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ACTION OF IONOPHORE A23187 ON THE STRENGTH OF CONTRACTION AND TRANSMEMBRANE ACTION POTENTIAL OF GUINEA PIG PAPILLARY MUSCLE

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UDC 615.33(A23187).015.4:612.172

The calcium ionophore A23187, in a concentration of 2.5 μM, caused a two- to threefold increase in the strength of the contraction of the isolated papillary muscle of the guinea pig heart, stimulated at a frequency of 0.2 Hz. The ionophore A23187 reduced the resting voltage of the preparation in the intertrial interval and reduced the total duration of contraction. The increase in the strength of contraction was not accompanied by any change in the amplitude or duration of the transmembrane action potential. In the presence of the ionophore the ascending phase of a single contraction cycle showed a discontinuity separating the development of the twitch into two phases; differentiation of the twitch gave two positive maxima. The substance D-600, which blocks the calcium current, reduced the duration of the action potential and inhibited the second phase of twitch development, but caused no change in the magnitude or rate of the first phase of contraction. It is suggested that under the influence of the ionophore the component of the twitch which is not blocked by D-600 is caused by liberation of calcium from the sarcoplasmic reticulum.

KEY WORDS: ionophore; calcium; twitch; action potential.

The carboxyl antibiotic A23187, which has the property of forming electrically neutral complexes with bivalent cations, acts as a carrier of Ca^{2+} through the cell membrane [5]. In the first investigations to study the effect of this ionophore on the myocardium, its positive inotropic action was not found [6]. Later, however, the ionophore A23187 was shown to increase the strength of myocardial contraction in warm-blooded animals, evidence of an increase in the intracellular calcium concentration in the presence of the ionophore [3, 4]. On the other hand, data on the effect of A23187 on the duration and level of the plateau phase of the transmembrane action potential of isolated Purkinje fibers were given in [2].

It was thus not decided whether the positive inotropic action of ionophore A23187 is connected with changes in the electrical activity of the myocardial cells. The object of this investigation was to study that problem.

EXPERIMENTAL METHOD

Experiments were carried out on isolated papillary muscles from the hearts of guinea pigs (300-350 g). The papillary muscle was isolated from the left or right ventricle and placed in a transparent plastic chamber

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(Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Zakusov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 6, pp. 690-693, June, 1978. Original article submitted January 20, 1978.

0007-4888/78/8506-0754$07.50

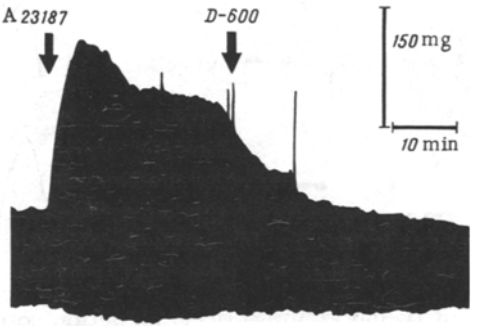

Fig. 1. Action of ionophore A23187 (2.5 mM) on strength of contraction of guinea pig capillary muscle. Frequency of stimulation 0.2 Hz (arrows marked addition of ionophore to chamber and addition of compound D-600, 2.5 mg/liter).

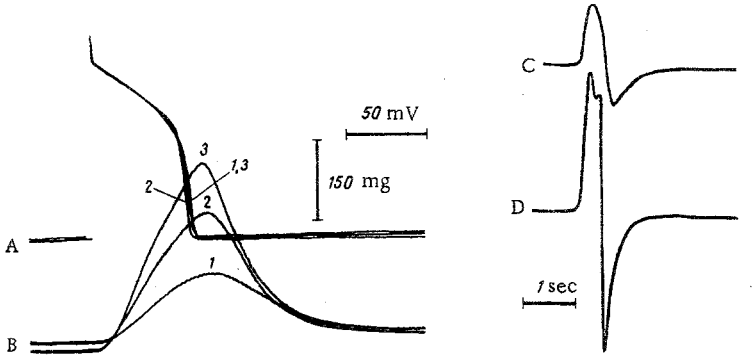

Fig. 2. Effect of ionophore A23187 on action potential (A) and single twitch of muscle (B). 1) Control; 2) A23187 acting for 2 min, 3) for 10 min; C) differentiation of twitch curve in normal Tyrode solution; D) differentiation 10 min after addition of ionophore. Time marker 200 msec.

