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CHARACTERISTIC CONTRACTILE RESPONSE TO THE CALCIUM IONOPHORE, A23187, IN GUINEA-PIG VAS DEFERENS

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1 In the guinea-pig isolated vas deferens, the calcium ionophore, A23187, initially caused a phasic contraction followed by rhythmic activity.

2 Treatment with atropine, phentolamine, tetrodotoxin and reserpine modified neither of the components of the contraction induced by the ionophore. Verapamil and nifedipine abolished the rhythmic activity but had no effect on the phasic contraction.

4 In a calcium-free solution, both components of the contraction were abolished.

5 It is suggested that A23187 is able to cause contraction by a direct action on the smooth muscle of guinea-pig vas deferens.

Introduction

The antibiotic ionophore, A23187, has been reported to increase the calcium permeability of biological and artificial membranes (Reed & Lardy, 1972; 1973). It is also well known that A23187 causes a contraction of various smooth muscles (Pressman, 1973; Swamy, Ticku, Triggle & Triggle, 1975; Mandrek & Golenhofen, 1977; Watson, 1978). Only a limited study of the effect of the ionophore on smooth muscle of the vas deferens has been made; Swamy *et al.* (1975) failed to demonstrate a contractile response to A23187 in the guinea-pig vas deferens. However, in the present experiments it has been observed that A23187 caused a contractile response of the guinea-pig isolated vas deferens. The present paper describes the nature of the A23187-induced contraction in guinea-pig vas deferens.

Methods

Vasa deferentia were dissected from male guinea-pigs weighing 250 to 400 g. The serous membrane was carefully stripped off. The preparation was suspended in an organ bath with 20 ml of Ringer solution of the following composition (mM): NaCl 137, KCl 5.4, $CaCl_2$ 2.5, $MgCl_2$ 1.0, $NaHCO_3$ 12 and glucose 5.5, bubbled with 95% O_2 and 5% CO_2, pH about 7.2. Calcium-free solution refers to a normal Ringer-solution from which calcium chloride was omitted. The bath temperature was kept at 32°C. The mechanical response was recorded isotonically under 0.5 g load via an isotonic transducer (Natsume, Tokyo). For reserpine-treated preparations the guinea-pigs had been previously injected intraperitoneally with reserpine (4 mg/kg) on 2 consecutive days and used on the 3rd day. A23187 was dissolved with the minimum volume of acetone and then diluted with water just before use. The final concentration of acetone did not exceed 0.1% which had little effect on the contractile activity. The following drugs were used: A23187 (Eli Lilly), reserpine (Apoplon, Daiichi), atropine sulphate (Nutritional Biochemicals Corp.), tetrodotoxin (Sankyo), phentolamine mesylate (Regitin, CIBA), (−)-verapamil hydrochloride (Knoll) and nifedipine (Bayer).

Results

Treatment of guinea-pig vas deferens with the antibiotic ionophore, A23187, at 2×10^{-5} M, caused contraction consisting of two components; an initial phasic contraction followed by rhythmic activity (Figure 1a). The rhythmic activity was maintained for more than 120 min after its onset. At the lower concentration of 10^{-5} M, A23187 failed to cause the initial phasic contraction, but still produced rhythmic activity. At concentrations lower than 5×10^{-6} M, A23187 caused no contraction.

Treatment with reserpine (4 mg/kg daily for 2 days, i.p.) had no apparent effect on either of the components of the contraction induced by A23187 (2×10^{-5} M) (Figure 1b). Applications of atropine

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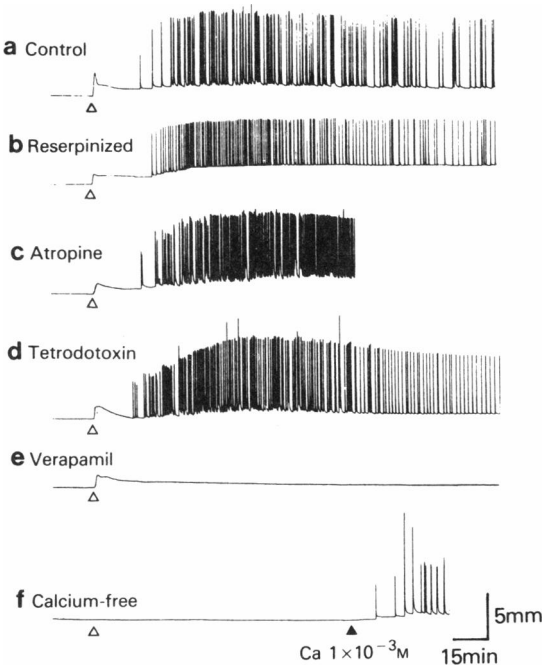

