J. Physiol. (1981), **316**, *pp.* 109–125
With 8 text-figures
Printed in Great Britain

CALCIUM AND THE α-ACTION OF CATECHOLAMINES ON GUINEA-PIG TAENIA CAECI

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(*Received 4 March 1980*)

SUMMARY

1. The involvement of calcium in the α-action of adrenaline on guinea-pig taenia caeci was studied by measuring the changes in membrane potential and muscle contraction, using the sucrose-gap method, and by determining the ^{42}K efflux, in the presence of a β-blocker (propranolol, $1{\cdot}8 \times 10^{-6}$ M).

2. In the presence of extracellular calcium, the hyperpolarization caused by adrenaline (3×10^{-6} M) was sustained during the period of its application (5 min), both in active preparations (at 36 °C) and in quiescent muscle (22 °C). In the absence of calcium, adrenaline caused a transient hyperpolarization which was smaller at 36 °C than at 22 °C and passed off within 5 min, while adrenaline was present.

3. Both the sustained and the transient hyperpolarization were associated with an increase in ^{42}K efflux which had a similar time course. ^{42}K flux measurements were made in depolarized tissue (52·8 mM-potassium), in which the effect was consistent and more pronounced than in polarized muscle (2·8 mM-potassium).

4. The transient hyperpolarization which is resistant to calcium removal and EGTA (0·1–2·0 mM) could be evoked only once but, following a short exposure to calcium (2·5 mM) for 20 sec and readmission of calcium-free medium, it was restored.

5. The sustained and the transient hyperpolarization and the increase in ^{42}K efflux were abolished by the α-antagonist phentolamine (10^{-5} M); their amplitude was dependent on the adrenaline concentration in the range 10^{-7} to 3×10^{-6} M, and both responses persisted in the absence of sodium or chloride.

6. The hyperpolarization and the increase in ^{42}K efflux were greater at higher external calcium concentrations (0·3–2·5 mM).

7. Cobalt (0·6 mM), D600 ($2{\cdot}5 \times 10^{-5}$ M) and the bee toxin apamin (10^{-7} M) reduced the α-response.

8. In the presence of apamin, in calcium-containing solution, the sustained hyperpolarization caused by adrenaline was preceded by, or converted to, depolarization, spike discharge and contraction.

9. The depolarizing effect of adrenaline in the presence of apamin persisted in sodium-free or chloride-free medium, but was blocked in the absence of calcium and diminished by cobalt and D600.

10. It is concluded that the α-response of guinea-pig taenia caeci consists of two components, both involving calcium. First, the activation of α-receptors increases

calcium entry, which leads to the opening of potassium channels, a sustained hyperpolarization and inhibition of muscle activity. Secondly, in the absence of external calcium, a transient hyperpolarization is revealed, presumably due to the release of bound calcium from a limited cellular store which can be replenished by addition of external calcium, and this leads to an increase in potassium permeability.

INTRODUCTION

The role of calcium as a mediator in producing stimulation or inhibition of smooth muscle was recently considered in a well-documented review (Bolton, 1979). In order to explain the modulation of muscle tension by stimulating drugs, both potential-sensitive and receptor-operated calcium channels have been described. Interaction of the agonist with the receptor may also release calcium from some site associated with the receptor, a process which is independent of extracellular calcium. The simultaneous existence of both mechanisms, producing an increase in intracellular calcium by opening calcium channels and dislodging bound calcium from a pool, was demonstrated in an elegant series of experiments carried out on parotid gland cells by Putney (1976*a*, *b*, 1977).

Activation of α-receptors may either stimulate smooth muscle, as in blood vessels, or hyperpolarize and relax, as in the gastro-intestinal tract. To account for the different responses two types of receptor-operated channels are thought to exist, with different ion permeabilities. The inhibiting action, as observed in taenia caeci of the guinea-pig, can be understood from the increase in potassium permeability leading to hyperpolarization of the muscle cells (Jenkinson & Morton, 1967; Shuba & Klevets, 1967; Bülbring & Tomita, 1969). It has been recognized that extracellular calcium is required for the inhibitory α-action in taenia and it has been suggested (Bülbring & Tomita, 1977*b*) that calcium contributes to the α-action by acting on some internal site to modulate potassium permeability. A similar role of internal calcium in regulating potassium permeability has also been proposed in other tissues (Meech, 1974; Putney, 1976*a*; Haylett, 1976; Banks, Brown, Burgess, Burnstock, Claret, Cocks & Jenkinson, 1979). For the taenia the question remains as to whether the internal calcium is raised by an increased influx during α-receptor activation or by liberation of calcium from some pool.

In this study, a transient component, independent of extracellular calcium, and a sustained component, dependent on external calcium, could be distinguished. The observations indicate that the transient phase is triggered by dislodging bound calcium from a limited store and that the sustained component is associated with a continuous entry of calcium, both leading to a change in potassium permeability and in membrane potential. The pathway used by calcium to enter the muscle cell is discussed.

METHODS

Tissue preparation. Guinea pigs of either sex (250–300 g) were stunned and bled. Strips of taenia caeci about 0·8 mm thick and 40 mm long were used throughout. The preparations were equilibrated in Krebs solution for at least 1 hr before the experimental procedure was started. Tissues were blotted and weighed at the end of the flux experiments to determine the wet weight.

Solutions. The composition of the solutions used is listed in Table 1. In chloride-free solution

TABLE 1. Solutions used*

	Na^+	K^+	Ca^{2+}	Cl^-	Mg^{2+}	HCO_3^-	$H_2PO_4^-$	La^{3+}	Hepes	Glucose	O_2 (%)	CO_2 (%)
Krebs	140·0	2·8	2·5	150·2	1·2	22·7	1·2	—	—	11·5	95	5
Calcium-free	134·6	2·8	—	149·8	6·2	22·7	1·2	—	—	11·5	95	5
Sodium-free	—	2·8	2·5	205·8	96·0	22·7†	—	—	—	11·5	95	5
Chloride-free	140·0	2·8	2·5	—	1·2	22·7	1·2	—	—	11·5	95	5
High-potassium	90·0	52·8	2·5	150·2	1·2	22·7	1·2	—	—	11·5	95	5
Lanthanum	136·5	2·8	—	171·7	1·2	—	—	10	4	11·5	100	—

