in vitro* was measured isometrically by integrating tension against time, and contractions were recorded on a kymograph using a servo-recorder. Ouabain caused a persistent contraction both of the spontaneously contracting and of the electrically stimulated uterus, the response depending upon the presence of calcium. After ouabain had induced a contraction of the spontaneously active uterus, relaxation to the baseline after washing out the ouabain was succeeded by a period of inactivity. Since the uterus could respond as usual to stimulation during this period, ouabain appeared to be selectively depressing spontaneous contractions. High concentrations of progesterone suppressed uterine activity and the response to ouabain. Ouabain reduced the susceptibility of the uterus to depression by anoxia.

The cardiac glycosides affect the uterus of animals of various species. Sugimoto (1913) reported stimulation of the non-pregnant guinea-pig uterus with small doses of strophanthin, and Ransom (1920) found that tincture of strophanthin sensitized the cat myometrium to calcium. Rothlin & Raymond-Hamet (1934), however, reported that digitalin suppressed the motor effect of adrenaline on the non-pregnant rabbit uterus. On the human uterus, Norris (1961) found that digoxin and ouabain increased both tone and the frequency of contractions *in vitro*, while digoxin significantly relieved the symptoms of sufferers from dysmenorrhoea in a double-blind trial. It seemed of interest, therefore, to investigate further the mode of action of cardiac glycosides on the uterus.

METHODS

Virgin guinea-pigs of 500 to 700 g were used. To ensure standard hormonal conditions the animals were used during natural oestrus and were also given 25 μg of oestradiol monobenzoate by subcutaneous injection 24 or 48 hr before experiments. Except for the transducer, the apparatus used has been described previously (Styles & Sullivan, 1962). Isometric uterine contractions were recorded with a transducer, the output voltage from which was coupled to a servo-recorder and integrator motor. A kymograph tracing of contractions was obtained with the servo-recorder, and uterine activity was measured in g sec with the integrator. In previous experiments the uterine horn was attached to an isometric lever which moved the operating rod of a linear potentiometer transducer (Styles & Sullivan, 1962). These have been replaced by a differential capacitance transducer because of the limited resolution of the potentiometer transducer (0.0254 mm). The capacitance transducer formed part of the circuit shown in Fig. 1, and consisted of a metal cantilever blade situated between fixed plates approximately 0.254 mm from the blade and forming a differential capacitor, which was

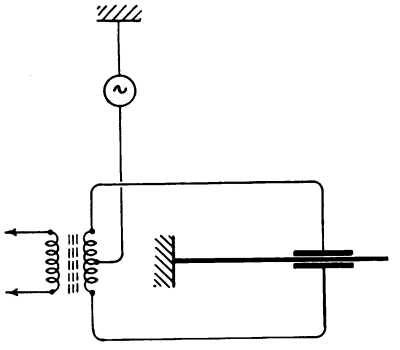

Fig. 1. Basic circuit arrangement of the differential capacitance transducer.

balanced when no force was applied to the end of the cantilever. Equal currents then flowed in the two halves of the primary winding of a transformer connected across the fixed plates of the transducer and there was then no secondary e.m.f. Application of a force (uterine muscle tension) moved the blade, unbalanced the differential capacitor and produced a secondary e.m.f. The signal was amplified by a two-stage transistor amplifier and fed to a phase-sensitive detector. The output from the detector was connected directly to the servo-recorder and integrator units. Sensitivity of the transducer was only limited by the mechanical stability of the differential capacitor, which was inherently high because of the mechanical symmetry of the assembly. The aluminium alloy cantilever blade was 60 mm long, 25 mm wide and 1.6 mm thick, and had a stiffness of 0.001 mm per g. With forces in the range 0 to 30 g, adequate freedom from baseline drift, a virtually isometric measurement and a linear relation between applied force and electrical output were obtained. The transducer was shielded from the water bath by an aluminium plate to prevent condensation, which tended to cause baseline drift.

