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CALCIUM IN RELATION TO THE ACTIONS OF OUABAIN AND ADRENALINE ON THE HEART

BY

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Isolated pairs of rabbit auricles have been observed in media containing 6, 24 or 75 mM-potassium, with corresponding reductions in sodium concentration. In 24 mM-potassium, adrenaline restored beating and excitability, as did calcium chloride, but ouabain had no effect. In 75 mM-potassium, adrenaline had no effect; calcium chloride caused a contracture; ouabain had no direct effect, but auricles which had been beating in the presence of ouabain contracted promptly on transfer to 75 mM-potassium. Left auricles, which do not beat spontaneously, were less sensitive to calcium and to ouabain. The results showed a membrane stabilizing action of calcium and an action on muscular contraction, and suggested that cardiac glycosides acted by causing accumulation of calcium at the activator site in the tissue.

Sodium, potassium and calcium are all well known to influence the excitability and force of contraction of cardiac muscle, but their interactions have been recognized as complex since the earliest descriptions by Ringer (1883). As expected from the observations and theory of Hodgkin & Huxley (Hodgkin, 1951), potassium is particularly related to the resting membrane potential (Vaughan Williams, 1959), and sodium to the action potential (Weidmann, 1955). Calcium particularly affects the force of contraction (Ringer, 1885; Weidmann, 1959), though it also has complex effects on the excitability of the tissue (see Shanes, 1958, for review). Their effects are not independent: for instance, reduction in the external concentration of sodium increases the force of contraction (Daly & Clark, 1921), probably because the uptake of calcium occurs competitively with the uptake of sodium (Lüttgau & Niedergerke, 1958). Drugs such as cardiac glycosides and adrenaline, which increase both excitability and force of contraction of hearts, might act by modifying the influence of these ions. Synergism between digitalis and calcium is well known clinically (Edens & Huber, 1916; Bower & Mengle, 1936; Cohen, 1952) and experimentally (Clark, 1912; Baker, 1947), and is associated, at least for ouabain, with increased accumulation of radioactive calcium in media containing ^{45}Ca (Sekul & Holland, 1960; Thomas, 1960a; Holland & Lüllmann, 1962). If, as seems likely, calcium acts as a link between excitation and contraction (Heilbrunn & Wiercinski, 1947; Sandow, 1952; Niedergerke, 1956), inotropic activity might well result from an increase in the amount of calcium available for such coupling. One way of testing this hypothesis is to eliminate the first step in the normal process of exciting a contraction by producing a sustained depolarization of the tissue, and using such a situation for studying the effects of ions and drugs. Responses of plain and striated muscle have been studied in this way (Evans, Schild & Thesleff, 1958; Axelsson &

Thesleff, 1958) with depolarization produced by replacing some or all of the sodium in the medium by potassium. We report here observations on rabbit auricles both in moderately (24 mM) and very (75 mM) high concentrations of potassium, with and without corresponding reductions in the concentration of sodium. At the higher potassium concentration, calcium produced a sustained but reversible contraction, and its effect was imitated by previous treatment with ouabain though not by adrenaline. At the lower potassium concentration, calcium apparently repolarized the auricles, as it restored excitability or spontaneous beating: this effect was imitated by adrenaline but not by ouabain. Some of these findings have already been communicated briefly (Walker & Weatherall, 1963 ; Weatherall, 1963).

METHODS

Isolated pairs of rabbit auricles or single left auricles were prepared as described by Carslake & Weatherall (1962) and set up to contract isotonically at 30° C in an organ-bath of 70 ml. capacity. Contractions were recorded on a kymograph with a light spring-loaded writing lever. Stimuli were applied from a pulse generator (Bernstein, 1950), through a platinum hook which transfixed the auricle: the return lead made contact with the tissue through the bath fluid. The salt solutions used are shown in Table 1. In some experiments, changes in ionic composition were made by adding up to 1.3 ml. of 1.4 M-potassium chloride or calcium chloride to the 70 ml. bath, so that the concentrations of these ions were increased by up to 20-mM with a slight (<2%) decrease in the concentrations of other ions. High concentrations of calcium were always produced in this way. Concentrations of potassium up to 30 mM were produced either in this way or (for 24 mM-potassium) by using one of the standard solutions shown in Table 1.

Changes in the composition of auricles and in potassium exchange were measured as described previously (Rayner & Weatherall, 1959 ; Carslake & Weatherall, 1962).

