EFFECT OF BLOCKING OF GLYCOLYSIS IN THE VESSEL WALL ON TWITCH ACTIVITY OF SMOOTH MUSCLES

A. I. Solov'ev and S. A. Bershtein

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KEY WORDS: twitch activity of smooth muscles; automatic control; glycolytic oscillator.

The mechanisms lying at the basis of periodic twitch contractions of smooth muscles (SM), including those of blood vessels, has recently been studied particularly intensively. It has been suggested that membranes of smooth-muscle cells contain so-called T- and S-systems for activating contraction, and forming twitch and slow contractions respectively [8]. On the basis of differences in the sensitivity of these systems to agents blocking calcium permeability, it has been concluded that they differ in their chemical properties; the question of their metabolic nature and, correspondingly, of the metabolic processes which lie at the basis of twitch and slow contractions of SM still remains unanswered. According to one point of view, the metabolic basis for cellular oscillations is the time course of glycolysis [3, 4]. Data have been obtained to show that the glycolytic oscillator determines rhythmic activity of *Physarum* cells [9]. Oscillations of glycolysis have been found in skeletal muscle [10] and myocardial [7] cell extracts.

The aim of the present investigation was to identify the metabolic mechanism of generation of rhythmic twitch contractions in vascular smooth muscles (VSM).

EXPERIMENTAL METHOD

Experiments were carried out on isolated segments of rat portal vein, placed in a constant-temperature chamber and perfused with Krebs' solution at 37°C. Contractions of VSM were recorded by a 6MKh3S mechanotron transducer on a self-recording EPP-09M electronic potentiometer. VSM was stimulated by square dc pulses 20 msec in duration, 10 V in amplitude, and with a frequency of 16 Hz. Monoiodoacetate was added to the perfusion fluid in a concentration of 1 mM and sodium pyruvate in a concentration of 10-20 mM. The pH of the perfusion fluid was monitored before and after the addition of these substances by a pH-340 meter. Shifts of pH on addition of 1 mM monoiodoacetate were not more than 0.1-0.2 pH unit, and in most cases did not require additional correction with buffer solutions.

EXPERIMENTAL RESULTS

In most experiments after perfusion of VSM for 15-20 min with Krebs' solution containing monoiodoacetate, an inhibitor of the final stages (conversion of 1,3-diphosphoglyceraldehyde into 1,3-diphosphoglyceric acid) of glycolysis, the amplitude and frequency of their twitch contractions were reduced by half or more, and in some experiments rhythmic contractions of VSM were completely suppressed during the first 10 min of perfusion. During this same period the responses of VSM to electrical stimulation were usually preserved, although the tendency for their amplitude to decrease was gradually strengthened (Fig. 1). During prolonged (for 1 h or more) perfusion with buffer solution containing monoiodoacetate, as a rule not only rhythmic twitch contractions, but also slow contractions of VSM evoked by electrical stimulation were completely inhibited. Under these conditions, the action of monoiodoacetate became irreversible.

The intensity of poststimulation depression of automatic contraction of VSM was sharply increased by the action of monoiodoacetate, evidently on account of inhibition of glycolysis in the earliest period of action of monoiodoacetate (Fig. 2).

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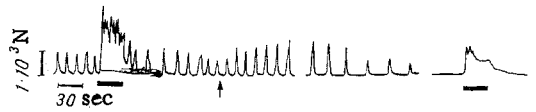

Fig. 1

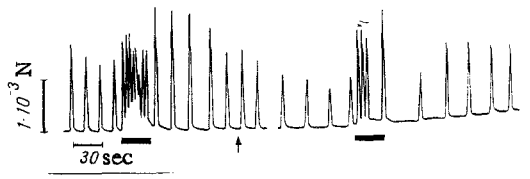

Fig. 2

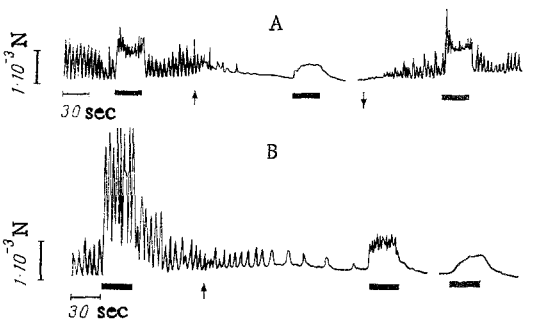

Fig. 3

Fig. 1. Effect of monoiodoacetate on spontaneous and evoked contractions of SM of rat portal vein. Arrow indicates beginning of perfusion with Krebs' solution containing monoiodoacetate (1 mM). Gaps in curve correspond to time interval of 3 min.

Fig. 2. Intensity of poststimulation depression of automatic contractions of rat portal vein SM under the influence of monoiodoacetate. Gap in curve corresponds to interval of 5 min. Legend as to Fig. 1.