As previously described (Styles & Sullivan, 1962), one horn of the uterus was set up in a Perspex organ-bath of 10 ml. capacity, inside which two silver wire electrodes were fixed into grooves 6.5 cm apart and connected to leads from a stimulator. The upper end of the uterine horn was attached to the transducer. Warmed modified Krebs solution (NaCl 154, $NaHCO_3$ 5.95, KCl 5.65, $CaCl_2$ 2.54, KH_2PO_4 1.18 and $MgSO_4$ 1.18 mM; dextrose 1 g/l.) entered the lower end of the organ-bath through side tubes and was perfused continuously, except when drugs were added to the bath, flowing out over the top into the water bath in which the organ-bath was kept at constant temperature. A mixture of 5% carbon dioxide and 95% oxygen was bubbled continuously through the organ-bath.

The uterus was fixed in the organ-bath and then stretched to "resting length," at which it functioned optimally (Csapo, 1954). The spontaneously active uterus was studied at 37° C. During electrical stimulation, spontaneous activity could be abolished by reducing the temperature of the bath and the calcium content of the Krebs solution. The automatic stimulator gave 50 cycles/sec sinusoidal a.c. of predetermined duration, and a stimulus strength of 15 V over periods of 5 sec repeated at intervals of 1 min was found to be optimal. The integrator was adjusted to zero when the uterus was relaxed, and uterine activity was recorded over periods of 5 min. The apparatus was calibrated by attaching weights to the cantilever blade of the transducer at the point of attachment of the uterine horn, and recording the integrator readings for 5 min periods. A graph was then plotted of weight in g against integrator readings and used to derive the integrated tension in g sec.

Ouabain (strophanthin G) was the cardiac glycoside chosen because it is water-soluble. Aqueous solutions were prepared from the pure compound, and usually 0.2 ml. volumes of various concentrations were introduced into the lower end of the organ-bath through a fine polyethylene tube. In some experiments ouabain was added to the perfusing Krebs solution.

RESULTS

The response of the guinea-pig uterus to ouabain

The uterine horn set up in the organ-bath contracted in response to intermittent electrical stimulation. Doses of ouabain added to the organ-bath and left in for 5 min caused an increase in tone, the uterus continuing to respond to individual stimuli. This is shown in Fig. 2, in which ouabain (5 μg/ml.) was added at the first arrow. Washing out began 5 min later at the second (inverted) arrow, and was repeated

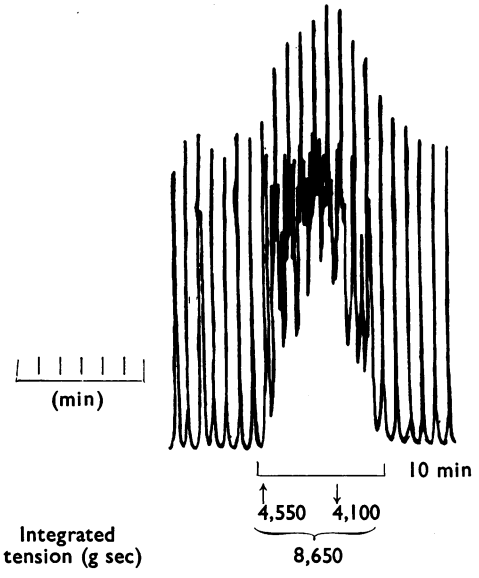

Fig. 2. Contractions of electrically-stimulated uterus. Kymograph tracing showing the response of the uterus to ouabain (5 μg/ml.) added at first arrow and washed out at second arrow until the baseline of uterine tone was regained. The integrated tension while ouabain was in the organ-bath (4,550 g sec) is referred to as the initial response. The integrated tension from addition of ouabain until the baseline was regained (8,650 g sec) is referred to as the total response.

until the uterus had relaxed to baseline 10 min after ouabain had been added. The integrated tension recorded while ouabain was in the organ-bath is referred to as the initial response. When the integrated tension recorded during the washing-out period is added to this, the sum is referred to as the total response. Both are plotted separately on the log concentration/response graph shown in Fig. 3, in which drug concentrations ranged from 0.25 to 10 μg/ml. The curve for the total response is much steeper than that for the initial response, indicating that contraction of the uterus becomes increasingly prolonged, despite repeated washings, as the drug concentration is increased.

When ouabain (0.5 μg/ml.) was left in the organ-bath for 40 min, there was no diminution in the response. The integrated tension developed did not vary significantly during this time.