TABLE 1

SALT SOLUTIONS USED FOR ISOLATED AURICLES

All solutions contained (mM) Ca 1·7, Mg 1·2, HCO_3 24·8, H_2PO_4 1·2, SO_4 1·2, dextrose 5·6 and the tabulated additional constituents

Medium	Additional constituent (mM)			
	Sodium	Potassium	Chloride	Sucrose
Standard	144·5	5·9	127·8	0
127 mM-sodium, 24 mM-potassium	126·8	23·6	127·8	0
75 mM-sodium, 75 mM-potassium	75·5	74·6	127·8	0
75 mM-sodium, 6 mM-potassium	75·5	5·9	59·1	140·0
145 mM-sodium, 75 mM-potassium	144·5	74·6	196·5	0
Standard hypertonic	144·5	5·9	127·8	140·0

RESULTS

Auricles in high potassium media

When the standard medium in which auricles were immersed was replaced by one containing more than 11 mM-potassium, or when potassium chloride was added to the organ-bath so that the concentration of potassium exceeded this level, the auricles stopped beating within 30 sec (or within 5 sec in 75 mM-potassium and remained relaxed for at least the next 15 min. In 75 mM-potassium they were also electrically inexcitable, but in 24 mM-potassium they continued to follow an electrical stimulus for 3 to 4 min, or resumed beating when stimulated up to 6 min after

exposure to the new medium. The pacemaker is known to be less sensitive than other parts of atrial muscle to increased concentrations of potassium (Mello & Hoffmann, 1960), and it seems likely that the persistent very small beat was due to the activity of cells in this specialized region. On restoring a normal medium, contractions began again and rapidly regained their original magnitude, more rapidly after a period in 24 mM-potassium than after an equal time in 75 mM-potassium.

Actions of calcium in media of different potassium concentrations. In these experiments the calcium concentration was increased by adding 1.4 M-calcium chloride to the bath, usually in sufficient quantity to raise the calcium concentration from 1.7 to 21.7 mM. None of the observed effects of calcium chloride followed addition of similar quantities of magnesium chloride, so the change in chloride concentration was not responsible for the observed effects. About 45 to 50 sec after adding the usual amount of calcium chloride to the standard medium a precipitate, presumably of calcium carbonate, began to appear and increased in density until it largely obscured the tissue in the bath. The concentration of calcium ions in solution was therefore diminishing. The precipitation was associated with a small change of *p*H, about 0.05 unit in 3 to 5 min: most of the effects observed occurred within 1 to 2 min and they are therefore not a result of changes in the hydrogen ion concentration of the bath. When half as much calcium chloride was added, some precipitation still occurred, and the effects on the tissue were less. The precipitate remained as a suspension until the bath was washed out, unless edetic acid was added, when it disappeared at once.

Three kinds of effect were observed on adding calcium chloride. In the ordinary medium (5.9 mM-potassium) the force of contraction increased and the rate decreased until large contractions occurred somewhat irregularly several seconds apart and then ceased altogether (Fig. 1). The auricles remained inactive and relaxed, and showed no sign of altering in tone for at least several minutes. In media containing 15 to 25 mM-potassium, addition of calcium chloride initiated beating and restored excitability, consistently if the potassium concentration was less than 20 mM, but only sometimes if it was higher. No systematic search was made for the upper

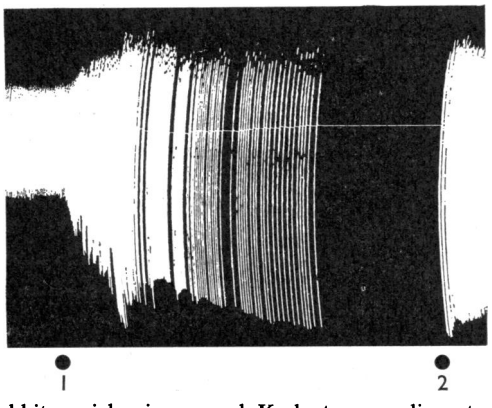

Fig. 1. Response of rabbit auricles in normal Krebs-type medium to extra calcium chloride (21.7 mM-calcium). (1) Calcium chloride added; (2) washout 26 min later.

limit at which restoration of beating was possible ; the highest observed concentration was 31 mM-potassium. In media containing 50 or 75 mM-potassium with a corresponding reduction of sodium concentration, addition of calcium chloride caused a slow contraction, or contracture, of the auricles, beginning sometimes at once and sometimes with up to 60 sec delay and continuing to develop for many minutes (Fig. 2). The contracture was not usually followed to completion, but it

