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DEPOLARIZING EFFECTS OF THE IONOPHORES X-537A AND A23187 AND THEIR RELEVANCE TO SECRETION

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The ionophore X-537A depolarized frog skeletal muscle fibres about 10 mV in 10 min and a further 30 mV or so over the next 50 minutes. With A23187, depolarization was relatively feeble and delayed in onset but was hastened when calcium was removed from the bathing medium. The results support conjecture that some functional responses to these ionophores are attributable, in part, to depolarization.

Introduction The ionophores X-537A and A23187, which are known to transfer calcium ions across cell membranes, elicit calcium-dependent secretion from various cells and this has generally been considered support for the view that calcium is a mediator in stimulus-secretion coupling. However, the effectiveness of the two ionophores as secretagogues does not parallel their reported abilities to transfer calcium ions. Thus, while both ionophores stimulate catecholamine output from perfused cat adrenals, X-537A is much more potent (Cochrane, Douglas, Mouri & Nakazato, 1975) even though it is the less effective calcium ionophore (Pressman, 1972; Pfeiffer, Reed & Lardy, 1974). Similarly, X-537A strongly stimulates vasopressin release from isolated neurohypophyses while A23187 does not (Nakazato & Douglas, 1974). Since depolarization is known to provide an adequate stimulus for secretion in chromaffin cells and neurohypophyseal terminals and because X-537A, unlike A23187, has the ability to transport monovalent cations, it has been suggested that the greater effectiveness of X-537A as a secretagogue in these systems may be related to an ability to depolarize (Nakazato & Douglas, 1974; Cochrane *et al.*, 1975). The purpose of the present experiments was to examine the effects of X-537A and A23187 on membrane potential. For convenience we have used frog skeletal muscle cells.

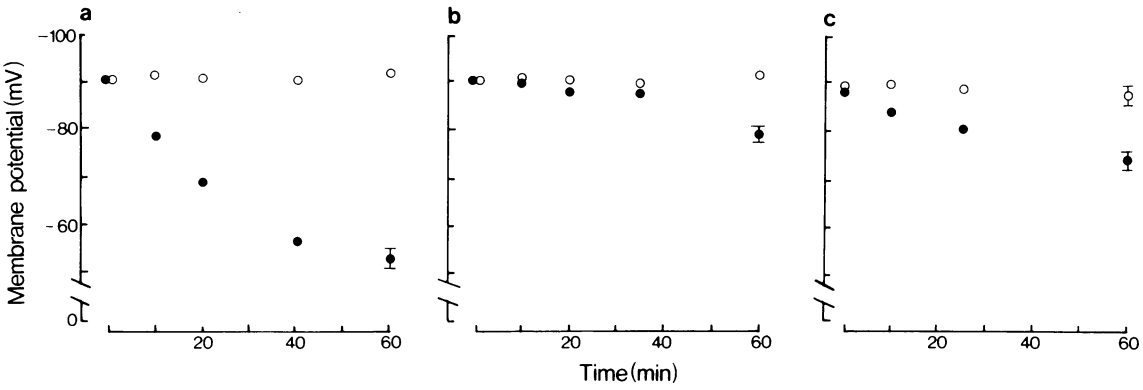

Figure 1 Depolarizing effects of the ionophores X-537A and A23187 on muscle fibres and the effect of calcium deprivation; (○) control potentials, (●) potentials in the presence of ionophore. Dimethylsulphoxide (0.2%) was present in all instances. (a) X-537A (10 μg/ml) in Ringer solution. (b) A23187 (10 μg/ml) in Ringer solution. (c) A23187 (10 μg/ml) in Ca-free Ringer solution. Muscles were exposed to Ringer or Ca-free Ringer solution for 40 min before introducing the ionophore. Each symbol represents the mean potential of many fibres (10-70) from different muscles. The standard error, where larger than the symbol, is indicated by vertical lines. Differences between sample means were tested for significance by Student's non-paired *t*-test and where equality of sample variances did not hold a modification of this test was used according to Snedecor & Cochran (1967). Except for the values at time zero and those in (b) at 10, 20, and 35 min, all values in the presence of the ionophores are significantly different from their corresponding controls ($P < 0.01$).

Methods Membrane potentials were recorded *in vitro* from frog (*Rana pipiens*) sartorius muscle fibres bathed in Ringer or Ca-free Ringer solution at about 22° C using 3 M KCl micropipettes. The Ringer solution had the following composition (mM): NaCl, 120; KCl, 2.5; $CaCl_2$, 1.8; Tris(tris (hydroxymethyl) aminomethane), 1.0, adjusted to pH 7.3. For Ca-free Ringer solution, $CaCl_2$ was omitted. Bathing media containing X-537A and A23187 were prepared from stock solutions in dimethylsulphoxide (DMSO). The DMSO concentration never exceeded 0.2% which was without effect on membrane potential (Figure 1).

Some muscles were fixed in 10% formalin and stained paraffin sections examined by light microscopy.