* Concentrations are in mM, unless otherwise stated.
† As choline bicarbonate.

sodium chloride was replaced by the impermeant sodium isethionate and the other chlorides by sulphates. In sodium-free solution sodium bicarbonate was replaced by choline bicarbonate. The calcium-free solutions contained 0·4 mM-EGTA (ethylene glycol-bis(β-aminoethyl ether)*N*,*N*-tetraacetic acid) and 6·2 mM-magnesium. Atropine ($1{\cdot}4 \times 10^{-6}$ M) and guanethidine ($2{\cdot}5 \times 10^{-6}$ M) were added to all solutions to block the cholinergic receptors and the release of catecholamines from nerve terminals. The pH of the lanthanum solution was adjusted by addition of sodium hydroxide. Drugs used were EGTA (Sigma), atropine (Merck), guanethidine (Ciba), phentolamine (Ciba), propranolol (ICI), sodium isethionate (Fluka), apamin (Serva), D600 (Knoll), L-adrenaline (Sigma); the other chemicals used were of analytical grade and obtained from Merck.

Electrophysiological measurements. The sucrose-gap method was used to measure changes in membrane potential and mechanical activity simultaneously (Bülbring & Tomita, 1969). Potential changes across the sucrose-gap were measured by means of calomel electrodes making contact with the test solution and with the reference solution (isotonic potassium chloride) and recorded via a pre-amplifier.

Flux measurements. After equilibration in Krebs solution for 1 hr the preparation was transferred to ^{42}K- or ^{45}Ca-containing solution (from the Radiochemical Centre Amersham, England). Efflux measurements were performed after loading the preparation for at least 1 hr in Krebs solution or in high-potassium solution (52·8 mM) containing the isotope; the temperature was the same as used in the actual efflux experiment. After this loading period the preparations were mounted and superfused with the solution of choice (flow: 1·0 ml./min) in a continuous-flow apparatus (Brading, 1967). Collection of the ^{42}K samples at 2 min intervals was started 20 min after the loaded preparation had been mounted.

Influx experiments (^{45}Ca) were carried out in Krebs solution or in high-potassium solution (52·8 mM). The preparations were preincubated in Krebs solution before the loading period was started. In order to limit ^{45}Ca leakage and minimize the contribution of externally bound ^{45}Ca, the preparations loaded with ^{45}Ca were washed for 10 min at 1 °C with an isotonic sucrose solution containing lanthanum (10 mM) (Brading, 1978).

Tissues containing ^{45}Ca were dissolved in toluene (1 ml.; 0·5 N-quaternary ammonium hydroxide in toluene; Packard) and their radioactivity determined in a liquid scintillation counter after adding scintillation fluid (10 ml.). The ^{42}K effluent activity was measured using a gamma nuclear counter. Efflux rate is expressed as the counts lost per 2 min as a percentage of the mean isotope content of the tissue during that time.

Mean values ± S.E. of the mean are presented; values are considered significant at $P < 0{\cdot}05$ (Student's *t* test for paired values referring to the changes in membrane potential and for unpaired values with respect to the flux measurements).

RESULTS

The effect of adrenaline

In spontaneously active taenia preparations superfused with Krebs solution at 36 °C, adrenaline caused hyperpolarization of the muscle cells, suppression of spike activity and relaxation (Fig. 1*A*), confirming previous observations (Bülbring & Tomita, 1969). The ^{42}K efflux observed in 2·8 mM-potassium solution was enhanced in the presence of adrenaline (Fig. 1*B*; Table 3), which is in accord with the observations on depolarized muscle (Jenkinson & Morton, 1967). In order to study the involvement of calcium, the adrenaline response was evoked in calcium-free solution, containing EGTA (0·4 mM) to bind residual extracellular calcium, and the magnesium concentration was raised (6·2 mM) to prevent the membrane from leaking (Bülbring & Tomita, 1970). Under calcium-free conditions spontaneous activity was abolished. Adrenaline caused a small transient hyperpolarization (Fig. 1*C*), which has been noticed previously (Bülbring & Tomita, 1977*b*); it was not accompanied by a consistent change in ^{42}K efflux in a solution containing the normal potassium concentration ratio (Fig. 1*D*; Table 3).

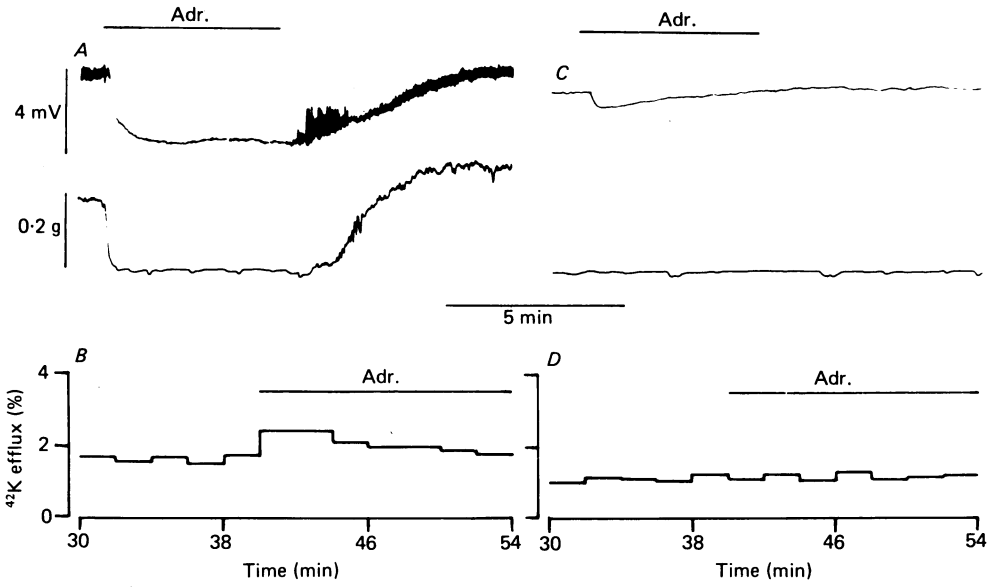

Fig. 1. Changes in membrane potential, in muscle contraction and in ^{42}K efflux caused by adrenaline (3×10^{-6} M) at 36 °C in the presence (*A*, *B*) and absence (*C*, *D*) of extracellular calcium (2·5 mM). Adrenaline was added after 20 min (*C*) or 40 min (*D*) superfusion of the preparation with calcium-free medium containing 0·4 mM-EGTA and 6·2 mM-magnesium. Note that the hyperpolarization (downward deflexion) is accompanied by relaxation of the muscle (downward deflexion) and by an increase in ^{42}K efflux (expressed as a percentage of the tissue content) in calcium-containing medium.