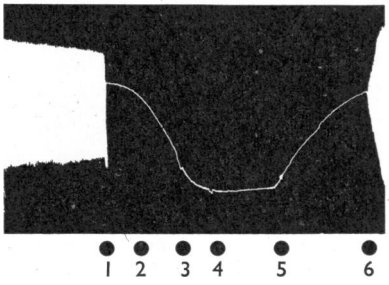

Fig. 2. Contracture of rabbit auricles in 75 mM-potassium and 75 mM-sodium on adding calcium chloride, and failure of recovery while anoxic. (1) Transfer to 75 mM-potassium and 75mM-sodium; (2) addition of calcium chloride (21.7 mM-calcium); (3) transfer to normal medium gassed with a mixture of 95% nitrogen and 5% carbon dioxide; (4) wash; (5) in normal medium gassed with 95% oxygen and 5% carbon dioxide; (6) wash. The interval between (1) and (2) was 2 min.

was often allowed to develop to the size of contractions of the auricles before they stopped in the high potassium medium, and this process took about 5 to 10 min. The contracture was reversible on washing out, and in time the auricles recovered apparently completely and resumed normal beating.

Responses in 75 mM-sodium and in 75 mM-potassium. In order to maintain a normal tonicity, the 75 mM-potassium medium also differed from the usual medium in having a reduced concentration of sodium (75 mM). Since both the effectiveness of calcium in increasing contractility and the uptake of calcium are greater when the external sodium concentration is low (Wilbrandt & Koller, 1948 ; Niedergerke & Harris, 1957 ; Niedergerke, 1959, 1963), the effects of low sodium and high potassium concentrations were examined separately. In a medium containing low sodium and normal potassium concentrations (75 mM-sodium and 6 mM-potassium), with osmotic activity maintained by adding sucrose (140 mM), auricles continued to beat with an initial but not sustained increase in amplitude of contractions and a decrease in rate. Addition of calcium chloride then caused a fresh increase in amplitude and further slowing, with arrest of beating within 1 min. The auricles could still be excited electrically, and responded to a train of stimuli with contractions of decreasing amplitude (Fig. 3). Nonstimulated auricles sometimes remained relaxed, and sometimes increased in tone in this combination of low sodium, normal potassium and high calcium concentrations. The increase in tone could be induced consistently in 75 mM-sodium and 6 mM-potassium by rendering the tissue anoxic by gassing with a mixture of 95% nitrogen and 5% carbon dioxide instead of 95% oxygen and 5% carbon dioxide (Fig. 4) and it is possible that variable oxygenation was responsible for the variable increase in tone observed otherwise. A contracture

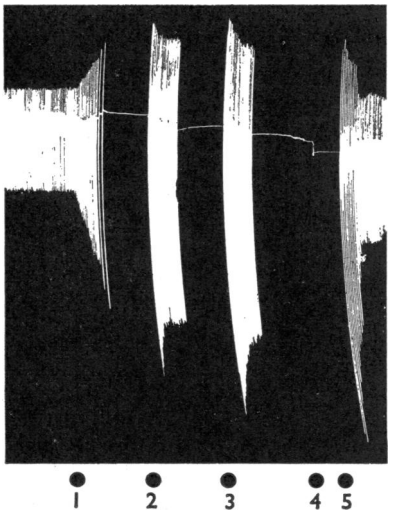

Fig. 3. Response of rabbit auricles in 75 mM-sodium and 6 mM-potassium to extra calcium chloride (21.7 mM). (1) Calcium chloride added, causing increase in amplitude and slowing and arrest of beat; (2), (3) two periods of electrical stimulation at 2 shocks/sec (note increased tone at end of each period); (4) normal medium restored; (5) beating resumed spontaneously. The interval between (1) and (2) was 2 min.