Results Some 10 min after treating muscle fibres in Ringer solution with X-537A (10 μg/ml), membrane potential had fallen about 10 mV and it continued to fall progressively thereafter (Figure 1a). After 2 h, we could not record any membrane potentials greater than −20 mV. By contrast, the membrane potential of fibres bathed in Ringer solution containing A23187 (10 μg/ml) was affected little for 30 min, although by 60 min it had fallen about 10 mV (Figure 1b).

A23187 has sometimes been introduced to tissues bathed with Ca-free media to facilitate its absorption by cell membranes. We have found that calcium deprivation hastens the depolarizing effect of A23187 (Figure 1c).

Although our main concern here is with depolarization, other effects of the ionophores that we have observed deserve mention. During the first 15 min of exposure to X-537A, some fibres were seen to twitch and by 40 min many appeared swollen. After 2 h the surface of the muscle, as viewed through the dissecting microscope, was no longer smooth but had a granular appearance. Many fibres had irregular regions of contracture and some seemed ruptured. Histological examination of muscles exposed to X-537A for 2 h showed that most of the surface fibres were ruptured. Damage was less in fibres lying deeper in the muscle. With A23187 no twitching occurred. After 2 h some fibres had a beaded appearance and showed peristalsis-like movements and some seemed to be ruptured. Histological examination showed many ruptured fibres mainly at the muscle surface. The damage was less, however, than with X-537A.

Discussion The strong and abrupt depolarization produced by X-537A is probably attributable, at least in part, to this drug's ability to transport monovalent cations (Pressman, 1972; 1973) and thereby facilitate movement of sodium and potassium ions down their concentration gradients. In lipid bilayers X-537A has been shown to increase membrane conductance to monovalent and divalent cations up to 10^4 times (Célis, Estrada-O & Montal, 1974). After this manuscript had been submitted, Devore & Nastuk (1975) reported a similar depolarizing effect of X-537A on frog cutaneous pectoris muscle which they conclude is due to the inward transport of Na^+ ions. Because A23187 has little ability to transport monovalent cations, especially at physiological pH (Pfeiffer *et al.*, 1974), its relatively small and delayed depolarizing effect has probably a different explanation. One possibility is that A23187 complexes with and depletes the membrane-bound calcium needed for maintenance of normal membrane permeability and potential: that calcium deprivation hastened the depolarizing effect of A23187 supports this interpretation. A similar action may contribute to depolarization in response to X-537A. Additional effects of the ionophores that could lead to depolarization are: (1) uncoupling of oxidative phosphorylation (Reed & Lardy, 1972a; Wong, Wilkinson, Hamill & Horng, 1973), with consequent reduction of the energy supply needed to maintain ionic gradiants, and (2) a rise in the cytoplasmic concentration of calcium ions by transfer of calcium from extracellular or intracellular sources (Caswell & Pressman, 1972; Reed & Lardy, 1972b; Scarpa, Baldassare & Insei, 1972). A rise in intracellular calcium has been reported to depolarize squid axon (Grundfest, Kao & Altamirano, 1954; Hodgkin & Keynes, 1956).

How the ionophores depolarize and why X-537A is more effective than A23187 is of less concern to us here than the functional consequences of this pattern. It is evident that the relatively strong depolarizing effect of X-537A could account for its greater effectiveness in eliciting secretion from neurohypophyseal terminals and chromaffin cells as earlier suspected (Nakazato & Douglas, 1974; Cochrane *et al.*, 1975): depolarization, in the presence of calcium, provides an adequate stimulus for secretion in both these preparations. Depolarization could also, for the same reason, account for the strong effects of X-537A on transmitter release from cholinergic (Kita & van der Kloot, 1974) and adrenergic neurones (Thoa, Costa, Moss & Kopin, 1974). The fibrillation we observed in muscles exposed to X-537A could be due to release of acetylcholine from motor nerve terminals while the absence of fibrillation in response to A23187 may reflect its inability to adequately depolarize these terminals.

The finding that A23187 depolarized more rapidly when calcium was omitted from the bathing medium may explain the contrasting

effects of this ionophore on isolated neurohypophyses in different experiments. Nakazato & Douglas (1974) found that A23187 had little or no stimulant effect on neurohypophyses bathed throughout with a calcium-containing solution. Russell, Hansen & Thorn (1974), on the other hand, noted a sharp rise in vasopressin output when calcium was added to neurohypophyses bathed for 70 min in a calcium-free medium with A23187 present for the final 40 minutes. Under these conditions the neurohypophyseal terminals may well have been depolarized. This once more raises the possibility (Douglas, 1968) that calcium influx alone may not provide an optimal stimulus for secretion. Clearly, depolarizing actions, and the changes in membrane properties they involve, must be considered as possible factors in functional responses to these ionophores. It remains to be seen whether the damage to the fibres we observed after prolonged exposure to the ionophores indicate a general action on cell membranes or an effect peculiar to muscle fibres that may arise from intense contraction.

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