KINETICS OF RELAXATION OF SMOOTH MUSCLE OF THE GUINEA PIG TAENIA COLI. EFFECT OF PAPAVERINE AND POLARIZATION

A. A. Galkin and E. N. Timin

UDC 612.731-064

Experiments were carried out on isolated strips of guinea pig taenia coli under double sucrose gap conditions. The effect of papaverine on the kinetics of relaxation of polarized and depolarized smooth muscle was investigated after contraction induced by electrical stimulation. The depolarized muscle was stimulated by a long hyperpolarizing pulse and contraction developed as an off-response (OR). The experiments showed that: 1) the relaxation phase of OR of the depolarized muscle is more protracted than relaxation of spontaneous contractions of the muscle in Krebs' solution; 2) papaverine accelerates the relaxation phase of OR of the depolarized muscle; 3) papaverine in Krebs' solution does not accelerate the relaxation phase of induced contractions; 4) hyperpolarization and additional depolarization accelerate the relaxation phase of OR of the depolarized muscle; 5) hyperpolarization after preliminary treatment with papaverine does not accelerate the relaxation phase of OR of the depolarized muscle. It is suggested that slow electrically excitable calcium channels, blocked by papaverine, are present in the membrane of smooth-muscle cells. The flow of Ca through these slowly inactivated channels prolongs the descending phase of OR of the depolarized muscle.

KEY WORDS: depolarized smooth muscle; off-response; rate of relaxation; papaverine; hyperpolarization.

Papaverine causes relaxation of depolarized smooth muscle [3, 6, 7]. A previous investigation [1] showed that no increase in tone can be obtained after preliminary treatment with papaverine (10^{-5}-$3 \cdot 10^{-5}$ g/ml) despite a tenfold or even 100-fold increase in the Ca concentration in the external solution. On the basis of this fact it was postulated that papaverine blocks calcium channels in the surface membrane. It was shown in the same investigation that the relaxation phase of contractile off-responses to prolonged hyperpolarization is accelerated by the action of papaverine. The question arises, is this acceleration of muscle relaxation the result of more active removal of Ca from the myoplasm or is it the result of weakening of the inward Ca flow? The answer to this question is very important both for explanation of the mechanism of action of papaverine and for an understanding of the mechanisms of coupling of excitation and contraction in smooth muscle.

EXPERIMENTAL METHOD

Experiments were carried out on isolated strips of guinea pig taenia coli (1.5-2 cm long, 300-500 μ thick). To stimulate the muscle and record the membrane potential simultaneously, a double sucrose gap method was used. The design of the experimental chamber was described in [2]. The following solutions were used (concentrations in mM): a) normal Krebs' solution: NaCl 120.7, KCl 5.9, NaH_2PO_4 1.2, $NaHCO_3$ 15.5, $MgCl_2$ 1.2, $CaCl_2$ 2.5, glucose 11.5; b) potassium solution: KCl 120, NaCl 47.7, $NaHCO_3$ 3.6, $CaCl_2$ 0.4, glucose 11.5. The pH of all solutions was 7.3. The experiments were carried out at room temperature and the solutions were not oxygenated (the reasons for these conditions are described in [2]).

EXPERIMENTAL RESULTS AND DISCUSSION

The strip of taenia coli was placed initially in Krebs' solution. Its automatic contractions are shown in Fig. 1a. After addition of papaverine (10^{-5} g/ml) spontaneous spike

A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow.
(Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Fedorov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No. 10, pp. 447-450, October, 1978. Original article submitted March 23, 1978.

 0007-4888/78/8610-1338$07.50

activity and contractions disappeared, but short depolarizing stimuli evoked contraction of the muscle. The rate of relaxation after automatic and evoked (by papaverine) contractions was the same. This is clear from Fig. 1c, where the descending phases are plotted on a semilogarithmic scale (the descending phase was considered to start from the time of maximal tension of the muscle; each curve is normalized with respect to amplitude).