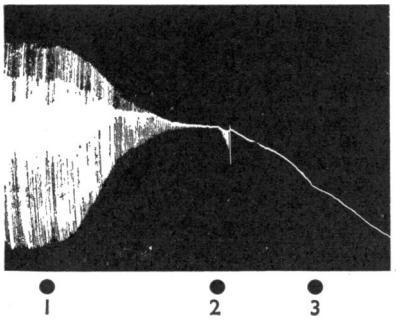

Fig. 4. Effect of anoxia on response of auricles in 75 mM-sodium and 6 mM-potassium to extra calcium chloride (21.7 mM). (1) Medium gassed with a mixture of 95% nitrogen and 5% carbon dioxide; (2) calcium chloride added; (3) gassed with 95% oxygen and 5% carbon dioxide. The interval between (1) and (2) was 4 min.

was also readily elicited by calcium chloride after a short period in 75 mM-sodium with no potassium present, in which condition the cell membrane is less stable than in 6 mM-potassium (Hoffman & Cranefield, 1960) and in which calcium entry is much increased (Thomas, 1960b). In any event, it appeared that reducing the sodium concentration of the medium was not by itself sufficient to produce a contracture.

When the concentration of potassium was raised without reducing that of sodium (145 mM-sodium and 75 mM-potassium), beating stopped promptly, and very little contracture occurred spontaneously or on adding calcium chloride. The failure

to respond with a contracture was not due to the high tonicity of the medium, because calcium chloride was effective in a hypertonic medium containing 75 mM-sodium, 75 mM-potassium and 140 mM-sucrose (Fig. 5). It therefore appeared that two changes were necessary for the contracture to occur: a reduction in the external sodium concentration, and an event which could be achieved by anoxia or by removal of external potassium, but was most reliably attained in a high concentration (75 mM) of potassium. As we describe later, a third condition necessary for contracture to occur is previous activity of the tissue. Very little response occurred to calcium in 75 mM-sodium 75 mM-potassium medium by left auricles which were not previously stimulated and had not been beating spontaneously.

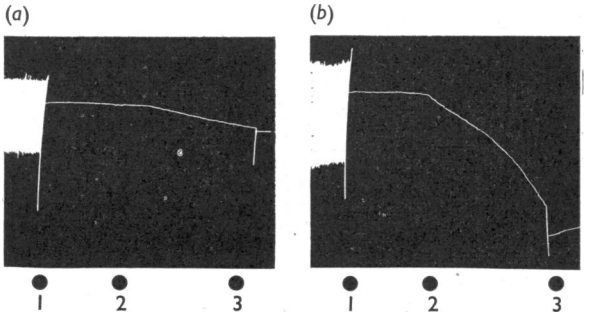

Fig. 5. Effect of sodium concentration on contracture induced by calcium chloride in 75 mM-potassium media. (*a*): (1) transfer to 75 mM-potassium and 145 mM-sodium; (2) addition of calcium chloride (21.7 mM-calcium); (3) washout. (*b*): as (*a*), but 75 mM-potassium, 75 mM-sodium and 140 mM-sucrose at (1). The intervals between (1) and (2) in each record were 2 min.

Anoxia. During the experiments in low sodium normal potassium media, it appeared that the contracture occurred more readily if the bath was not oxygenated or was poorly oxygenated. The effect of lack of oxygen was examined more systematically by gassing for various periods and in various circumstances with a mixture of 95% nitrogen and 5% carbon dioxide instead of 95% oxygen and 5% carbon dioxide. Auricles in the normal medium continued to beat for several minutes in this condition, but the force of contraction diminished considerably, particularly during the second and third minutes of anoxia. Recovery occurred about as quickly on restoring oxygen. In 75 mM-sodium 75 mM-potassium medium, anoxia caused a variable, moderate increase in tone, even though no extra calcium was added. A more striking consequence of anoxia was apparent if a contracture was induced with calcium, and the auricle was restored to a normal medium. In the absence of oxygen, no relaxation occurred, even with repeated washing out of the bath and tissue (Fig. 2). On restoring oxygen, relaxation occurred promptly at about the rate observed if there had been no exposure to anoxia. On the other hand, restoration of oxygen while excess calcium was still present did not alter the rate of development of a contracture. It appeared therefore that oxygen influenced the calcium contracture in two ways. Lack of oxygen served instead of a high concentration of potassium to initiate a contracture in a low sodium medium, and

oxygen was necessary for recovery from the contracture once the external calcium concentration was reduced to normal.