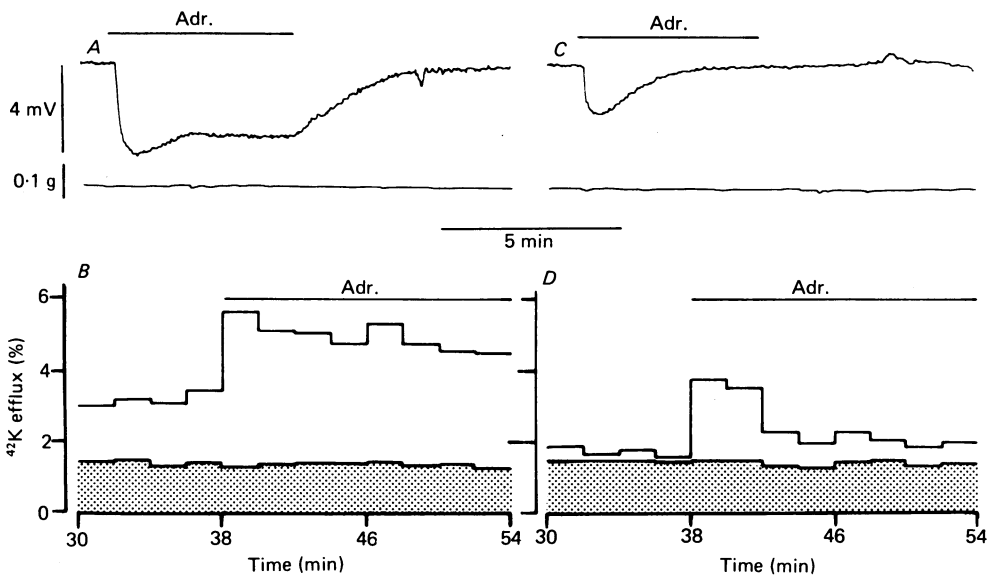

Fig. 2. Changes in membrane potential, in muscle contraction and in ^{42}K efflux caused by adrenaline (3×10^{-6} M) at 22 °C in the presence (*A*, *B*) and absence (*C*, *D*) of calcium (2·5 mM). The ^{42}K efflux was measured on depolarized muscle (52·8 mM-potassium). The same procedure was followed as described in Fig. 1. The ^{42}K efflux was not changed upon addition of adrenaline in the presence of phentolamine (10^{-5} M; stippled graph in *B* and *D*).

The experiment was repeated on quiescent preparations obtained at lower temperature (22 °C) to prevent fluctuations in ion fluxes caused by changes in spontaneous activity. Adrenaline caused a sustained hyperpolarization in the presence of calcium and, under calcium-free conditions, the hyperpolarization was again transient in nature. The amplitude of the transient hyperpolarization was high compared with that at 36 °C (Fig. 2*C*; Table 2). Since the increase in ^{42}K efflux caused by adrenaline was not consistent in polarized preparations (Hüter, Bauer & Goodford, 1963) the ^{42}K efflux experiments were carried out on depolarized muscle (Jenkinson & Morton, 1967). A reproducible increase in ^{42}K efflux was obtained in calcium-containing medium (Fig. 2*B*) and a transient rise in efflux was observed in calcium-free medium (Fig. 2*D*) when this experiment was carried out on depolarized muscle (52·8 mM-potassium, 22 °C; Table 3), and this procedure was followed for the rest of the ^{42}K efflux experiments. In the presence of high potassium (52·8 mM) adrenaline still caused a small hyperpolarization (less than 10% of the value found in 2·8 mM-potassium Krebs solution) accompanied by relaxation of the muscle, indicating that the same mechanisms are involved as in non-depolarized muscle.

The hyperpolarization caused by adrenaline sometimes showed an initial maximum in calcium-containing medium before reaching a constant level; this was also apparent in the ^{42}K efflux (Fig. 2*A* and *B*). In the presence and in the absence of extracellular calcium the hyperpolarization reached its maximum in about 60 sec (calcium-containing medium: 70 ± 5 sec, $n = 54$; calcium-free medium: 53 ± 3 sec, $n = 57$). The maximum amplitude of the hyperpolarization and of the increase in ^{42}K efflux (in depolarized muscle) evoked by adrenaline in the absence of calcium (20 min) was about 65% of that observed in the presence of calcium (Table 2).

Adrenaline interacts with both α- and β-receptors in taenia caeci (Bülbring & Tomita, 1969). The β-action was blocked by the continuous presence of propranolol ($1{\cdot}8 \times 10^{-6}$ M) in all experiments. The transient response evoked in calcium-free medium and the sustained response observed in the presence of extracellular calcium were both abolished after blocking the α-receptors by phentolamine (10^{-5} M); its effect on potassium efflux is shown in Fig. 2*B* and *D*. The potassium efflux was diminished by phentolamine in all experiments; more experimental data are required to explain this change. The potassium efflux was not enhanced in the presence of adrenaline and its antagonist. This observation indicates that the changes in membrane potential and potassium efflux evoked by adrenaline were due to interaction with the α-receptors. The amplitude of the sustained as well as the transient hyperpolarization was dependent on the concentration of the agonist, as shown in the dose–response curves (Fig. 3).