Contractions of the muscle in depolarizing hyperpotassium solution were evoked by electrical stimulation. Hyperpolarizing pulses, 11 sec in duration, were applied to the muscle and contraction developed as off-responses. Full details of these off-responses (OR) are described in [2]. OR of a muscle in depolarizing solution and OR after addition of papaverine to the depolarizing solution are demonstrated in Fig. 1b. Relaxation phases of OR are plotted on a semilogarithmic scale in Fig. 1c. Clearly the descending phases of contractions in Krebs' solution, in Krebs' solution with papaverine, and in depolarizing solution with papaverine had straight-line sections, indicating the monoexponential character of the process. It must be specially emphasized that the gradients of these straight-line segments were equal. The time constants of muscle relaxation under the above conditions were, respectively: $\tau_{Krebs} = 4.5 \pm 0.53$ sec (n = 13), $\tau_{Krebs+pap} = 4.1 \pm 0.45$ sec (n = 5), and $\tau_{KCl+pap} = 5.3 \pm 0.78$ sec (n = 12). The differences between them are not statistically significant. The kinetics of relaxation of the muscle in depolarizing solution without papaverine was complex in character. In most experiments there were no straight-line sections of the semilogarithmic curves. In cases when the relaxation curves did have a straight-line section, the time constants were: $\tau_{KCl} = 18.4 \pm 0.94$ sec (n = 40).

The differences in the rates of relaxation of the muscle in potassium solution and Krebs' solution was evidently due to polarization of the membrane. To test this hypothesis experiments were carried out in which a hyperpolarizing and depolarizing current was applied in the relaxation phase (Fig. 2). Two superposed original records of OR in potassium solution are shown in Fig. 2a. The broken line indicates OR during the relaxation phase of which hyperpolarization was applied. Descending phases of OR are plotted in Fig. 2b on a semilogarithmic scale during application of hyperpolarizing currents of different strengths or a depolarizing current in the relaxation phase. Clearly both additional depolarization and hyperpolarization accelerated relaxation of the muscle. In response to strong hyperpolarization the rate of relaxation came close to that in Krebs' solution. If papaverine was added to the depolarizing solution, hyperpolarization was ineffective (Fig. 3).

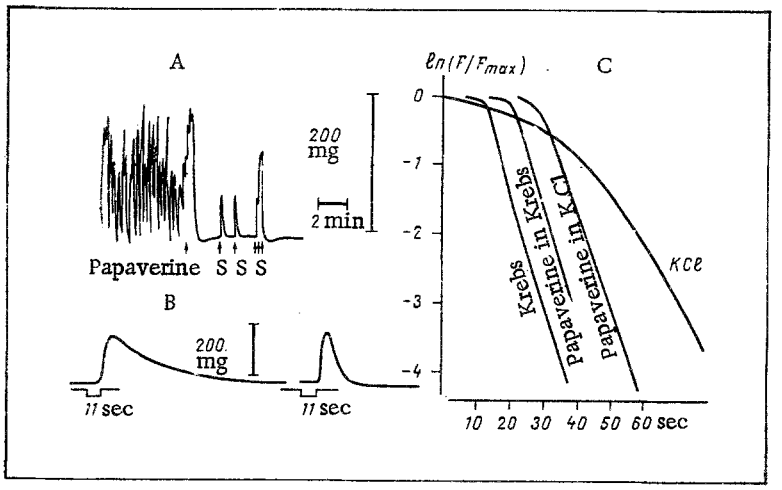

Fig. 1. Effect of papaverine on rate of relaxation of normally polarized and depolarized muscle. a) Spontaneous and evoked (after treatment with papaverine) contractions of muscle in Krebs' solution. Time of application of papaverine marked by arrow. S) Application of short depolarizing stimulus; b) OR in depolarizing solution without papaverine (left) and in presence of papaverine (right); c) relaxation phases plotted on semilogarithmic scale. F) Force developed by muscle; F_{max}) amplitude of maximal contraction.