Fig. 3. Effect of monoiodoacetate (1 mM) + pyruvate (15 mM) (A) and of pyruvate alone (15 mM) (B) on spontaneous and evoked contractions of rat portal vein SM. Arrows indicate beginning and end of perfusion by corresponding solutions. Gaps in curves correspond to time interval of 1 min (A) and 5 min (B).

One explanation of the gradual inhibition of both rhythmic twitch contractions and evoked slow contractions of VSM by monoiodoacetate may be that blockade of glycolysis, which periodically supplies substrate for the Krebs' cycle and the respiratory carrier chain, ultimately leads to suppression of energy formation processes in the Krebs' cycle also. Accordingly, in another series of experiments, VSM was perfused with buffer solutions containing monoiodoacetate together with pyruvate, a substrate of the Krebs' cycle. The intensity of responses of VSM under these circumstances to electrical stimulation remained practically unchanged throughout the experiment (for 3-4 h). Moreover, the inhibitory effect of monoiodoacetate which was observed became reversible under these conditions. However, inhibition of rhythmic twitch contractions of VSM not only was not prevented but, on the contrary, they were completely suppressed after only 3-5 min of perfusion, i.e., much earlier than when only monoiodoacetate was present in the perfusion fluid (Fig. 3A). One possible explanation of this may be that pyruvate, which oxidizes NADH, inclines glucose catabolism toward lactic acid formation, and an increase in the lactic acid concentration ought, by a negative feedback mechanism, to lead to inhibition of glycolysis. Assuming that automatic myogenic contractile activity of VSM is determined by glycolysis, this ought to lead to inhibition of rhythmic twitch activity of smooth-muscle cells.

During perfusion of VSM by buffer solutions with the addition of pyruvate only, marked inhibition of rhythmic twitch contractions of VSM was observed, but in some experiments they were completely suppressed, whereas their responses to electrical stimulation were fully preserved (Fig. 3B).

A previous study in the writers' laboratory [1] showed that if oxygenation of VSM was limited (pO_2 about 30 mm Hg) the intensity of their twitch contractions was sharply reduced and their synchronization with the electrical manifestations of twitch activity disturbed. This phenomenon is evidently based on a decrease in excitability of VSM membranes [2] and a decrease in the number of intercellular contacts of "nexus" type [6], leading to a disturbance of the spread of excitation in the smooth-muscle layer. The fact that although the rhythm of changes in membrane potential was practically completely preserved, the frequency of twitch contractions was significantly reduced under these conditions, deserves attention. Oscillations of activity of VSM cells are thus evidently not determined completely by the time course of aerobic metabolism. Changes in the intensity of oxidative phosphorylation due to oxygen deficiency undoubtedly limit the manifestations of automatic contractile activity, but they are evidently not the factor which triggers rhythmic contractions of smooth-muscle cells.

Remarkable synchronization in fluctuations in NADH concentration in the glycolytic oscillator and spontaneous changes in tension of SM of the portal vein has recently [8] been demonstrated. When the membrane voltage of intestinal SM, generating slow waves, was clamped spontaneous inward currents were recorded [5]; in our opinion, these inward currents are connected with the activity of a cytoplasmic oscillator which was not identified by the authors cited.

The glycolytic oscillator in vascular smooth-muscle cells thus plays the role of trigger mechanism for the generation of rhythmic twitch contractions. It can be postulated that glycolysis supplies the mechanisms of Na^+-and K^+-transport and of pacemaker potential formation with energy, and energy formation in the Krebs' cycle is closely connected with processes determining the magnitude of the tension developed by SM.

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EFFECT OF DESTRUCTION OF THE PARAVENTRICULAR AND MEDIOBASAL HYPOTHALAMUS ON PAIN SHOCK IN RABBITS

E. V. Golanov, V. V. Yasnetsov, S. B. Parin, and L. V. Kalyuzhnyi

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It was shown recently that injection of antagonists of opioid peptides improves the state of animals with endotoxic [7], hypovolemic [4], and electrically induced pain shock [2]. This suggests that opioid peptides participate in the pathogenesis of shock states. The paraventricular nuclei of the hypothalamus are known to contain relatively high concentrations of enkephalins [8] and this region is known to take part in the regulation of functions of the pituitary [1], which produces opioid peptides [8]. The only concentrations of β-endorphinergic cells in the brain have been identified in the mediobasal hypothalamus [3].

The object of the investigation described below was accordingly to study the effect of destruction of the paraventricular and mediobasal hypothalamus on the course of pain shock in rabbits.

EXPERIMENTAL METHOD

Experiments were carried out on 15 noninbred rabbits of both sexes weighing 2.2-2.8 kg. The arterial pressure (BP) of the animals (by a direct method), the heart rate (HR) and respiration rate (RR) were recorded. A state of shock was induced by electrical stimulation with a sinusoidal current through two electrodes, one located actually on the sciatic nerve, the

Laboratory of Physiology of Pain, P. K. Anokhin Research Institute of Normal Physiology, Academy of Medical Sciences of the USSR, Moscow.

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