The influence of sodium and chloride in the α-action

Although it is believed that the contribution of sodium and chloride to the α-action of adrenaline on taenia is limited (Bülbring, Goodford & Setekleiv, 1966; Jenkinson & Morton, 1967; Ohashi, 1971; Bülbring & Tomita, 1977*a*), these ions may be essential in the transient response elicited in calcium-free solution. Therefore, the effect of adrenaline was investigated after reversing the chloride or sodium concentration gradient across the cell membrane by omitting these ions from the superfusion fluid. Replacement of sodium by magnesium reduced the ^{42}K efflux, especially in the

presence of calcium (Table 3); this is consistent with the change in membrane resistance noted previously (Bülbring & Tomita, 1977*a*). The ^{42}K efflux was increased by adrenaline to a lesser extent than in Krebs solution, both in the absence and presence of calcium (Table 2). Nevertheless, the size of the hyperpolarization elicited by adrenaline in calcium-containing solution was not significantly changed, something also mentioned by Bülbring & Tomita (1977*a*, *b*). The hyperpolarization elicited by the agonist in calcium-free, sodium-free medium was even greater than that in Krebs

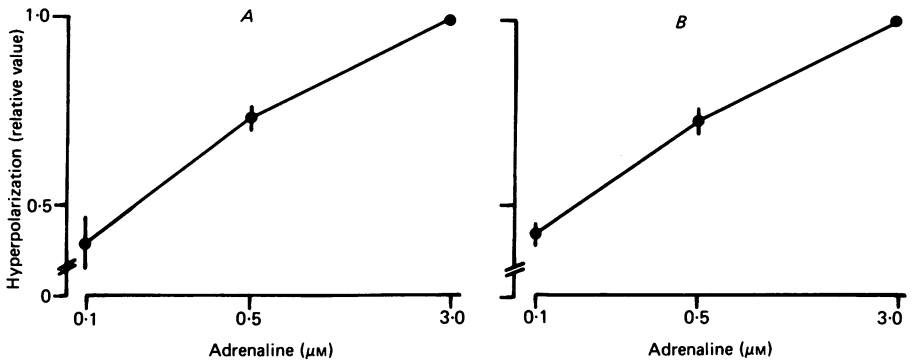

Fig. 3. The hyperpolarization produced by different adrenaline concentrations at 22 °C in the presence of calcium (2·5 mM; *A*) and in calcium-free medium (*B*). The hyperpolarization is expressed as a relative value (*A*: 1·00 = 7·5±0·9 mV, $n = 4$; *B*: 1·00 = 3·7±1·8 mV, $n = 4$).

solution (Table 2). Chloride may contribute to the α-action at 36 °C (Bülbring & Tomita, 1969; Ohashi, 1971). However, the sustained adrenaline response was not changed by reducing the extracellular chloride concentration under our experimental conditions (22 °C); both the hyperpolarization (Table 2) and the increase in ^{42}K efflux were similar in chloride-free and Krebs solution (Table 3). On the other hand, the transient hyperpolarization and the transient increase in ^{42}K efflux evoked by the agonist in calcium-free solution were diminished in the absence of chloride (Tables 2 and 3). These results indicate that the availability of sodium or chloride is not necessary to evoke the hyperpolarization and that the main factor is an increase in potassium permeability.

Calcium and the α-action

The observations described so far have shown that the α-response consists of two components, one of which requires extracellular calcium and the other which could be evoked in calcium-free medium. The latter might be due to some residual calcium. However, prolonged superfusion of the preparation with calcium-free solution (50 min) containing EGTA (0·4 mM), or increasing the EGTA concentration to 2 mM did not affect the initial, transient α-response (Tables 2 and 3). These results exclude a contribution by residual extracellular calcium.

Following the initial transient hyperpolarization produced by adrenaline in the absence of extracellular calcium, the membrane potential returned to its pre-adrenaline

TABLE 2. Changes in membrane potential caused by adrenaline (3×10^{-6} M) under different experimental conditions

Solution	Conc. (M)	Temp. (°C)	Exposure time (min)	Hyperpolarization caused by adrenaline in the presence of Ca^{2+}			Exposure time (min)	Hyperpolarization caused by adrenaline in the absence of Ca^{2+}		
				mV	Rel. values	n		mV	Rel. values	n
Krebs	—	36	20	5·4 ± 1·8	—	5	20	1·5 ± 0·8	—	5
Krebs	—	22	20	4·8 ± 0·5	—	43	20	3·1 ± 0·2	—	35
EGTA	$0{\cdot}4 \times 10^{-3}$	22	—	—	—	—	20	3·4 ± 0·4	1·00	3
EGTA	$2{\cdot}0 \times 10^{-3}$	22	—	—	—	—	20	2·8 ± 1·5	0·80 ± 0·29	3
Na^+	$140{\cdot}0 \times 10^{-3}$	22	20	3·6 ± 0·9	1·00	4	20	2·8 ± 0·7	1·00	4
Na^+	0	22	20	3·2 ± 0·9	0·90 ± 0·25	6	20	7·1 ± 1·8†	2·53 ± 0·80†	4
Cl^-	$150{\cdot}2 \times 10^{-3}$	22	20	7·4 ± 2·5	1·00	5	20	5·4 ± 1·7	1·00	4
Cl^-	0	22	20	8·5 ± 0·9	1·15 ± 0·12	5	20	2·3 ± 0·3†	0·42 ± 0·05†	4
Krebs	—	22	20	3·9 ± 1·1	1·00	7	20	1·9 ± 0·5	1·00	5
Co^{2+}	$0{\cdot}6 \times 10^{-3}$	22	20	4·5 ± 0·5	1·16 ± 0·06	3	20	2·0 ± 0·2	1·06 ± 0·11	3
Co^{2+}	$0{\cdot}6 \times 10^{-3}$	22	35	2·8 ± 0·2*	0·71 ± 0·04*	4	60	0·8 ± 0·1†	0·40 ± 0·05†	3
Krebs	—	22	20	3·9 ± 0·7	1·00	6	20	3·0 ± 0·6	1·00	6
D600	$2{\cdot}0 \times 10^{-5}$	22	20	3·4 ± 0·3	0·86 ± 0·07	4	20	3·2 ± 0·4	1·08 ± 0·12	3
D600	$2{\cdot}0 \times 10^{-5}$	22	35	2·5 ± 0·2*	0·64 ± 0·06*	3	60	2·0 ± 0·2†	0·67 ± 0·07†	5
Krebs	—	22	20	4·2 ± 0·9	1·00	4	20	2·4 ± 0·9	1·00	4
Apamin	$1{\cdot}0 \times 10^{-7}$	22	20	0·8 ± 0·8*	0·04 ± 0·04	4	20	0·2 ± 0·2†	0·10 ± 0·07†	4

The hyperpolarization evoked by adrenaline (5 min exposure) in the absence of calcium was obtained after superfusion of the preparation for 20 min with calcium-free solution containing EGTA (0·4 mM) and magnesium (6·2 mM). Relative values are expressed as the ratio of the maximal hyperpolarization observed in the control (taken as unity) and that measured after changing the external ion concentration or after application of drugs (the exposure time is listed).