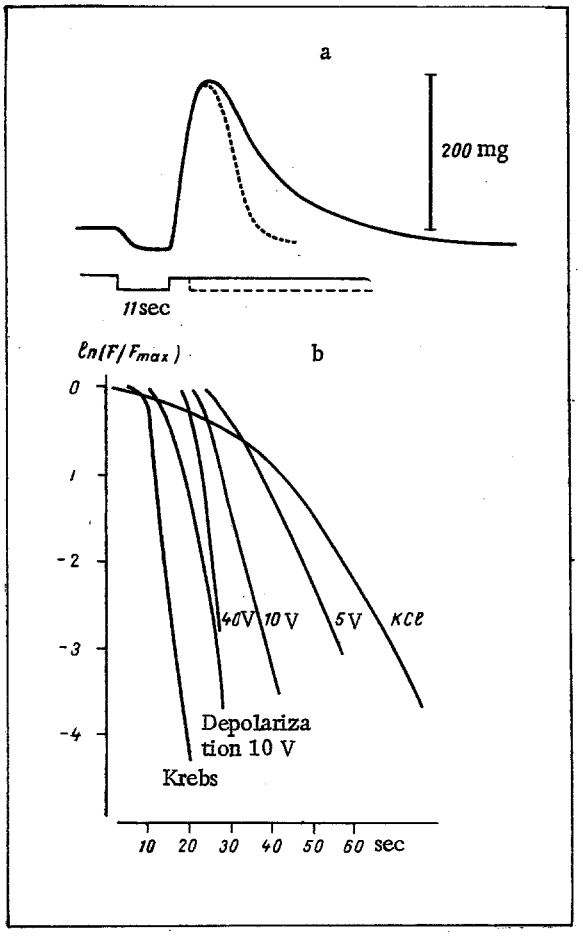

Fig. 2

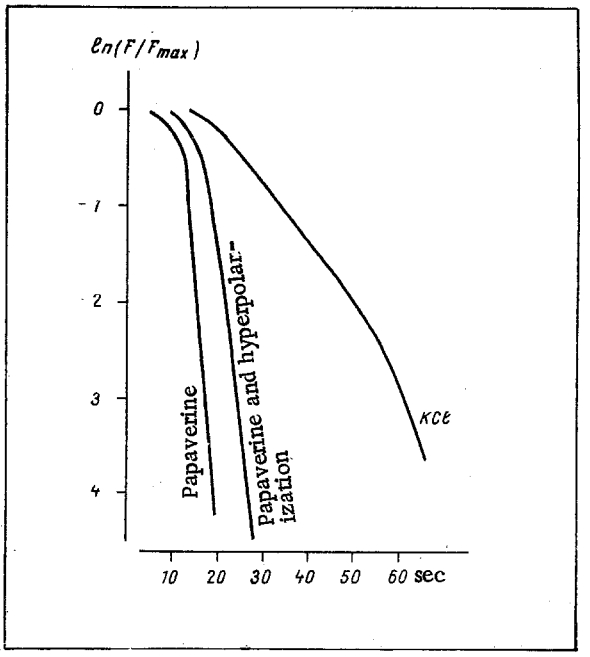

Fig. 3

Fig. 2. Effect of polarizing currents on descending phase of OR. a) Superposed original recordings of two OR. Broken line denotes OR during relaxation phase of which hyperpolarization was applied; b) descending phases of OR on semilogarithmic scale during application of depolarizing and hyperpolarizing stimuli in relaxation phase. Numbers on curves denote strength of stimuli (in V). Relaxation phase of automatic contraction in Krebs' solution shown for comparison.

Fig. 3. Descending phases of OR in presence of papaverine. Hyperpolarization does not accelerate muscle relaxation.

Relaxation of the muscle reflects a decrease in the free Ca concentration in the myoplasm. The data given above suggest that besides the outflow of Ca from the myoplasm (either into the external medium or into the intracellular reserves), during relaxation of the depolarized muscle Ca ions enter the cell from the external medium through the surface membrane. Such a flow of Ca through the membrane could take place if slowly inactivated (with a time constant of the order of tens of seconds) calcium channels were present in it. The existence of slow calcium channels of this type has been demonstrated in cat motoneurons [5] and molluscan neurons [4]. If the existence of slowly inactivated calcium channels in the membrane of smooth-muscle cells is postulated, it explains why hyperpolarization and additional depolarization accelerate relaxation. Hyperpolarization closes the activation gates of these channels and depolarization accelerates closure of the inactivation gates. Both these factors reduce the calcium permeability of the membrane. In Krebs' solution, when the fiber is polarized, at rest the inward Ca flow is negligible and the rate of relaxation is high. To explain the action of papaverine it can be suggested that it blocks the slow calcium channels, when the Ca flow, delaying relaxation, is blocked and the rate of relaxation coincides with the rate of relaxation of the muscle in Krebs' solution. Under these circumstances the rate of relaxation ceases to depend on the membrane potential.

