absence of a depriming effect of nonachlazine on the pain syndrome formed by impulses in C-group afferents [3]. Pain of both central and peripheral origin is known to lead to exhaustion of the noradrenalin reserves in the brain tissue as a result of its increased liberation, a deficiency of its precursors, and disturbance of processes of catecholamine synthesis [2, 7].

The results thus evidently demonstrate the relative affinity of nonachlazine for the heart with particular reference to its intervention in the adrenergic processes of the heart during the response to stress.

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ACTION OF IONOPHORE A 23187 ON THE FORCE OF CONTRACTION AND SLOW ACTION POTENTIAL OF GUINEA PIG PAPILLARY MUSCLE

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The principal property of ionophores, including the calcium ionophore A 23187 discovered in 1972 [12], is their ability to form complexes with cations and to catalyze their passage from a polar medium into a nonpolar medium [10, 13]. Meanwhile, according to a recently expressed hypothesis [5, 6], Ca ions which participate in the formation of the slow inward calcium current must bind beforehand with the sarcolemma, i.e., they must move from the aqueous phase into the lipid phase. It can thus be postulated that if the quantity of Ca^{++} bound to the cell membrane of the myocytes is increased by the action of an ionophore, the potential-dependent inflow of Ca^{++} through the sarcolemma must increase. The investigation described below was carried out to test this hypothesis.

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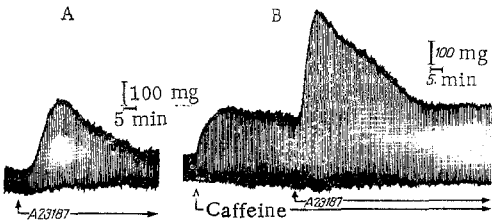

Fig. 1

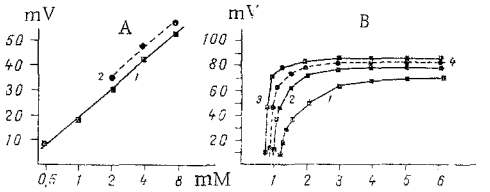

Fig. 2

Fig. 1. Action of 2 μM ionophore A 23187 on force of contraction of papillary muscle in control (A) and in presence of 2 mM caffeine (B). Injection of drugs indicated by arrows.

Fig. 2. Dependence of overshoot of SAP on Ca^{++} concentration in perfusion fluid (A) and dependence of amplitude of SAP on strength of stimulation (B). A: 1) Control (n = 10), 2) during action of ionophore (n = 4). Abscissa, Ca^{++} concentration in perfusion solution (in mM); ordinate, overshoot of SAP (in mV). B (n = 4): 1) 1 mM Ca^{++}, 2) 2 mM Ca^{++}, 3) 4 mM Ca^{++}, 4) 2 mM Ca^{++} + 2 μM A 23187. Abscissa, strength of stimulation in thresholds in the presence of 2 mM Ca^{++} (9V, 5 msec); ordinate, amplitude of SAP. Size of dots on graph corresponds to standard error of the mean.

EXPERIMENTAL METHOD

The papillary muscle from the right ventricle of a guinea pig's heart was placed in a perfusion chamber (volume 0.5 ml, rate of perfusion 10 ml/min). One end of the muscle was fixed to the bottom of the chamber and the other end was connected to a 6MKh1S mechanical-to-electrical transducer (USSR) to record the force of contraction. Transmembrane potentials were recorded with glass microelectrodes. The preparation was stimulated by square pulses 5 msec in duration; the strength of the stimuli varied from 1 to 6 thresholds. Flat Ag-AgCl electrodes, not touching the preparation, were used for stimulation. Before the beginning of the main experiments the preparation was adapted for 1.5-2 h during electrical stimulation (1 Hz) and perfusion with normal Tyrode solution of the following composition (in mM): Na^+ 144.6, K^+ 2.7, Mg^{++} 1.2, Ca^{++} 2, HCO_3^- 25, $H_2PO_4^-$ 1.2, Cl^- 127.5, glucose 10; the pH of the perfusion solution after oxygenation with carbogen (95% O_2 + 5% CO_2) was 7.4. The main experiments were conducted during stimulation with a frequency of 0.1 Hz. To investigate the slow action potential (SAP) the sodium channels were inactivated by increasing the K^+ concentration in the Tyrode solution from 2.7 to 18 mM without compensation of osmolarity. The experiments were conducted at temperatures of 25 and 37°C (± 1°C) and the following preparations were used: ionophore A 23187 (from Eli Lilly, USA), caffeine-sodium benzoate (from Medprom, USSR), isoproterenol (from Sigma, USA), and izoptin (verapamil) (from LEK, Yugoslavia). The experimental results are given in the form M ± m.