* Significantly different from values obtained in calcium-containing Krebs solution.

† Significantly different from values obtained in calcium-free Krebs solution.

TABLE 3. Maximal changes in ^{42}K efflux caused by adrenaline (3×10^{-6} M) under different experimental conditions

Solution	Conc. (M)	Temp. (°C)	^{42}K efflux in absence of adrenaline (% isotope activity in tissue) Ca^{2+}-containing medium	n	^{42}K efflux in absence of adrenaline (% isotope activity in tissue) Ca^{2+}-free medium	n	Maximum ^{42}K efflux in presence of adrenaline (% isotope activity in tissue) Ca^{2+}-containing medium	n	Maximum ^{42}K efflux in presence of adrenaline (% isotope activity in tissue) Ca^{2+}-free medium	n	Increase in ^{42}K efflux in presence of adrenaline (% of pre-adrenaline value) Ca^{2+}-containing medium	Increase in ^{42}K efflux in presence of adrenaline (% of pre-adrenaline value) Ca^{2+}-free medium
K^+	$2{\cdot}8 \times 10^{-3}$	36	1·62±0·17	5	1·68±0·26	5	2·03±0·17	5	1·79±0·24	5	27± 8	8± 3
K^+	$2{\cdot}8 \times 10^{-3}$	22	1·40±0·12	6	1·31±0·15	6	1·77±0·16	6	1·36±0·19	6	28± 8	3± 3
K^+	$52{\cdot}8 \times 10^{-3}$	22	2·90±0·23	10	1·42±0·12	19	4·80±0·32	10	2·41±0·27	19	72±15	60± 4
EGTA	$0{\cdot}4 \times 10^{-3}$	22	—	—	1·42±0·12	19	—	—	2·41±0·27	19	—	60± 4
EGTA	$2{\cdot}0 \times 10^{-3}$	22	—	—	1·88±0·10	2	—	—	3·11±0·60	2	—	65±21
Na^+	0	22	0·66±0·09	4	0·61±0·08	4	0·98±0·24	4	1·24±0·27	4	47± 7	203±44
Cl^-	0	22	2·42±0·19	4	1·69±0·08	4	4·52±0·59	4	2·35±0·42	4	85± 9	37±19
Co^{2+}	$0{\cdot}6 \times 10^{-3}$	22	1·56±0·10	4	1·06±0·08	4	3·14±0·31	4	1·76±0·21	4	102±27	69±24
D600	$2{\cdot}0 \times 10^{-5}$	22	1·29±0·19	4	1·01±0·15	4	2·51±0·17	4	1·63±0·26	4	112±42	61± 2
Apamin	$1{\cdot}0 \times 10^{-7}$	22	2·06±0·07	2	1·35±0·31	3	2·39±0·12	2	1·59±0·33	3	19± 3	16± 2

The calcium medium contained 2·5 mM; EGTA (0·4 mM) and magnesium (6·2 mM) were added to the calcium-free solution. Adrenaline was added for 14 min after 38 min superfusion of the preparation with the test solution containing 52·8 mM-potassium (except in the first two experiments; 2·8 mM-potassium). The mean efflux rate in the absence of adrenaline was estimated during the 8 min preceding application. The maximum ^{42}K efflux in the presence of adrenaline was obtained in the 2 min period following application of the agonist.

value within about 4 min, even though adrenaline was still present. Moreover, this response could be evoked only once, a second dose being ineffective (Fig. 4*A* and *B*). Short admission of calcium (2·5 mM) for 20 sec followed by a washing period of 20 min with calcium-free solution was sufficient to restore the adrenaline response (Fig. 4*C*). This observation implies the existence of a calcium pool, available and necessary for evoking the α-response in the absence of external calcium. The amount of calcium

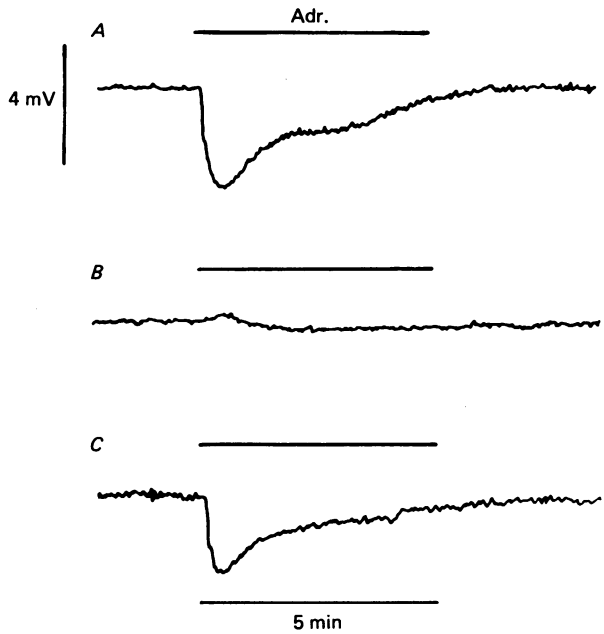

Fig. 4. The effect of adrenaline (3×10^{-6} M) at 22 °C after a short application of calcium. The transient hyperpolarization in calcium-free solution is shown. Continuing recording *A*, adrenaline was added again to the calcium-free solution in *B*. Note that the α-response as reflected by a transient hyperpolarization could not be evoked. Calcium (2·5 mM) was applied for 20 sec between *B* and *C* and the preparation was superfused again with calcium-free medium for 20 min before adrenaline was added to evoke the α-response (*C*).

located in this compartment must, in view of the transient nature of the α-response, be limited. Moreover, it is depleted by a single adrenaline application. Hence, it is unlikely that this compartment is located intracellularly, because these stores are believed to contain substantial amounts of calcium (Popescu, 1977). The calcium compartment involved in the α-action can be readily filled with extracellular calcium, but it is not replenished with calcium from the cytoplasm. Thus, this compartment is most likely located in the membrane as part of the membrane structure. Following depletion of this compartment by applying adrenaline (3×10^{-6} M) for 5 min, different degrees of replenishment could be obtained by adding different calcium concentrations for 10 min periods (Fig. 5). When, 20 min after readmission of calcium-free (EGTA) solution, adrenaline was applied again, it acaused a hyperpolarization whose amplitude was related to the calcium concentration used to replenish the compartment (Fig. 5*A–C*). Similarly, the sustained hyperpolarization was related to the extra-

cellular calcium concentration (Fig. 5*D–F*) and the ^{42}K efflux was decreased at 1·0 mM-calcium to 40 % and at 0·3 mM-calcium to 32 % ($n = 2$) of the efflux observed in 2·5 mM-calcium solution, in accord with the reduced hyperpolarization. Thus the increase in potassium permeability may be taken as a measure of the amount of calcium entering the cell as well as of the amount of calcium released from a cellular compartment upon α-receptor activation.

