ROLE OF INTRACELLULAR AND EXTRACELLULAR CALCIUM IN ACTIVATION OF SEROTONIN-INDUCED CONTRACTION OF PULMONARY ARTERIAL SMOOTH MUSCLE

V. A. Buryi, A. V. Gurkovskaya,
N. I. Gokina, and M. F. Shuba

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Experiments with antagonists of receptor-controlled and voltage-dependent Ca channels have shown that serotonin-induced contraction of smooth-muscle cells (SMC) of the rabbit pulmonary artery is almost entirely dependent on the inflow of Ca^{++} ions into the cell from the extracellular medium [5]. However, these results cannot rule out the possibility that Ca^{++} ions released either from internal juxtamembranous depots, if this release is somehow coupled with an inflow of Ca^{++} ions from the external medium, or from the sarcoplasmic reticulum of the cell, participate in the response to serotonin.

The aim of this investigation was to study contractile responses to serotonin in calcium-free medium and the effect of calcium antagonists and caffeine on these responses.

EXPERIMENTAL METHOD

Experiments were carried out on circular muscle strips from the rabbit pulmonary artery. The experimental method was the same as in previous investigations [1]. Calcium Kreb's and Ringer–Locke's solutions contained 1 mM EGTA and 6 mM $MgCl_2$. The results are represented in the form of the mean value ± the standard deviation ($M \pm \sigma$; n denotes the number of observations).

EXPERIMENTAL RESULTS

In calcium-free solutions containing EGTA serotonin in a concentration of 10^{-6} M induced contractions of the muscle strips (Fig. 1, 2), which rose to a maximum of 50.2 ± 2.9% of the initial value (n = 16) and then fell to 17.7 ± 1.6% relative to the steady-state level (n = 16). Readdition of serotonin after rinsing the strip with calcium-free solutions led only to a very small (3.1 ± 1.2% of the initial value, n = 10), slowly rising contraction. If, however, washing of the strip with calcium-free solution was preceded by application of Ca-containing solution even for a short time (about 10 min), its response to serotonin was completely restored.

The results of these experiments show that Ca^{++} ions released from a certain EGTA-resistant source, replenished by Ca^{++} ions from the extracellular medium, also participate in the activation of serotonin contraction. To determine the localization of the serotonin-sensitive source of Ca^{++} ions, we used Ca^{++}-ion antagonists — verapamil and Cd^{++} ions. In concentrations of 10^{-6} and 10^{-3} M respectively these antagonists virtually completely abolished contractions induced by the hyperpotassium solution, and reduced by about half the contraction to serotonin in Ca-containing solution (Fig. 1, 3). In calcium-free solution verapamil and cadmium suppressed the contractile response to serotonin more effectively than in normal solution, the phasic component of the contractile response being more sensitive to the blockers than the tonic component (Fig. 1, 4). These results suggest that Ca^{++} ions, released from an extracellular serotonin-sensitive source, enter the cell through Ca channels. The binding sites of Ca^{++} ions also are evidently located mainly near voltage-dependent Ca channels, controlled by serotonin receptors. However, these experiments do not rule out the possibility that Ca^{++} ions released from intracellular sources may participate in the contractile response to serotonin provided that this release is coupled with Ca inflow through Ca channels. The possible mechanism of this coupling may be what is known as

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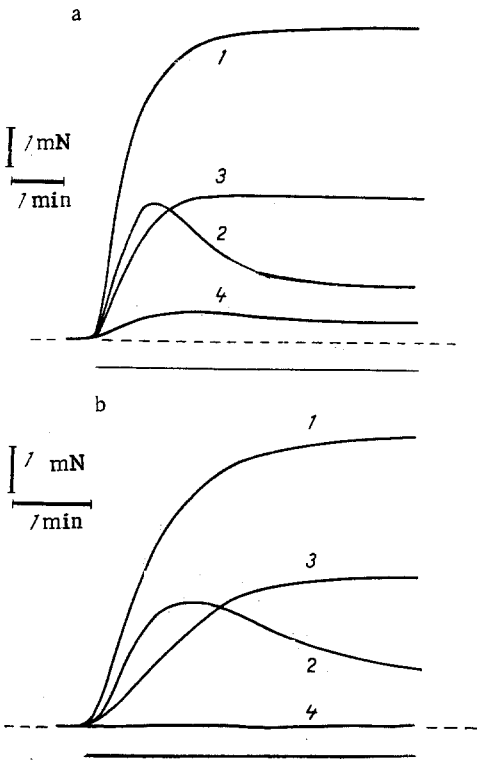

Fig. 1. Action of verapamil and Cd^{++} ions on contractile responses of muscle strips induced by serotonin in normal and calcium-free solution containing EGTA. a) Contractile responses to serotonin (10^{-6} M): 1) in normal Kreb's solution, 2) at 15th minute of action of calcium-free solution containing EGTA, 3) at 20th minute of action of verapamil (10^{-6} M) in normal Krebs' solution, 4) at 10th minute of action of verapamil in calcium-free solution; b) contractile response to serotonin: 1) in normal Ringer–Locke solution, 2) at 10th minute of action of calcium-free solution, 3) at 10th minute of action of Cd^{++} ions (10^{-3} M) in normal solution, 4) at 10th minute of action of Cd^{++} ions in calcium-free solution. Continuous horizontal line — time of action of serotonin, broken line — initial level of muscle tension.