(capacity 20 ml) filled with Tyrode's solution of the following composition: NaCl 118.4 mM, KCl 4.9 mM, $CaCl_2$ 2.5 mM, $NaHCO_3$ 25 mM, KH_2PO_4 and $MgSO_4$ 1.2 mM, glucose 10 mM. After oxygenation of the solution in the chamber with carbogen (95% O_2 and 5% CO_2) its pH was 7.4. The muscle was fixed at one end to bipolar stimulating electrodes and at the other end to a mechanical-electrical transducer (of the 6MKhIS, USSR, type). The preparation was stimulated by square pulses 10 msec in duration and with a frequency of 0.2 Hz. The experiments were carried out at 20°C. Simultaneously with the mechanical activity of the papillary muscle the transmembrane action potential and the first derivative of the contraction strength were recorded. The action potential was derived by glass microelectrodes (resistance 10-20 MΩ), amplified by means of a type MZ-4 (Japan) microelectrode amplifier, and recorded with a C-5A polaroid camera from the screen of a Tektronix 5103 N/D-13 oscilloscope (USA). Parallel with the recording on the oscilloscope, the strength of contraction and its first derivative were recorded for 1 h continuously on automatic writers with a time constant of under 0.1 sec.

The ionophore A23187 was dissolved in methyl alcohol to a concentration of 2 mM; 25 μl of this solution was added directly to the chamber. The final concentration of the ionophore was thus 2.5 μM. In control experiments the action of methanol itself on the mechanical and electrical activity of the muscle was studied.

The action of D-600 (2 mg/liter), which blocked the calcium current, was studied in special experiments in the presence of the positive inotropic action of the ionophore.

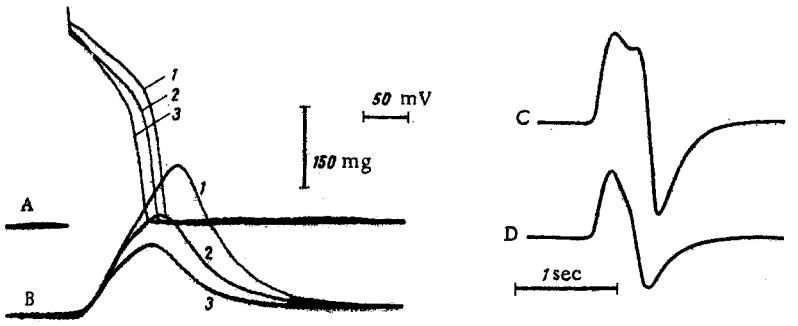

Fig. 3. Effect of D-600 (2.5 mg/liter) on action potential (A) and contraction (B) during action of ionophore. 1) A23187 acting for 28 min; 2) D-600 acting for 5 min in presence of A23187; 3) D-600 acting for 20 min in presence of ionophore. C) Differentiation of twitch before addition of D-600; D) differentiation 20 min after beginning of action of D-600.

EXPERIMENTAL RESULTS AND DISCUSSION

Addition of the ionophore to the chamber containing the preparation caused a gradual increase in the amplitude of contractions of the papillary muscle (Fig. 1). The maximal response to addition of ionophore A23187 developed after 4-6 min and reached 200-300% of the original strength of contraction. After reaching the maximum, the strength of contraction began to fall, but remained higher than its initial value for the 60 min of the experiment. Besides its positive inotropic action on the strength of contraction, A23187 also reduced the resting tension of the muscle and thus led to a greater relaxation in the intertrial interval (Fig. 1).

The effect of the ionophore on the transmembrane action potential and the shape of the single twitch are illustrated in Fig. 2A, B. For 10 min A23187 caused virtually no change in the shape or duration of the action potential (Fig. 2A). As control experiments showed, a very small decrease in the duration of the action potential during the first few minutes after addition of the ionophore can be explained by the effect of the solvent. The action of the ionophore on a single contraction cycle was manifested as an increase in the strength and rate of development of contraction and a decrease in its total duration (Fig. 2B). The positive inotropic effect of A23187 is thus not connected with any change in the transmembrane action potential.