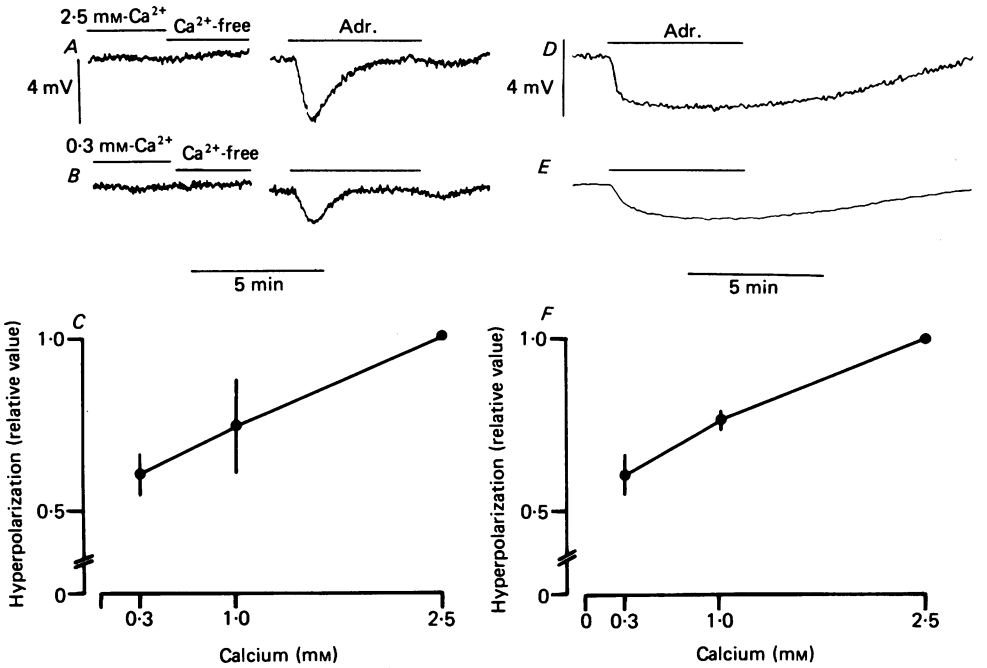

Fig. 5. The hyperpolarization evoked by adrenaline (3×10^{-6} M) at 22 °C in calcium-free medium (*A*, *B*, *C*) and in the presence of different calcium concentrations (*D*, *E*, *F*). Adrenaline was applied 20 min after readmission of calcium-free solution, preceded by a superfusion period (10 min) with 2·5 mM-calcium (*A*) or 0·3 mM-calcium (*B*). The relation between the hyperpolarization (expressed as a relative value: 1·00 = 1·4 ± 0·5 mV, $n = 4$) evoked by adrenaline and the calcium concentrations (preceding the calcium-free period) is shown in *C*. The adrenaline responses evoked in the presence of 2·5 mM-calcium and 0·3 mM-calcium respectively are shown in *D* and *E*. The hyperpolarization (expressed as a relative value: 1·00 = 2·4 ± 0·8 mV, $n = 4$) observed at different calcium concentrations is plotted in *F*.

Calcium antagonists and the α-action

It is clear from the experiments carried out by Bülbring & Tomita (1977*b*) and the present experiments that extracellular calcium is required to obtain a sustained α-response. Activation of the α-receptors leads to an increase in calcium influx in the parotid gland (Putney, 1976*a*) and in a variety of smooth muscle (Wahlström, 1973; Godfraind, 1976; Casteels, Kitamura, Kuriyama & Suzuki, 1977). Influx experiments carried out on polarized muscle (Krebs solution containing 2·8 mM-potassium) or on depolarized muscle (52·8 mM-potassium) did not show a detectable change in ^{45}Ca

content ($P < 0{\cdot}05$, $n = 6$) after incubation of the preparations with adrenaline for 5, 7 and 10 min, respectively.

Another approach to studying the contribution of calcium entry to the α-action was to investigate the effect of calcium antagonists which are believed to reduce calcium entry. Cobalt (as cobalt chloride) (Marier, Putney & Van de Walle, 1978) and D600 (Mayer, Van Breemen & Casteels, 1972) were used. Fig. 6*A* shows the sustained

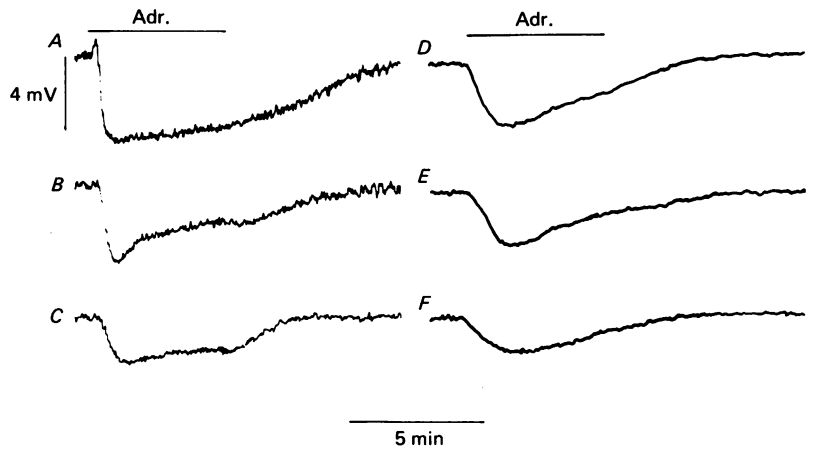

Fig. 6. The effect of adrenaline (3×10^{-6} M) on taenia caeci at 22 °C in the presence of D600 (2×10^{-5} M). The hyperpolarization produced by adrenaline in the presence of calcium (2·5 mM) is shown in *A*. The adrenaline response (*B*) declined after reaching an initial maximum in the presence of D600 (20 min) and a further reduction, especially of the initial maximum, is observed in the second response (*C*) evoked after 35 min treatment with D600. Another preparation was superfused for 10 min with calcium-containing Krebs solution between *D*–*E* and *E*–*F* before exposure to calcium-free solution. Records *D*–*F* were taken 20 min after removal of calcium. The adrenaline response evoked under calcium-free conditions in the presence of D600 (20 min; *E*) is comparable with that in the absence of D600 (*D*); the next adrenaline response (60 min D600) is markedly reduced in amplitude (*F*).