Recovery from contracture

After a contracture, well oxygenated tissues restored to a normal medium relaxed at first quickly, later more slowly, and after a time which appeared to be independent of the amount of relaxation, started beating. The course of relaxation was roughly exponential, with a half-time of about 4 min, and did not differ much in a variety of experiments. The time until beating started varied greatly, and tended to be greater with longer immersion in 75 mM-potassium medium, with longer exposure to high concentrations of calcium, and in repeated experiments on the same pair of auricles. A group of three experiments directed to measuring the relative importance of these effects (Table 2) showed that the time in 75 mM-potassium had

TABLE 2

TIME FOR REAPPEARANCE OF BEATING

The figures under " Time to recover " not in brackets are the times in minutes before visibly detectable beating occurred after restoring the auricles to a normal medium. Each experiment, consisting of three treatments, was done with a different pair of auricles. The figures in brackets show the orders in which the treatments were tested. The difference between short and long immersions in 75 mM-potassium is significant ($P=0{\cdot}02$)

Time (min) in		Time (min) to recover			
75 mM-Na, 75 mM-K	High Ca	Expt. 1	Expt. 2	Expt. 3	Mean
7	5	28 (1)	9 (2)	21 (3)	19·3
15	5	39 (3)	32 (1)	38 (2)	36·3
15	13	45 (2)	51 (3)	28 (1)	41·3

a large and statistically significant effect ($P=0.02$), and that the effect of the other factors was small and not significant in this small series. Beating always started with very small contractions and gradually became more prominent. The amplitude could be increased promptly by adding either adrenaline (2 to 5 μM) or, rather unexpectedly, calcium chloride (6 to 22 mM). These substances were effective also in initiating a substantial beat in auricles recovering from a calcium-induced contracture, even in the first few minutes of relaxation. Since the auricles were at this stage recovering from exposure to excess potassium as well as excess calcium, this effect of calcium chloride is presumably comparable to the effect seen after arrest in 20 mM-potassium, an effect which is also imitated by adrenaline. It is probably important that it can be produced by extra calcium in the medium even when the tissue as a whole might be expected to be getting rid of previously accumulated calcium.

Actions of ouabain

Ouabain increased the amplitude of contraction in a normal medium but, as is well known, the effect developed slowly, over a period of several minutes. It did not have any evident effect in media containing either 24 or 75 mM-potassium, in which media auricles were inactive. If, however, auricles were allowed to beat in a medium containing ouabain (for example 2 μM for 15 min), their transfer to

75 mM-potassium resulted in an immediate steep contracture, even though no extra calcium was added (Fig. 6). The contracture which resulted resembled that produced by calcium in the absence of ouabain in several ways. The time course of recovery on washing out was similar. Relaxation on washing out did not occur in the absence of oxygen and could be prevented apparently indefinitely by gassing with a mixture of 95% nitrogen and 5% carbon dioxide. The development of the contracture could be arrested promptly by adding edetic acid to the bath, and

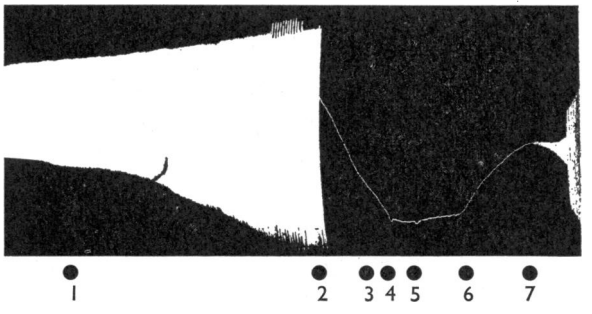

Fig. 6. Contracture of rabbit auricles after beating in ouabain. (1) Ouabain added to bath to give 2 μM; (2) transfer to 75 mM-potassium and 75 mM-sodium; (3) gassed with a mixture of 95% nitrogen and 5% carbon dioxide; (4), (5) washout with normal medium; (6) gassed with 95% oxygen and 5% carbon dioxide; (7) washout. The interval between (1) and (2) was 15 min.

reactivated by then adding sufficient calcium chloride to neutralize the chelating agent (Fig. 7). All these observations suggested that the final mechanism of contracture was the same, and that ouabain was acting by causing an accumulation of calcium in the tissue at a site where it was released when the external potassium concentration was raised sufficiently. As ouabain has been shown to increase the readily exchangeable fraction of calcium in auricles (Lüllmann & Holland, 1962), and as the rate of entry of labelled calcium is considerably increased in beating auricles (Winegrad & Shanes, 1962), it seemed likely that ouabain would be less