A kymograph tracing of spontaneous uterine contractions is shown in Fig. 4 (*a*). The mean duration of rest periods between contractions was 1.8 min. Ouabain

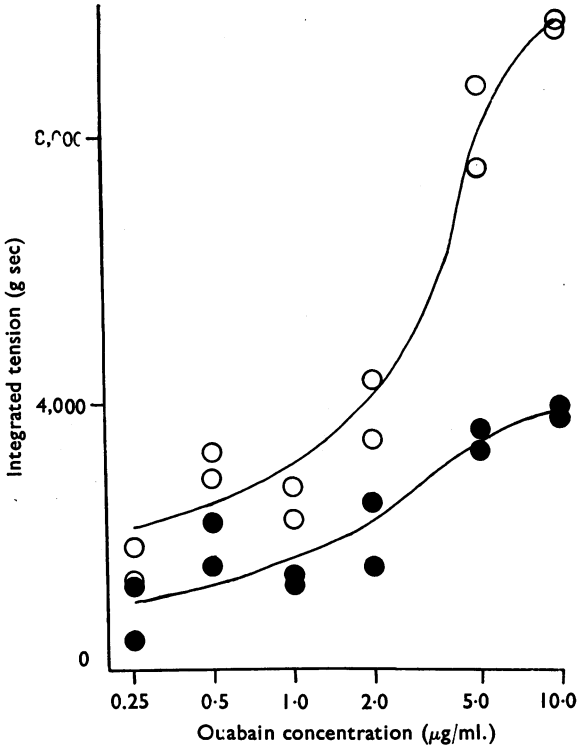

Fig. 3. Ouabain concentration/response curves for electrically-stimulated uterus. The ouabain concentrations are on a log scale. Filled circles represent initial response (while ouabain was in the organ-bath); open circles represent total response (until relaxation to baseline).

(2 μg/ml.), left in the organ-bath for 5 min, caused a contraction lasting 12 min (Fig. 4*b*); the total response was 11,560 g sec. It will be seen, however, that relaxation was followed by a period of inactivity lasting for 12 min before spontaneous activity started again with an atypical contraction. To determine whether the uterus could still respond to ouabain during this period of inactivity, ouabain was again added in the same concentration as soon as the uterus had relaxed after washing out the first dose. A contraction immediately developed (Fig. 4*c*), the duration and total integrated tension of which were of the same order as those which followed the first dose. However, the inactive period after washing out the second dose had increased to 20 min (as a result of the cumulative effect of repeating the dose). When spontaneous activity was subsequently re-established (Fig. 4*d*), the mean duration of the rest periods was 4.3 min.

Stimulation of the uterus before and after treatment with ouabain

To see whether the uterus was still able to respond fully to other drugs besides ouabain during the period of inactivity, submaximal concentrations of various drugs were added to the organ-bath before ouabain and again during the inactive phase after ouabain. The results in Table 1 show that the integrated tension developed