α-response in the presence of calcium. Between *A* and *B*, D600 was added, 20 min before the addition of adrenaline. At the onset the hyperpolarization was as large as the control, but it gradually faded. Prolonged treatment with D600 caused further reduction (*C*). The transient α-response in the absence of calcium is shown in *D*. When the refilling of the cellular calcium store (by a 10 min exposure to calcium-containing solution) preceded the application of the calcium antagonist, the transient α-response to adrenaline was similar to that in the control (*E*). However, when refilling of the calcium compartment was carried out in the presence of the calcium antagonist, the transient α-response was markedly diminished (*F*). The observations suggest that the release of calcium from the cellular compartment is not affected by calcium antagonists (Fig. 6*B* and *E*), but, as refilling of the cellular calcium store becomes incomplete, the limitation of calcium entry diminishes the sustained as well as the transient component of the α-response (*C* and *F*). Cobalt and D600 produced the same effects. The maximal amplitude of the hyperpolarization caused by adrenaline after 20 min exposure to the calcium antagonist and that of the second response evoked

after 35 min (calcium-containing medium) or 60 min (calcium-free medium) are presented in Table 2. Both calcium antagonists reduced ^{42}K efflux during the resting state and both diminished the increase in ^{42}K efflux caused by adrenaline (Table 3).

The bee toxin apamin is believed to prevent the opening of potassium channels in liver cells (Banks *et al.* 1979) and in the taenia caeci (Maas & den Hertog, 1979). The spontaneous spike activity of the taenia was often increased by apamin, while the hyperpolarization caused by adrenaline was diminished and preceded by a depolarization. In the experiment shown in Fig. 7 the immediate effect of adrenaline,

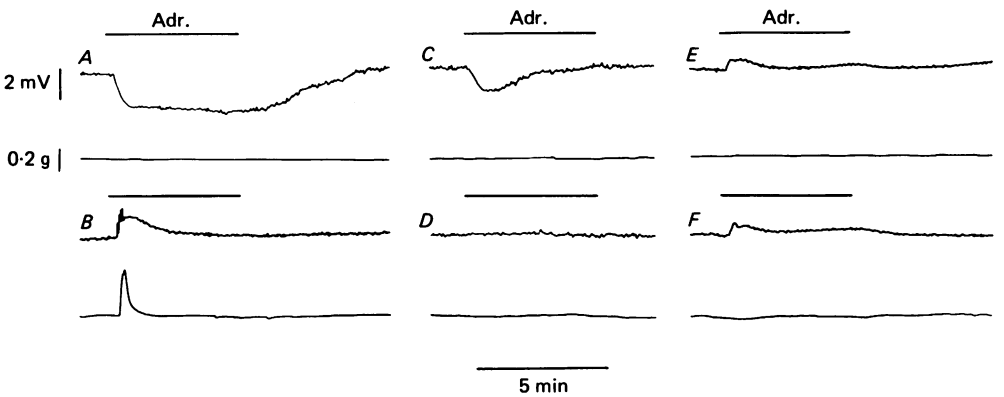

Fig. 7. The effect of apamin (10^{-7} M) on the adrenaline response (3×10^{-6} M) evoked in the presence and absence of calcium (2·5 mM) at 22 °C. The sustained hyperpolarization observed in calcium-containing medium upon addition of adrenaline (*A*) was converted into a depolarization in the presence of apamin (20 min; *B*), while the transient adrenaline response (*C*) obtained in calcium-free solution (20 min) was abolished (*D*). The adrenaline depolarization (*B*) in Krebs solution containing apamin was reduced by D600 (2×10^{-5} M; 20 min, *E*; 35 min, *F*). The actual sequence of the experiment was *A*, *C*, *B*, *D*, *E* and *F*.

after 20 min pretreatment with apamin (*B*), was a depolarization with increased spike discharge and contraction. This excitatory phase was always followed by a repolarization, not exceeding the pre-adrenaline potential in this experiment. The excitatory phase, reflected by membrane depolarization and muscle contraction, that was observed in all experiments upon addition of adrenaline in the presence of apamin, was followed by a repolarization, sometimes exceeding the pre-adrenaline value and consequently resulting in hyperpolarization of the cells (one out of four experiments). The average value of the maximal depolarization measured in four different experiments was 6·2 mV (±1·1 mV) and that of the maximal hyperpolarization was 0·8 mV (±0·8 mV; Table 2). Muscle contraction was also observed upon addition of adrenaline in the presence of apamin under high-potassium conditions (52·8 mM) followed by relaxation; changes in membrane potential could not be measured accurately under these circumstances.

The initial excitation upon addition of adrenaline was not seen in calcium-free medium (Fig. 7*D*), and the transient hyperpolarization was much reduced or abolished (Fig. 7*D*; Table 2). The enhancement of the ^{42}K efflux associated with the α-action in calcium-containing or calcium-free medium was inhibited by apamin (Table 3). Thus, with a smaller increase in potassium permeability in the presence

of apamin, the hyperpolarization was either partly or wholly (Fig. 7*B*) converted into depolarization. This depolarization persisted in the absence of external sodium or chloride, it was diminished by calcium antagonists (Fig. 7*E*, *F*) and abolished by removing external calcium (Fig. 7*D*). These results support the assumption that activation of the α-receptor increases calcium entry.