Figure 1 Effects of various treatments on the contractile response to A23187 in guinea-pig isolated vas deferens: (△) represents application of A23187 (2×10^{-5} M). (a) Control; (b) reserpine (4 mg/kg daily, i.p. for 2 consecutive days); (c) atropine (10^{-6} M); (d) tetrodotoxin (10^{-6} M) and (e) verapamil (10^{-6} M) applied in the solution 15 min before application of A23187; (f) calcium-free solution was introduced 30 min before application of A23187 and then calcium (1×10^{-3} M) (at ▲) was applied 120 min after the A23187-application.

(10^{-6} M) (Figure 1c), tetrodotoxin (10^{-6} M) (Figure 1d) or phentolamine (10^{-6} M) failed to inhibit the response to A23187 (2×10^{-5} M). On the other hand, application of (−)-verapamil (10^{-6} M) (Figure 1e) or nifedipine (10^{-7} M) abolished the rhythmic activity, but did not affect the phasic contraction induced by A23187 (2×10^{-5} M). Moreover, as seen in Figure 1f, A23187 (2×10^{-5} M) failed to cause any mechanical activity of the vas deferens in a calcium-free solution. Subsequently in the presence of calcium (1×10^{-3} M) A23187 was able to elicit rhythmic activity.

Discussion

It is known that the calcium ionophore, A23187, causes a contraction in various smooth muscles such as intestinal (Swamy, *et al.*, 1975; Mandrek & Golenhofen, 1977) and vascular smooth muscles (Pressman, 1973; Watson, 1978). On the other hand, Swamy *et al.* (1975) found that the smooth muscle of the vas deferens failed to show any contractile response to A23187 even at 5×10^{-5} M. In the present experiments, however, it has been shown that the same ionophore (2×10^{-5} M) produces a characteristic contractile response; an initial phasic contraction followed by rhythmic activity. Although the reason for the discrepancy between the present and the other experiments is not evident, it may be explained by the different experimental conditions employed, for in the present experiments, the ionophore was dissolved in acetone before use and the serous membrane of the vas deferens was removed from the tissue, whereas in the previous experiments they were not. In particular, the removal of the serous membrane may cause the tissue to be more sensitive to the agents than in the preparation with its membrane, as reported by Bentley & Sabine (1963).

However, A23187 was able to elicit visible contraction in the cold-stored (for 3 days) preparation of guinea-pig vas deferens (Swamy *et al.*, 1975). This might be explained by the increased sensitivity of the muscle to the agent brought about by cold storage (Shibata, 1969; Carrier & Shibata, 1977).

A23187, and another ionophore, X537A, have been found to release endogenous catecholamines (Thoa, Costa, Moss & Kopin, 1974; Ito, Nakazato & Ohga, 1978). In the vas deferens also, X537A elicited a transient phasic contraction which was inhibited by phenoxybenzamine and reserpine treatment (Swamy *et al.*, 1975). A similar inhibitory action of phentolamine and reserpine was also observed on the response to X537A in guinea-pig vas deferens (unpublished results). On the other hand, the contractile response to A23187 was not affected by treatment with reserpine, phentolamine or atropine. These results eliminate the possibility of involvement of an adrenergic or cholinergic mechanism in the development of a contractile response to A23187. Furthermore, tetrodotoxin, a fast Na^+ channel inhibitor, also had no effect on the response to this ionophore. On the other hand, verapamil or nifedipine, so-called Ca^{2+}-influx blockers, abolished the rhythmic contraction but had no effect on the phasic contraction. Moreover, in a calcium-free solution, both of the components of the ionophore-induced contraction were abolished. These results suggest that in guinea-pig vas deferens, A23187 is able to cause contraction by a direct action on the smooth muscle. Also, extracellular Ca^{2+} ion, in particular Ca^{2+} ion crossing the sarcoplasmic membrane, is an essential factor in the production of the contractile effect of the ionophore. It is therefore concluded that verapamil- or nifedipine-sensitive Ca^{2+} influx may play an important role in the development of the rhythmic contraction but not of the phasic contraction induced by A23187.

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