EXPERIMENTAL RESULTS

Addition of 2 μM A 23187 to normal Tyrode solution increased the force of contraction of the myocardium by 3.12 ± 0.47 times (n = 5) at 25°C and by 1.88 ± 0.28 times (n = 4) at 37°C, in good agreement with data published previously [1, 3]. Exhaustion of the intracellular reserves of Ca^{++} as a result of prolonged exposure to caffeine [15] did not abolish the positive inotropic action of the ionophore (Fig. 1). Similar results were obtained in three other experiments.

An increase in the K^+ concentration caused rapid (in the course of 5-7 min) depolarization of the preparation from −89 ± 1 to −46 ± 1 mV (n = 20), and if 2 mM Ca^{++} was present in the solution, a SAP developed in response to stimulation [8]; the rate of rise of the action potential was reduced from 190 ± 15 to 13 ± 2 V/sec (n = 7) and its amplitude fell from 128 ± 2 to 77 ± 1.5 mV (n = 20). With an increase in the Ca^{++} concentration in the perfusion fluid between 0.5 and 8 mM the overshoot increased as a linear function; a tenfold increase in concentration caused an increase in the overshoot of 33.4 ± 0.8 mV, close to the value of the potential predicted by the Nernst equation: 30.6 mV in response to a tenfold increase in the Ca^{++} concentration at 37°C (Fig. 2A). Isoproterenol in a concentration of 4 μM caused an increase in the rate of rise of the SAP to 38 ± 5 V/sec (n = 4) and an increase in the overshoot of SAP to 48 ± 2 mV (n = 4). Verapamil (10 μM) completely blocked the SAP. It can be concluded from these observations that the SAP was determined by the slow inward calcium flow [4].

The amplitude of the SAP depended on the strength of stimulation of the preparation, and this dependence changed when the Ca^{++} concentration in the perfusion fluid changed (Fig. 2B): An increase in the Ca^{++} concen-

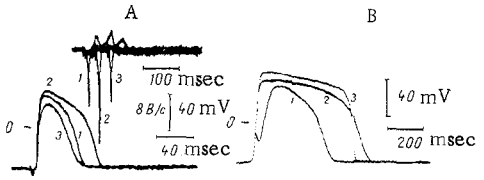

Fig. 3. Action of ionophore A 23187 on SAP (bottom traces in A and traces in B) and rate of rise of SAP (top traces in A) at 37°C (A) and at 25°C (B): 1) control, 2) 2 min of action, 3) 15 min of action.

tration led to a fall in the threshold of appearance of the SAP, its amplitude rose more sharply with an increase in the strength of stimulation, and the maximal amplitude was shifted to a higher level. As Fig. 2B shows, when the intensity of stimulation was six times above threshold, the maximal value of the amplitude was reached in all concentrations of Ca^{++}; it was during stimulation of this strength that the action of the ionophore on the SAP was studied.

The addition of 2 μM A 23187 to the perfusion fluid at 37°C led to a rapid rise in the overshoot and in the rate of rise of the SAP (Fig. 3A). The maximal increase in the overshoot was 5.2 ± 0.4 mV ($P < 10^{-4}$; $n = 14$) and it was reached by the second minute, after which the overshoot, the rate of rise, and the duration of the SAP began to decrease, and by the 15th minute they were below the control values. Recovery of these parameters was not observed until 1 h after rising with Tyrode solution not containing the ionophore. The maximal increase in the overshoot was independent of the Ca^{++} concentration in the perfusion fluid (Fig. 2A) and amounted to 4.9 ± 1.2 mV in the presence of 4 mM Ca^{++} ($P < 10^{-4}$; $n = 4$) and 5.3 ± 1.2 mV in the presence of 8 mM Ca^{++} ($P < 10^{-4}$; $n = 4$). The change in the dependence of SAP on the strength of stimulation under the influence of 2 μM of the ionophore corresponded to an apparent increase in the Ca^{++} concentration in the perfusion fluid of 1.5-1.6 times (Fig. 2B).