The increase in the strength of contraction of the capillary muscle during the action of the ionophore was accompanied by a characteristic change in the shape of the single twitch, namely the appearance of a discontinuity during the phase of development of the twitch (Fig. 2B). Differentiation of the curve of this twitch in its positive part gave two components, corresponding to two phases of the rate of development of the twitch (Fig. 2G, D). Confirmation that each of these components of the twitch reflects an independent process of addition of calcium ions to the contractile system of the cell was given by experiments in which D-600, which blocks the calcium current, was used. Addition of D-600 (the time of addition is shown in Fig. 1) 27 min after the beginning of action of the ionophore shortened the duration and lowered the level of the plateau phase of the action potential and, at the same time, reduced the duration and amplitude of contraction (Fig. 3A, B). The phase of contraction after the discontinuity, on account of which the amplitude and duration of contraction were reduced, was more sensitive to the blocking action of D-600. This was reflected in the curve of differentiation of the twitch as blocking of the second component of the positive phase, despite only small changes in the magnitude of the first component (Fig. 3C, D). This result thus suggests that the second phase of the biphasic twitch is due to the calcium current through the cell membrane during the action potential.

The positive inotropic action of ionophore A23187, as we know, is not the result of liberation of endogenous catecholamines [3, 7]. However, the mechanism of action of this ionophore on the strength of contraction has not been finally explained, for it can not only transport Ca^{2+} through the sarcolemma [5], but can also liberate it from the sarcoplasmic reticulum. Since A23187 transports Ca^{2+} inside the cell, it is used as a working tool by means of which the role of a change in intracellular Ca^{2+} in the physiological properties of cells can be studied. For instance, the study of the effect of A23187 of the transmembrane action potential of Purkinje fibers showed that this ionophore, in a concentration of 20 μM, causes hyperpolarization of the membrane and reduces the duration and level of the plateau phase [2]. However, the results of the present investigation show that the increase in the strength of contraction under the influence of A23187 is not accompanied by any change in the action potential. These differences can probably be explained by the fact that a much lower concentration of the ionophore (2.5-5 μM) was used in the present experiments.

Besides the reduction in the resting tension and the duration of contraction, the positive ionotropic action of the ionphore was also accompanied by changes in the shape of the twitch in response to the stimulus. A discontinuity appeared in the phase of development of contraction and, correspondingly, the positive phase of the first derivative of the contraction was split into two components. According to data in the literature, a biphasic contraction can be obtained in sodium-free solution with an increased Ca^{2+} level through the action of caffeine, [1]. The authors cited explain the nature of this contraction by the participation of two different Ca^{2+} fractions in the contractile act: 1) intracellular Ca^{2+} accumulated in the sarcoplasmic reticulum, 2) extracellular Ca^{2+} arriving via the calcium channel in the phase of depolarization of the cell membrane. This hypothesis is confirmed by inhibition of the second phase of contraction by agents blocking the calcium current (Mn^{2+}, La^{3+}, D-600) and the insensitivity of the first phase of contraction to these blocking agents. The discontinuity in the phase of development of contraction observed in the presence of A23187 is probably similar in nature to the biphasic contraction described above. This explanation is confirmed by experiments using D-600, which abolishes the second component of the twitch but has little effect on the first. The biphasic character of development of contraction under the influence of A23187 can probably be explained by a change in the relative contributions of the reticular and extracellular calcium in myocardial contraction.

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EFFECT OF BARBITURATES ON SELECTIVE SECRETION OF RAT SALIVARY GLANDS AFTER ADMINISTRATION OF ANIONS

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UDC 612.313.3.014.46

Secretion of labeled anions and their metabolites in the saliva of adult Wistar rats was studied. The salivary glands are characterized by high selectivity of secretion of materials. After subcutaneous injection of [^{14}C]acetate, [^{32}P]orthophosphate, [^{35}S]thiocyanate, and [^{131}I]iodide, in control animals under physiological conditions ^{14}C is concentrated in the mixed saliva 2.5-6 times more than in the blood, the ^{32}P level in the saliva is 1/5-1/20 of the blood level, and the ^{131}I and ^{35}S indices occupy an intermediate position between those of ^{14}C and ^{32}P. Penetration of labeled anions and their metabolites into mixed saliva from the blood was considerably altered in rats receiving barbital sodium (medinal): the relative activity of ^{14}C, ^{35}S, and ^{131}I in the saliva compared with the blood was lower in these rats than in the control animals, but the relative activity of ^{32}P in the saliva compared with the blood was higher than in the control rats.

KEY WORDS: secretion of saliva; labeled anions; selectivity; barbiturates.

Investigation of the effect of narcotics on selective secretion of anions by the salivary glands is not only of special interest in connection with the study of salivary gland function, but it is also important in the context of the study of the principles governing neurogenic influences on permeability of membranes and secretory

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(Presented by Academician of the Academy of Medical Sciences of the USSR S. V. Anichkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 6, pp. 693-696, June, 1978. Original article submitted November 18, 1977.

0007-4888/78/8506-0757$07.50