The authors are grateful to B. I. Khodorov for valuable discussion of the results.

LITERATURE CITED

1. A. A. Galkin, D. A. Sarkisyan, E. N. Timin, et al., Byull. Éksp. Biol. Med., No. 2, 177 (1977).
2. V. I. Pogadaev, E. N. Timin, and B. I. Khodorov, Biofizika, 21, 848 (1976).
3. S. Imai and K. Takeda, J. Pharmacol. Exp. Ther., 156, 557 (1967).
4. P. G. Kostyuk, O. A. Krishtal, and P. A. Doroshenko, Pflüg. Arch. Ges. Physiol., 348, 83 (1974).
5. L. Liebl and H. D. Lux, Pflüg. Arch. Ges. Physiol., 335, 80 (1975).
6. U. Peiper, L. Griebel, and W. Wende, Pflüg. Arch. Ges. Physiol., 330, 74 (1971).
7. G. Pöch and W. Umfahrer, Arch. Pharmak. Exp. Path., 293, 257 (1976).

EFFECT OF BRADYKININ AND MORPHINE ON SENSOMOTOR CORTICAL NEURONS IN RATS

V. M. Bulaev and E. V. Koplik

UDC 615.212.7.015.23:615.225.2

Acute experiments on rats showed that bradykinin, injected by microiontophoresis, activates sensomotor cortical neurons in rats. Morphine, administered in the same way, prevents the development of the bradykinin effect. Bradykinin, it is suggested, acts on opiate receptors in cerebral cortical neurons.

KEY WORDS: bradykinin; morphine; cerebral cortex; opiate receptors.

Bradykinin, if injected intra-arterially or intraperitoneally, induces a sensation of pain in man [4, 8] and a nociceptive response in animals [6]. The sensation of pain after intra-arterial injection of bradykinin is connected with stimulation of paravascular receptors [9] and efferent fibers [2], i.e., it is peripheral in origin. However, recent investigations have shown that bradykinin acts directly on the CNS, by activating interneurons in the posterior horns of the spinal cord after iontophoretic application [7, 11], and it has accordingly been postulated that bradykinin participates in impulse transmission in the spinal cord.

The writers showed previously that bradykinin, when injected intra-arterially, causes activation of sensomotor cortical neurons in rats and that morphine, when injected intravenously, abolishes this effect [1]. However, on the basis of the available results it has not proved possible to determine whether bradykinin and morphine have a direct action on cortical neurons, for the drugs in these investigations were injected systemically.

The object of the present investigation was to ascertain if bradykinin and morphine affect cortical neurons; the method of microiontophoresis, whereby drugs to be tested can be applied directly to single cortical neurons, was used for this purpose.

EXPERIMENTAL METHOD

Rats were anesthetized with urethane (1 g/kg). Sensomotor cortical unit activity was recorded extracellularly by means of multibarreled microelectrodes, filled with aqueous solutions of NaCl (3M), bradykinin (10 mM; pH 4.5), and morphine (0.05 M; pH 5). Bradykinin and morphine were applied to the neurons by cationic currents with a strength of 10-40 nA. The duration of injection varied from 20 to 200 sec. Unit activity was amplified by the MZ-4

Laboratory of Pharmacology of the Nervous System, Institute of Pharmacology, Academy of Medical Sciences of the USSR. Laboratory of Emotions and Emotional Stresses, P. K. Anokhin Institute of Normal Physiology, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Zakusov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No. 10, pp. 450-452, October, 1978. Original article submitted January 12, 1978.

0007-4888/78/8610-1341$07.50