At 25°C the effect of the ionophore on the parameters of SAP was more marked (Fig. 3B). By the second minute of action the overshoot was increased by 6.1 ± 0.9 mV ($P < 10^{-4}$; $n = 5$), and at the same time the duration of the SAP increased considerably. However, the maximal value of the overshoot was reached by the 15th minute of action, when it was increased by 15.5 ± 0.9 mV ($P < 10^{-4}$; $n = 7$); the SAP under these circumstances was appreciably shortened and the rate of repolarization sharply increased, indirect evidence of an increase in the outward K^+ currents. It can thus be concluded that ionophore A 23187 increases the overshoot of the SAP through an increase in the inward calcium current and not on account of a decrease in the outward K^+ currents. Further evidence in support of the same conclusion is an increase in the rate of rise of the SAP (Fig. 3A).

According to data in the literature, the action of ionophore A 23187 on the myocardium of the guinea pig is unconnected with liberation of histamine, serotonin, catecholamines, or prostaglandins or with stimulation of the corresponding receptors [3]; ionophore A 23187 likewise does not cause an increase in the intracellular cyclic AMP concentration [9]. Previous investigations of the action of A 23187 on the myocardium [1-3, 11] were based on the classical point of view according to which carboxyl ionophore antibiotics, the group to which A 23187 belongs, are mobile carriers, migrating through the membrane and facilitating the electrically neutral transport of Ca^{++} ions along the concentration gradient [7, 13]. It was shown in [3] that the positive inotropic action of A 23187 cannot be explained purely by an increase in the passive permeability of the sarcolemma, for if that were true it would lead to considerable discrepancy between the theoretically calculated change in the ratio between the inward and outward flows of $^{45}Ca^{++}$ under the influence of the ionophore [14] and the change in that ratio discovered experimentally [3]. The suggestion was put forward that A 23187 penetrates into the membrane of the sarcoplasmic reticulum, stimulating liberation of Ca^{++} from it [3, 9]. The results of the present investigation did not confirm this hypothesis, for caffeine, which exhausts intracellular reserves, was unable to prevent the positive inotropic action of the A 23187. The results of the present experiments show that this increase in the force of contraction can be partially explained by an increase in the potential-dependent and not only the passive, inflow of Ca^{++}.

The effect of ionophore A 23187 on the action potential was studied in another investigation [2], which showed that the ionophore, after acting at 37°C for 5 min, reduced the amplitude and duration of the plateau of the action potential of the Purkinje fibers. This was explained by an increase in the intracellular Ca^{++} concentration and the consequent activation of outward K^+-currents and by a decrease in the e.m.f. for the

inward calcium current [2]. After the action of the ionophore at 37°C for 5-10 min a decrease in the overshoot and duration of the SAP below the initial values also was observed. This inhibitory action of the ionophore was stabilized in the present experiments by the 10th-15th minute at 37°C and by the 30th-40th minute at 25°C. The changes in the parameters of the action potential observed under these circumstances may in fact be ascribed to an increase in the intracellular Ca^{++} concentration. At 25°C in the present experiments inhibition of SAP by the ionophore did not occur until the 15th minute of action of A23187, although the increase in the overshoot was accompanied by a sharp rise in the outward K-currents. A similar effect was observed until the third minute of action of the ionophere at 37°C. During the first minutes of its action the effect of the ionophore on electrical activity of the myocardial cells was thus manifested as an increase in the calcium current, which could be due to an increase in the quantity of Ca^{++} bound with the sarcolemma. The mechanism of this action of the ionophore can be represented as follows: Two molecules of ionophore, binding with Ca^{++}, replace the hydration membrane, and complexes of the ionophore with Ca^{++} formed in the aqueous phase move into the lipid phase [10]. Since these complexes are lipophilic [7], the energy barrier to the movement of Ca^{++} from a polar environment into a nonpolar environment is lowered and the access of Ca^{++} to the binding sites on the membrane is facilitated.

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