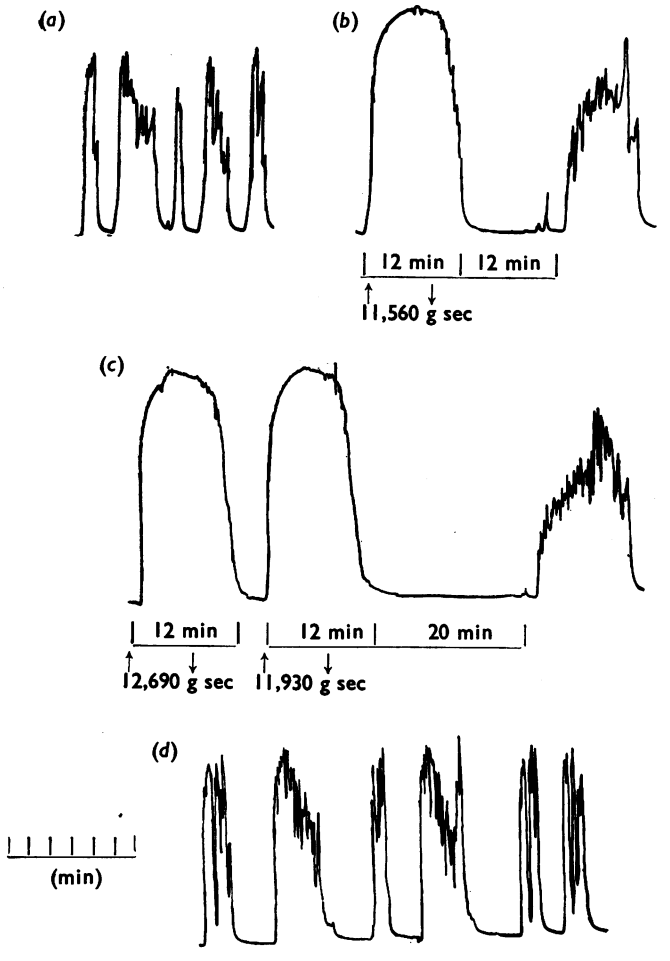

Fig. 4. Kymograph tracings of spontaneously contracting uterus showing the response to ouabain (2 μg/ml.) added at first arrows and washed out at second arrows. The total integrated tensions are shown. (*a*) Initial spontaneous activity. (*b*) Response to a dose of ouabain showing duration of contraction and subsequent period of inactivity. (*c*) Response to two consecutive doses of ouabain. (*d*) Period of spontaneous activity 30 min later.

in response to these drugs was not very different before and after ouabain. The response to electrical stimulation was similarly measured before and after ouabain, and, as shown in Table 1, did not alter much. This was to be expected since it had previously been found (Fig. 2) that after washing out a dose of ouabain the uterus had continued to respond to stimulation without any diminution of response.

Kymograph tracings are shown in Fig. 5 of the responses to oxytocin, acetylcholine, 5-hydroxytryptamine and ouabain in concentrations causing similar amounts of integrated tension while the drugs were in the organ-bath. During control periods of spontaneous activity, the mean period of quiescence between contractions was 1 min. After oxytocin, acetylcholine and 5-hydroxytryptamine, spontaneous activity

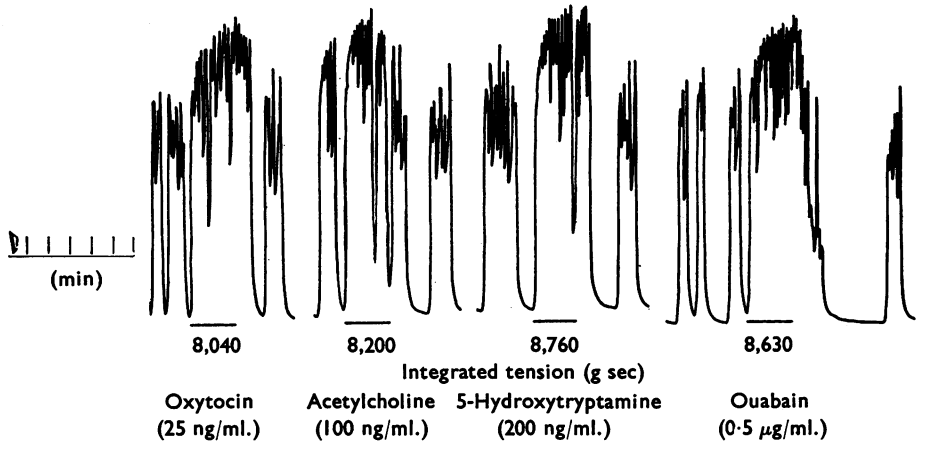

Fig. 5. Kymograph tracings of a spontaneously active uterus in the presence of concentrations of drugs causing similar amounts of integrated tension. Drugs were left in the organ-bath for 5 min (during the horizontal lines) and the integrated tensions developed during these times are shown. After washing out the ouabain, a period of inactivity ensued.

was resumed within 2 min of relaxation to baseline, but following ouabain only after 6 min.

Effect of ouabain in low-calcium Krebs solution

With both the electrically-stimulated and spontaneously contracting uterus, reduction of the calcium content of the Krebs solution from 2.54 to 1.27 mM did not significantly impair the response to ouabain, but with futher reduction the response rapidly declined. In calcium-free Krebs solution, all uterine activity ceased and there was no response to ouabain.