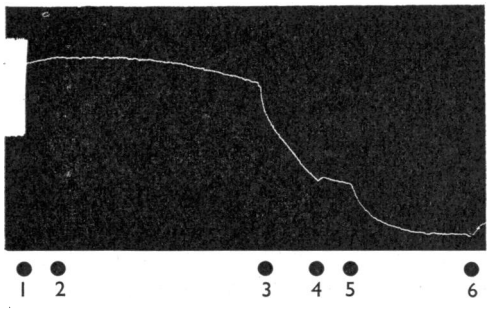

Fig. 7. Contracture of rabbit left auricle after period of inactivity in ouabain. Auricle initially electrically stimulated at 2 shocks/sec. (1) Stimulus off; (2) ouabain added to give 10 μM; (3) 15 min later, bath refilled with 75 mM-potassium and 75 mM-sodium medium; (4) 4 min later, edetic acid added to give 2 mM; (5) 2 min later, calcium chloride added to give 21.7 mM-calcium; (6) washout. The interval between (1) and (2) was 2 min.

effective if the auricles had been quiescent during their period of exposure to the drug. This was found actually to be the case. As Fig. 8 shows, even the effect of calcium chloride is less on a left auricle which has previously been quiescent than when it has been driven electrically. When a similar experiment is done in the presence of ouabain (2 μM), no contracture occurs if the auricle has been quiescent and the response to calcium chloride is as meagre as in the absence of ouabain. On the other hand, the auricle driven at 2 or 2.6 beats/sec in ouabain contracts promptly in 75 mM-sodium 75 mM-potassium medium without extra

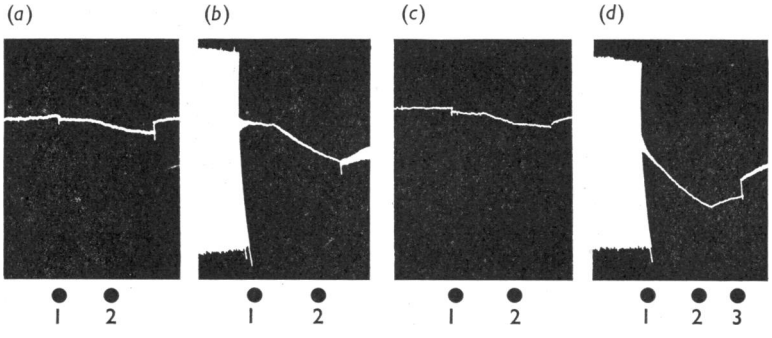

Fig. 8. Effect of beating and of ouabain on contracture of left auricle. (*a*): auricle quiescent for 10 min in normal medium; (1) 75 mM-potassium and 75 mM-sodium medium; (2) calcium chloride added to give 21.7 mM-calcium. (*b*): auricle stimulated at 2.6 shocks/sec for 10 min in normal medium; (1) and (2) as in (*a*). (*c*): auricle quiescent for 15 min in medium with 2 μM ouabain; (1) and (2) as in (*a*). (*d*): auricle stimulated at 2.6 shocks/sec for 10 min in medium with 2 μM ouabain; (1) as in (*a*), contracture occurred without addition of calcium chloride; (2) edetic acid added to 2 mM and contracture arrested; (3) washout. The interval between (1) and (2) in each record was 2 min.

calcium. If a high enough ouabain concentration is used for long enough, contracture occurs in a high potassium medium even though the auricle has been quiescent (Fig. 7): in general, a long exposure, a high rate of beating and a high concentration of ouabain all favour contracture, just as they also favour the inotropic action of ouabain on the heart in ordinary media (Sanyal & Saunders, 1958). At the other extreme, small contractures have been observed after exposure to only 0.2 μM-ouabain, provided that the auricles remained beating in this medium at a rate of 2 beats/sec for at least 2 hr. This exposure to ouabain is close to that likely to occur in its clinical use and suggests that the action is relevant to its therapeutic effect.

Actions of adrenaline

The action of adrenaline on auricles in a normal medium is very well known. It resembles the action of calcium in increasing the force of the beat, but differs in causing a sustained acceleration. In 127 mM-sodium 24 mM-potassium, or similar concentrations of potassium without reduction of sodium, and also in auricles recovering from a period in 75 mM-potassium, adrenaline (2 μM) acted like calcium in restoring excitability and spontaneous contractions. In fact it was the rather more effective of the two, acting more rapidly or after calcium had failed altogether

to restore beating. The effect of adrenaline was prolonged: in one experiment an auricle continued to beat well, though slowly, in 24 mM-potassium for 53 min in the presence also of 2 μM-adrenaline, and showed no sign of becoming less responsive before the experiment was stopped.

