It follows from the results described above that the blood clotting system of rats responds actively to injection of protein C_a: APTT is doubled and the plasminogen activator level is raised threefold during the first few minutes. It can be postulated that the anticoagulant reaction manifested as an increase in APTT follows a similar course in rats, dogs, cats, and monkeys. The response of the fibrinolytic system of rats differs from the response of cats and monkeys and is similar to that in dogs, although in rats the effect lasts longer.

We found a decrease of 50% in factor V activity, in agreement with experiments in vitro to study inactivation of human factor Va by protein C_a [14].

The blood clotting system of rats can thus be used as a model with which to study the role of protein C in the system regulating hemostasis.

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CELLULAR MECHANISMS OF TRANSIENT CONTRACTION OF CORONARY ARTERIAL SMOOTH MUSCLES IN HYPOXIA: ROLE OF INTRACELLULAR Ca^{++}

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Pacemaker activity of vascular smooth-muscle cells (SMC) is known to be depressed in hypoxia, and their maintained level of tension falls. The basic mechanisms of this phenomenon have been established [1, 4, 10] – a decrease in excitability and calcium conductivity [8] of the sarocolemma of SMC and a decrease in the sensitivity of their contractile proteins to Ca^{++} [2, 6]. In recent years however, evidence has been obtained [3, 9, 11, 13] that SMC of the coronary arteries respond to a fall in the level of oxygenation by a biphasic constrictor-dilator reaction. The mechanisms of transient hypoxic contraction (THC) of the coronary SMC in oxygen deficiency remain largely unexplained. For instance, the sources of Ca^{++}, activating the contractile system of SMC, have not been identified, and this remains an obstacle for the search for pharmacological agents capable of effectively blocking the devel-

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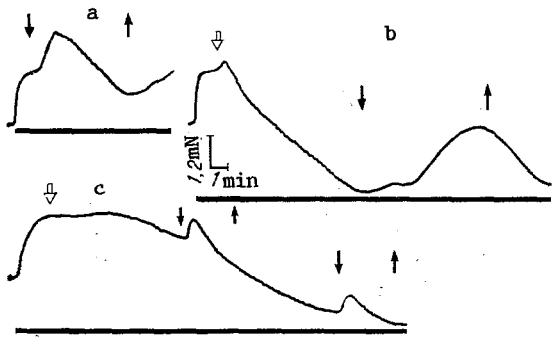

Fig. 1. Contractile responses of activated SMC of porcine coronary arteries to a fall in the level of oxygenation of Krebs' solution from 147 to 15 mm Hg during perfusion with normal (a) and calcium-free (b) solutions and solutions containing verapamil (c). Solid arrows indicate beginning and end of hypoxia, empty arrows indicate beginning of perfusion with calcium-free solution with EGTA (2 mM) and Krebs' solution with addition of verapamil (10^{-5} M). Straight lines beneath curves correspond to duration of transmural electrical stimulation (8 Hz, 20 msec, 30 V).

opment of hypoxic coronary spasm. The aim of the present investigation was to identify the subcellular structures in coronary arterial SMC responsible for THC formation.

EXPERIMENTAL METHOD

Experiments were carried out on isolated segments of epicardial porcine and canine coronary arteries 1-3 mm in diameter. Vascular preparations were placed in a constant-temperature perfusion chamber 0.6 ml in volume and subjected to passive stretching by a force of 10-15 mN and bathed with Krebs' solution (37°C, pH 7.4). Contractions of SMC were recorded by means of a 6MKh-4S mechanical to electrical transducer. Functional removal of the sarcolemma of SMC (skinning) was carried out with the aid of the detergent saponin, by the method described previously [2, 6]. The following preparations were used in the experiments: ATP and EGTA from "Calbiochem," USA; saponin from "Merck," West Germany; Tris-HCl, procaine, ouabain, and caffeine from "Serva," West Germay; verapamil from LEK, Yugoslavia; and nifedipine from "Germed," East Germany. The remaining reagents were of Soviet origin and of the chemically pure grade.

EXPERIMENTAL RESULTS

The experiments showed that coronary arterial SMC, whether preactivated (potassium chloride, serotonin, electrical stimulation) or not preactivated, respond to a decrease in the level of oxygenation of the Krebs' solution from 147 to 20-15 mm Hg by transient contraction with an amplitude of 1 to 4 mN and a duration of 1-3 min, after which they usually changed to vasodilatation (Figs. 1a and 2a). Hypoxic contraction of SMC was due mainly to Ca^{++} release from the sarcoplasmic reticulum (SPR), for it was reproduced in calcium-free solution with EGTA (2 mM; Fig. 1b) and was largely (up to 50-60%) preserved in the presence of verapamil and nifedipine (10^{-6}-10^{-5} M; Fig. 1c), blockers of the inward calcium current. THC also was completely suppressed by procaine (5 mM), which blocks release of Ca^{++} from SPR (Fig. 2b, d). In the modern view, verapamil penetrates into the myoplasm of SMC and can affect release of Ca^{++} from SPR. This same state of affairs evidently explains the small decrease in the amplitude of THC of the coronary arteries under the influence of verapamil (Fig. 1c).

The determination role of Ca^{++} ions stored in SPR in the development of THC was confirmed by the experiments on "skinned" SMC. If SPR in SMC was preserved intact, hypoxia led to the development of transient contraction of the skinned SMC (Fig. 3a). After exhaustion of the Ca^{++} reserves in SPR by caffeine (5 mM) and destruction of SPR by a high concentration of saponin, the constrictor phase of the response of SMC to hypoxia could not be reproduced and only relaxation of SMC was recorded (Fig. 3b, c).