Effect of ouabain in the presence of progesterone

Progesterone in aqueous solution was added to the organ-bath to give concentrations of 5, 10 and 20 μg/ml. Spontaneous uterine activity did not change during

TABLE 1

RESPONSE OF GUINEA-PIG UTERUS TO STIMULATION

Comparison of the response to stimuli applied before ouabain (*a*) with the response during the inactive period (*b*) after ouabain

Stimulus	Response (*a*) (g sec)	Ouabain (μg/ml.)	Response (*b*) (g sec)	$\frac{(b)}{(a)}$ %
Oxytocin (25 ng/ml.)	8,040	0·5	8,550	106
Acetylcholine (100 ng/ml.)	8,200	0·5	7,700	94
5-Hydroxytryptamine (200 ng/ml.)	8,760	0·5	7,180	82
Oxytocin (100 ng/ml.)	6,490	1·0	6,290	97
Acetylcholine (400 ng/ml.)	8,680	1·0	8,190	95
5-Hydroxytryptamine (800 ng/ml.)	5,940	1·0	5,540	93
Potassium chloride (2 mg/ml.)	4,575	2·0	4,060	90
Electrical stimulation	1,360	5·0	1,590	117
Oxytocin (100 ng/ml.)	3,605	5·0	4,500	125
Acetylcholine (200 ng/ml.)	5,490	5·0	4,850	89
5-Hydroxytryptamine (100 ng/ml.)	3,140	5·0	3,120	100

5 min periods of observation after adding progesterone, and the response to ouabain was not affected when it was then added to the organ-bath, in which the progesterone remained for a further 5 min. However, progesterone in higher concentrations (40 and 60 μg/ml.) abolished spontaneous contractions and depressed the response to ouabain (1 μg/ml.) by 80 to 95%. At a concentration of 80 μg/ml., progesterone suppressed the response to ouabain (1 μg/ml.) completely.

Effect of ouabain during anoxia

When the uterus was made anoxic by substituting a mixture of 5% carbon dioxide in nitrogen for 5% carbon dioxide in oxygen bubbling through the organ-bath, all

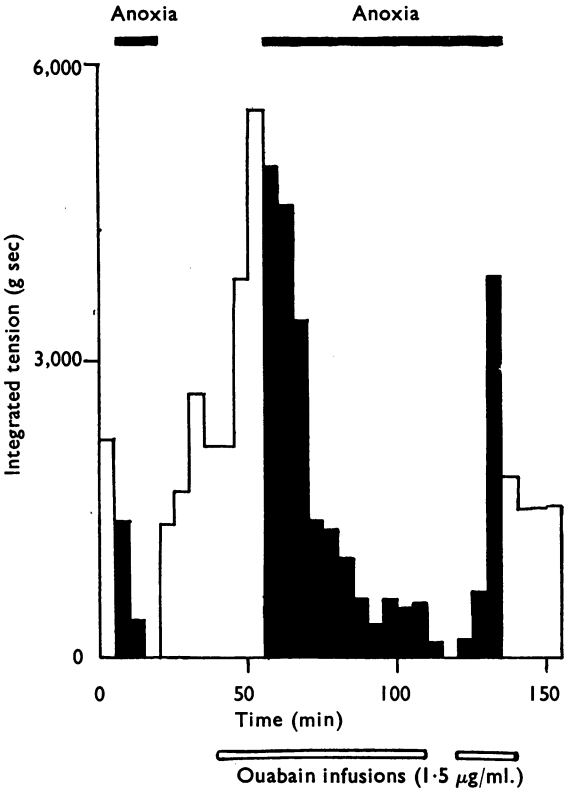

Fig. 6. Effect of ouabain infusion (1·5 μg/ml.) on the electrically-stimulated uterus in the presence and absence of anoxia. The ordinate gives the integrated tensions developed during successive 5 min periods.

spontaneous uterine activity was abolished. The response to electrical stimulation was also usually completely suppressed, though a small response persisted in some experiments.