In 75 mM-potassium media, the addition of adrenaline to the bath had no observable effect even in concentrations up to 340 μM. Nor did adrenaline cause driven left auricles to go into contracture in 75 mM-potassium media after treatment of the auricles for 5 to 15 min in normal media with up to 390 μM-adrenaline even when this concentration was also maintained in the high potassium medium. It is unlikely that these negative results were due to destruction of adrenaline in the well-oxygenated fluid: Stafford (1962) gives a half-time of inactivation of about 40 min in similar conditions, though with a lower (2 μM) concentration of adrenaline, and her observations are consistent with those described in the previous paragraph in which effects of 2 μM-adrenaline were maintained for nearly 1 hr without adding more adrenaline to the bath.

Actions of caffeine

As caffeine and related methylxanthines are well known to increase the force of cardiac contraction, and as caffeine causes contracture of skeletal muscle depolarized by high concentrations of potassium (Axelsson & Thesleff, 1958; Frank, 1960), we expected that it would do so also in our preparation. In fact, no response occurred on adding caffeine (0.4 mM) or aminophylline (2.9 mM), to auricles in 75 mM-sodium 75 mM-potassium medium, nor did these drugs affect auricles in the 24 mM-potassium medium. In the standard medium, in the same concentrations both drugs exerted the expected effects on rate and force of contraction.

Changes in ionic content of auricles

The large changes in ionic composition of the medium in which the auricles were immersed produced changes in the auricles themselves. Table 3 shows the changes in sodium and potassium content, and the net entry of ^{42}K in auricles which had equilibrated for 30 to 60 min in the standard medium before transfer to one or other of the special media for 5, 10 or 15 min. Part of the changes in composition of the auricles is presumably due to extracellular alterations in the new media. If the extracellular space does not change and remains at 32.9 ml./100 g of wet weight, as found at 30° C in the standard medium (Carslake & Weatherall, 1962), there is normally outside the cells about 280 μmoles of sodium and 12μmoles of potassium per g of dry weight. In 127 mM-sodium 24 mM-potassium medium, these quantities become 244 and 36 μmoles and in 75 mM-sodium 75 mM-potassium medium, 146 μmoles of each ion per g of dry weight. It is uncertain how rapidly the extracellular space equilibrates with the medium, and the process is probably not any simpler in auricles (Carslake & Weatherall, 1962) than in toad stomach, in which it has been studied more fully (Burnstock, Dewhurst & Simon, 1963). Therefore, any exact correction of the results in Table 3 would be speculative. Clearly the auricles gained potassium intracellularly as well as extracellularly, and faster in 75 mM-potassium than in 24 mM-potassium. The efflux of potassium was

TABLE 3

EXCHANGE OF POTASSIUM IN RABBIT AURICLES

Media are listed by abbreviations of those listed in Table 1. * In the experiments in 127Na, 24K the concentration of calcium was 13.6 mM throughout the period of ^{42}K uptake. In the other experiments, calcium chloride was added 2 min after transfer of auricles to the radioactive medium, and the concentration was then raised from 1.7 to 21.7 mM. Concentrations and entry are given in μmole/g of dry weight. Values are means or means with standard errors

	1·7 mM-calcium				13·6 and 21·7 mM-calcium*			
Medium	No. of auricles	Sodium (μmole/g)	Potassium (μmole/g)	Potassium entry (μmole/g/min)	No. of auricles	Sodium (μmole/g)	Potassium (μmole/g)	Potassium entry (μmole/g/min)
Standard	3 left 3 right	— —	379±15 331±10	3·0±0·18 4·5±0·54	2 left 2 right	390 375	330± 9 341±11	3·6±0·06 4·8±0·12
127Na, 24K for 15 min	4 left 4 right	— —	496±27 473±14	11·1±0·63 10·8±0·35	4 left 4 right	— —	471±15 471±13	10·2±0·21 10·1±0·28
127Na, 24K+adrenaline (3 μM) for 15 min	4 left 4 right	— —	505±18 476±12	12·1±0·60 12·6±0·29	— —	— —	— —	— —
75Na, 75K for 5 min	2 left 2 right	463 381	416±53 468±18	24·5±0·57 27·0±3·04	— —	— —	— —	— —
75Na, 75K for 10 min	3 left 3 right	277 261	540±22 522±24	28·6±1·50 28·9±0·79	3 left 3 right	261 277	523±25 458±20	23·2±1·31 24·8±2·36
75Na, 6K for 10 min	3 left 3 right	212 236	348±22 278±30	3·3±0·32 3·9±0·62	3 left 3 right	246 219	346±17 286±11	3·6±0·45 3·7±0·20