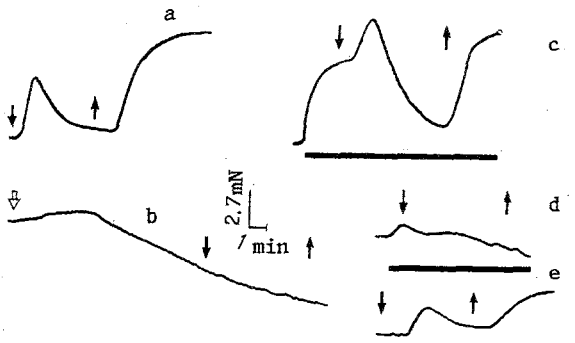

Fig. 2. Contractile responses of inactivated (a, b, e) and activated (c, d) SMC of porcine coronary artery to lowering of level of oxygenation of Krebs' solution from 147 to 15 mm Hg during perfusion with normal (a, b) solution and solution containing procaine (b, d) and 10 min after end of exposure to procaine (e). Empty arrow indicates beginning of perfusion with Krebs' solution with the addition of procaine (5 mM). Remainder of legend as to Fig. 1.

THC of SMC was sharply reduced (only the phasic component of the response remained) by selective blockade of glycolysis in SMC by monoiodoacetate together with sodium pyruvate (0.5 and 15 mM respectively). Previously the writers [5] showed that blockade of glycolysis in vascular SMC leads to a change in the direction of their response to hypoxia. For instance, portal vein SMC, which always relax during hypoxia, begin to contract, whereas SMC of the pulmonary artery, on the contrary, begin to relax. The connection between glycolysis and the character of response of SMC to hypoxia is evident, but the mechanisms of this connection have not yet been explained. It can be tentatively suggested that inhibition of THC of SMC during blockade of glycolysis is due to the fact that Ca^{++} release from SPR is an ATP-dependent process, whereas the ATP concentration in the myoplasm of SMC falls sharply in response to the combined action of monoiodoacetate and hypoxia.

Contraction of coronary SMC during hypoxia is not connected with inhibition of activity of the Na^{+}-K^{+}-pump. According to our data [7], the energy supply for the Na^{+}-K^{+}-pump in vascular SMC is provided by glycolysis and, consequently, it is an oxygen-independent process. THC of SMC in the present experiments was in fact preserved or even intensified by the action of the Na^{+}-K^{+}-ATP blocker ouabain (10^{-5} M), and the depolarizing and constrictor effects of ouabain still continued even after prolonged (over 15 min) incubation of SMC in a hypoxic medium.

What are the mechanisms of Ca^{++} release from SPR during hypoxia? Information has been obtained in recent years [12] to show that one of the secondary messengers in the mechanisms of pharmacomechanical coupling of excitation with contraction in vascular SMC is a metabolic product of phosphatidylinositol, namely inositol-1,4,5-triphosphate, which causes contraction of SMC due to release of Ca^{++} from SPR. We know that lithium ions inhibit the enzyme inositol-1-monophosphatase, which catalyzes the conversion of inositol monophosphate into free inositol, required for the synthesis of phosphatidylinositol, and thereby prevents the formation of inositol-1,4,5-triphosphate.

We showed that preliminary incubation of SMC in Krebs' solution with the addition of lithium hydroxybutyrate (10^{-3}-10^{-2} M) leads to a marked decrease in the strength of THC. The protective effect of lithium hydroxybutyrate is exhibited, moreover, only if it acts for a sufficiently long time on SMC. For instance, whereas at the 10th minute of incubation of the vascular preparation in a solution containing lithium hydroxybutyrate the amplitude of THC of SMC was reduced by only 10%, at the 30th and 50th minutes, the reduction was by 32 and 54% respectively. There was a particularly marked decrease in the duration of THC of SMC, which was 120, 100, and 60 sec at the 10th, 30th, and 50th minutes respectively (in the absence of lithium hydroxybutyrate the duration of THC averaged 192 sec). A particular feature of the response of coronary SMC to hypoxia after administration of lithium is the sharp increase in amplitude of the phase of hypoxic relaxation of SMC. For instance, whereas in the absence of lithium hydroxybutyrate the amplitude of hypoxic relaxation of SMC averaged

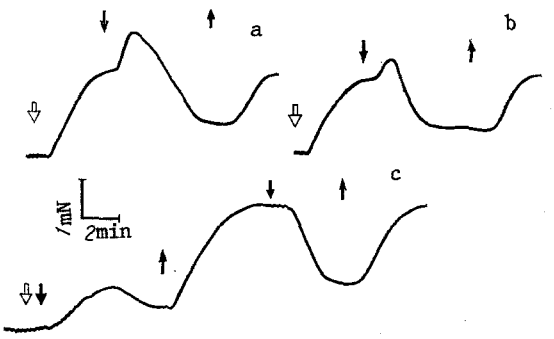

Fig. 3. Contractile responses of activated Ca^{++} of saponin-skinned SMC of porcine coronary artery to fall in level of oxygenation of buffer solution from 147 to 15 mm Hg. a) Control, b) after preliminary incubation of SMC for 5 min in buffer solution with caffeine (5 mM), c) SPR in SMC destroyed by a high concentration of saponin (absence of contractile response of SMC to caffeine served as the control of completeness of destruction of SPR). Solid arrows mark beginning and end of exposure to hypoxia, empty arrows – addition of Ca^{++} ions (10^{-6} M) to buffer solution.

28% of the amplitude of THC, at the 10th, 30th, and 50th minutes of incubation in a solution with lithium, it was 182, 215, and 415% respectively.

Transient contraction of SMC of the coronary arteries during hypoxia is thus due to the release of Ca^{++} from SPR, and it is evidently mediated through the formation of inositol-1,4,5-triphosphate in them when oxygenation is deficient.

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