When the uterus was anoxic it could still contract in response to doses of ouabain. Also, when ouabain was continuously infused at a low concentration, suppression by anoxia of the responses of the uterus to electrical stimulation was prevented (Fig. 6). After 10 min of anoxia, the uterus failed to respond to electrical stimula-

tion, but the normal response quickly returned when nitrogen was replaced by oxygen. The normal response was increased by the addition of ouabain (1.5 μg/ml.) to the Krebs solution perfusing the organ-bath. The uterus was then made anoxic once more, but it continued to respond, though more weakly, until ouabain-free Krebs solution was used, when the response ceased. However, when ouabain was added again, the response of the still-anoxic uterus to electrical stimulation was greatly enhanced. Finally, when the solution was re-oxygenated and the bath perfused with ouabain-free Krebs solution once more, the uterus responded consistently to electrical stimulation.

DISCUSSION

The most striking result noted in these experiments was the temporary suppression of spontaneous activity after washing out a dose of ouabain which had caused a contraction. This period of inactivity was prolonged by higher concentrations of ouabain and by the cumulative effect of repeated doses. The inactivity was not due to the inability of the uterus to contract, since there was little difference between the response to other drugs and to electrical stimulation before adding ouabain and during the period of inactivity. This after-effect was peculiar to ouabain among the drugs tested.

Schatzmann & Ackermann (1961) studied the effect of ouabain on the isolated taenia coli preparation of the guinea-pig. In a concentration of 1 μg/ml. ouabain caused a biphasic effect. In the first phase there was a sharp rise in tone, reaching a maximum about 10 min after the addition of ouabain. This was succeeded, in the second phase, by relaxation to baseline, which occurred about 25 to 30 min after adding ouabain. When the drug was then washed out there followed a period of inactivity lasting 20 to 60 min before spontaneous contractions returned.

Whereas the response of the taenia coli to ouabain was biphasic, in the experiments of this paper the uterus remained in tonic spasm for 40 min during which ouabain (0.5 μg/ml.) was in the organ-bath. However, with both the taenia coli and the uterus, after washing out ouabain there was a period of inactivity before spontaneous contractions were renewed. Schatzmann & Ackermann (1961) did not investigate whether the taenia coli could contract in response to stimulation during this inactive period, but they did report that action potentials were absent, returning when spontaneous contractions were resumed. They also found that ouabain caused slight depolarization and an increased frequency of action potentials during the first phase of the response to the drug, while, in the second phase as the muscle relaxed, action potentials gradually ceased. In the light of further experiments they suggested that the changes in excitability elicited by ouabain could be explained in terms of the inhibitory effect on active cation transport through cell membranes.

Since the uterus was capable of responding fully to stimulation during the inactive phase after washing out ouabain, it is tempting to suppose that the inactivity was due to a temporary suppression of the mechanism which normally initiates spontaneous contractions. Marshall (1959, 1962) has investigated this mechanism, and found that uterine cells randomly assume " pacemaker " activity and give rise to action potentials which initiate muscle contraction.

Experimental evidence indicates that calcium is the essential link between excitation and contraction in smooth muscle (Daniel, Sehdev & Robinson, 1962) and it was found that contraction of the uterus induced by ouabain was dependent upon the presence of calcium in the perfusing Krebs solution. Schatzmann & Ackermann (1961) observed that the initial excitatory effect of ouabain on the taenia coli was enhanced by calcium, and there is evidence that the action of ouabain on heart muscle is mediated by calcium (Holland & Sekul, 1961).

Kuriyama & Csapo (1961) found that the progesterone-dominated uterus is relatively inexcitable. When the parturient rabbit uterus was treated *in vitro* with progesterone (10 μg/ml.) the cell membrane became hyperpolarized, and both spontaneous and oxytocin-induced activities were suspended or suppressed. The experiments described here confirmed that progesterone suppressed spontaneous uterine contractions, but much higher concentrations were required to produce this effect in the guinea-pig uterus, namely 40 μg/ml. or more. These concentrations also depressed the response to ouabain.

Observations during periods of anoxia confirmed the findings of Norris (1961), using isolated strips of human uterus, that ouabain reduced the susceptibility of the uterus to depression by anoxia. This was evident from the observation that ouabain prevented suppression of the response to electrical stimulation by anoxia.

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