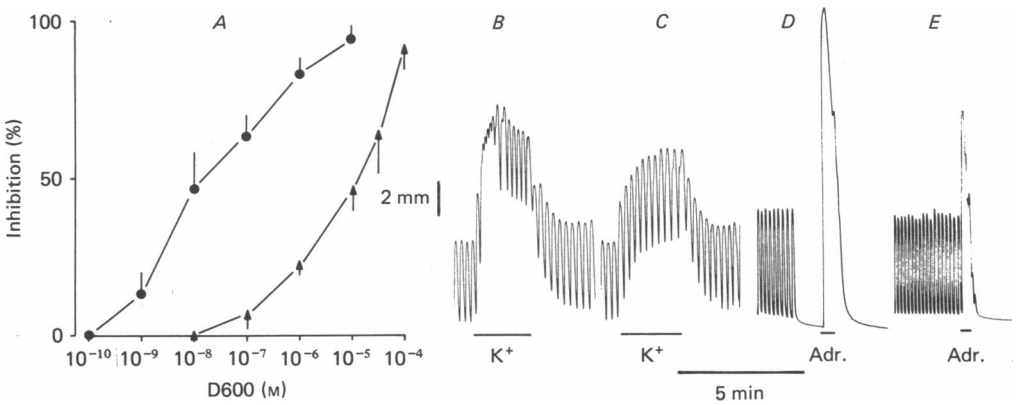

Fig. 8. The effect of D600 on the isotonic contraction evoked by potassium (10 mM) and adrenaline (3×10^{-6} M) respectively in the presence of apamin (10^{-7} M) at 22 °C. Inhibition of the potassium contraction by different concentrations of D600 (20 min) is shown in *A* (●), *B* (in the absence of D600) and *C* (10^{-8} M-D600). The reduction of the adrenaline contraction (in the presence of apamin) by D600 is shown in *A* (▲), *D* (in the absence of D600) and *E* (10^{-5} M-D600).

Voltage-dependent and α-receptor-operated calcium channels

The question arises whether the calcium channels opened after interaction of the agonist with the α-receptors are identical with the voltage-dependent calcium channels. Calcium entry through the voltage-dependent channels is increased during depolarization (Bolton, 1979; Van Breemen & Siegel, 1980). The sensitivity of the voltage-dependent calcium channels and of the α-receptor-operated channels to the calcium antagonist D600 was tested by comparing the contractions evoked by potassium (10 mM) with those evoked by adrenaline (3×10^{-6} M) in the presence of apamin (10^{-7} M). The first sign of inhibition of the adrenaline contraction by the calcium antagonist was seen at a concentration which caused about 70% inhibition of the potassium contraction. The potassium contraction was highly sensitive to the calcium antagonist because 50% inhibition was observed at 10^{-8} M-D600, while the receptor-operated channels had a lower sensitivity as reflected by a 50% inhibition of the adrenaline response at 10^{-5} M-D600 (Fig. 8). These results imply that the calcium entry assumed to occur in the presence of adrenaline and the enhanced calcium entry in depolarized muscle take place via different pathways.

DISCUSSION

The results show that the hyperpolarization caused by the α-action of adrenaline consists of a sustained component, dependent on extracellular calcium, and a transient component which can be evoked in the absence of extracellular calcium. Both responses are accompanied by enhancement of the potassium efflux and persist in the absence of chloride or sodium.

Evidence for the hypothesis that potassium channels are opened by calcium bound to the inner surface of the cell membrane has been obtained in a variety of cells: red blood cells (Whittam, 1968), *Helix aspersa* neurones (Meech, 1974; Meech & Standen, 1975), parotid gland cells (Putney, 1976*a*), liver cells (Bank *et al.* 1979) and taenia caeci (Bülbring & Tomita, 1977*b*). Although an increase in calcium uptake on activation of the α-receptors was not detectable when ^{45}Ca content was measured, the evidence nevertheless suggests that α-receptor activation may normally lead to an increase in potassium permeability through a calcium-dependent pathway.

Apamin has been shown to block the increase in potassium efflux produced by α-agonists, and in its presence receptor activation causes an initial depolarization of the membrane and contraction, followed occasionally by a small hyperpolarization. The depolarization was decreased by calcium antagonists and abolished in the absence of calcium. Hence, the depolarization evoked upon addition of adrenaline, when the hyperpolarization assumed to be caused by a change in potassium efflux is prevented by apamin, is most likely due to an increased calcium entry. The presence of extracellular calcium is probably necessary to maintain a continuous calcium entry to keep the potassium channels open. Limitation of this calcium entry by calcium antagonists reduced the change in potassium permeability, indicating a coupling between intracellular calcium and potassium permeability in taenia caeci.

When the taenia caeci was superfused with calcium-free medium the transient α-response could be evoked only once, but was restored after short exposure to calcium. Similar observations have been made with stimulating substances in parotid gland cells (Putney, 1976*a*, 1977), in arterial smooth muscle (Keatinge, 1972*a*, *b*; Van Breemen & Siegel, 1980) and in turtle aorta (Bozler, 1969). These results suggest the existence of a limited calcium pool which releases calcium upon activation of the α-receptor and is replenished upon application of extracellular calcium. The degree of refilling depends on the calcium concentration applied, as reflected by the subsequent adrenaline-induced hyperpolarization under calcium-free conditions, whose magnitude is determined by the amount of calcium released from this pool. This calcium store is most likely part of the membrane structure, as discussed in the Results.

The sustained component of the α-action is linked with a continuous calcium entry. The channels through which this entry occurs were found to be distinct from the voltage-dependent calcium channels, and appear to be receptor-operated channels of the kind also considered by Bolton (1979) and Van Breemen & Siegel (1980). These channels are inhibited by calcium antagonists, but are less sensitive than the voltage-dependent channels. The transient component of the α-action, caused by release of calcium from a membrane-bound pool, was not affected by calcium antagonists, but replenishment of this pool by extracellular calcium was sensitive to D600 and cobalt at concentrations which block the receptor-operated channels.

The fact that both the refilling of the calcium pool and the sustained component of the α-action show a similar sensitivity to calcium antagonists suggests that the pool can be filled by calcium entering through the receptor-operated channels. However, the calcium pool can also be replenished in the absence of an α-agonist, which implies that calcium can enter this pool by other pathways.

These results imply that activation of the α-receptor causes release of calcium from a membrane-bound pool, leading to the initial, transient potassium efflux and hyperpolarization of the muscle cells, followed by a continuous release of calcium from this pool depending on the availability of extracellular calcium and the rate of replenishment, which is reflected by the sustained component of the α-response.

The author is indebted to Miss Jacqueline Pielkenrood and Mr Jan van den Akker for performing part of the experiments and for technical assistance.

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