also accelerated. In three experiments on auricles loaded for 15 min in standard medium containing ^{42}K and transferred to 24 mM-potassium during efflux, the rate constant (mean and standard error) was 0.0277 ± 0.0016 min^{-1}, which is nearly double the normal rate for auricles which are not beating (Rayner & Weatherall, 1959). The net entry of ^{42}K and the net gain in total potassium were always modestly reduced by extra calcium when the external potassium concentration was high: in 5.9 mM-potassium where the potassium fluxes were smaller, extra calcium had no demonstrable effect. These observations agree well with the concept of calcium as a membrane stabilizer, decreasing the permeability especially of the depolarized membrane to potassium. In contrast, adrenaline in 24 mM-potassium accelerated the entry both of radioactive and total potassium: in right auricles the increment was associated with slow spontaneous beating but the gain occurred also in left auricles which were quiescent throughout the experiment.

DISCUSSION

These experiments were devised to show effects on cardiac muscle contraction when the muscle fibres were no longer regulated by an excitable membrane. As Evans *et al.* (1958) have shown, preparations of smooth muscle in media containing high concentrations of potassium and little or no sodium respond to drugs, and we have examined the behaviour of some cardiac muscle in similar conditions. The consequences of raising the external potassium concentration were quite different according to whether $[K_e]$ was raised to about 25 or 75 mM, and particularly the effects of calcium were different in the two circumstances.

In 25 mM-potassium the auricles did not beat and gradually became inexcitable. Both excitability and spontaneous activity were restored either by increasing the concentration of calcium in the medium or by adding adrenaline. Further experiments are necessary to show whether both substances are acting on the same process in the tissue, or whether one is acting by altering the distribution in the tissue of the other, or whether the mechanisms are independent. As changes in the sodium, potassium or chloride conductance might each or all be involved, the number of possibilities is considerable.

In 75 mM-potassium the situation is quite different and adrenaline is without effect. Calcium produces a slow contraction of the tissue, sustained as long as calcium is present in excess and reversible on washing out. This resembles the action of calcium injected intracellularly (Heilbrunn & Wiercinski, 1947), or externally at the onset of depolarization (Weidmann, 1959), and also resembles its action in smooth and striated muscle and in amphibian cardiac muscle in a high potassium low sodium medium (Evans *et al.*, 1958 ; Lüttgau & Niedergerke, 1958 ; Frank, 1960). It seems clear that the effect is associated with an increased entry of calcium into the tissue (Niedergerke, 1963). A similar slow contraction occurs immediately in 75 mM-potassium without addition of extra calcium in auricles which have been beating in ouabain. The ouabain-contracture imitates the calcium-contracture in being reversible when the tissue is transferred to a normal oxygenated medium, in persisting in an anoxic but otherwise normal medium, and in being arrested by edetic acid, and so is probably due to accumulation of calcium in the

tissue during the period of immersion in the presence of ouabain. Direct evidence of some such accumulation has been obtained by Holland and collaborators (Holland & Sekul, 1959; Lüllmann & Holland, 1962) and by Klaus & Kuschinsky (1962), who found an increase in size of the rapidly exchangeable fraction of auricular calcium under the influence of cardiac glycosides. The present experiments suggest that this calcium is directly concerned in causing or enhancing contraction of cardiac muscle, both in ordinary beating and during sustained depolarization as observed here.

The published data about ^{45}Ca turnover do not show unequivocally whether the increase in the rapidly exchangeable fraction is due to increased entry or diminished removal from the fraction. As cardiac glycosides allow hearts to beat for longer and restore beating when there is no calcium in the external medium (Konschegg, 1913; Loewi, 1917) the effect is probably in preventing removal, but direct experimental evidence on the point is necessary. Calcium competes with sodium for entry to heart muscle (Lüttgau & Niedergerke, 1958), and the two ion species may also leave the tissue by a common pathway, so that blockage of calcium extrusion could occur by the same mechanism as the well known inhibition of the sodium pump (Glynn, 1957; Caldwell & Keynes, 1959). This hypothesis can be tested quantitatively when sufficient experimental results are available, and difficulties in measuring ionic fluxes in multicompartmental systems (such as appear to exist for both sodium and calcium) are overcome.

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