Ca-induced Ca release [6]. To test this hypothesis, we studied the action of caffeine on serotonin-induced contraction. Caffeine in a concentration of 20 mM in normal Kreb's solution caused transient contraction, which was followed by relaxation of SMC. It is considered that transient contraction is the result of an increase in the intracellular concentration of Ca^{++} ions due to their release from the sarcoplasmic reticulum by caffeine [4]. In the absence of Ca^{++} ions in the external solution serotonin induced a much greater contractile response than caffeine (Fig. 2a, b, 2). However, after rinsing with calcium-free solution to remove the serotonin, caffeine caused only relaxation of the muscle strip (Fig. 2a, 3), possible evidence of complete emptying of the caffeine-sensitive source of serotonin, If, however, serotonin were added to the calcium-free solution 10 min after the action of caffeine, contraction developed, in which only the phasic component (Fig. 2b, 3) was significantly reduced (by 54.6 ± 3.9%, n = 4). Consequently, under the influence of serotonin Ca^{++} ions are released from at least two sources, one of which is sensitive to caffeine and is evidently located inside the cell in the sarcoplasmic reticulum. Since release of Ca^{++} ions from this source is somehow coupled with their arrival from the external medium, we attempted to explain whether Ca^{++} ions entering the cell constitute a secondary mediator, triggering Ca release from the intracellular source. For this purpose caffeine was added against the background of the action of a hyperpotassium solution, which induced an increased inflow of Ca^{++} ions into the cell through voltage-dependent Ca channels. As will be clear from Fig. 3b, the inflow of Ca^{++} ions into SMC did not cause release of Ca^{++} ions from the intracellular source, since the addition of caffeine during the action of the hyperpotassium

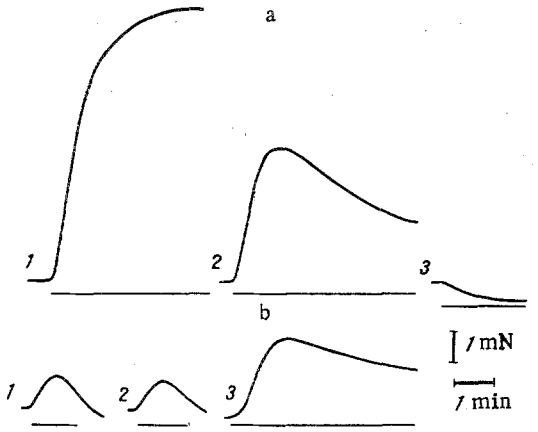

Fig. 2

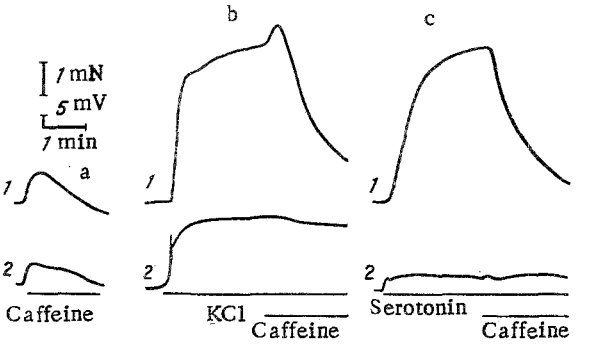

Fig. 3

Fig. 2. Responses of muscle strips to serotonin before and after action of caffeine. a) Responses of muscle fibers: 1) to serotonin (10^{-6} M) in normal Kreb's solution, 2) to serotonin at 10th minute of action of calcium-free solution, 3) to caffeine (20 mM) 10 min after washing to remove serotonin; 3) responses of muscle strips: 1) to caffeine (20 mM) in normal Kreb's solution, 2) to caffeine at 2nd minute of action of calcium-free solution, 3) to serotonin at 10th minute of action of calcium-free solution 8 min after action of caffeine. Here and in Fig. 3: horizontal line denotes time of action of substances.

Fig. 3. Contractile and electrical responses of muscle strips to caffeine (20 mM). a) In normal Krebs' solution; b) against background of response to hyperpotassium solution (60 mM), c) against background of response to serotonin ($2 \cdot 10^{-6}$ M). 1) Contractile response, 2) electrical.

solution led only to the same transient contraction as in normal solution. If, however, caffeine were added against the background of the action of serotonin, no contraction to caffeine developed (Fig. 3c). The results of these experiments suggest the existence of a certain secondary mediator, formed by the action of serotonin and capable of releasing Ca^{++} ions from a caffeine-sensitive source. Under these circumstances Ca^{++} ions entering through Ca channels evidently play the role of a cofactor, limiting formation of the secondary mediator. According to data in the literature, the most likely candidate for the role of this mediator may be inositol triphosphate, which is a hydrolysis product of phosphatidylinositol hydrolysis, activated by phospholipase C [3, 9]. It has been shown that inositol triphosphate releases Ca^{++} ions from the endoplasmic reticulum of many types of cells, including skeletal [11, 12] and smooth muscle cells [10, 13]. It has also been found that activation of many types of receptors [2, 9], including serotonin receptors [7, 8], is accompanied by hydrolysis of phosphoinositides as the result of stimulation of phospholipase C by the agonist. The role of Ca^{++} ions in these processes has been determined less clearly. It is mainly considered that under normal physiological conditions the concentration of Ca^{++} ions required for hydrolysis of phosphatidylinositol-4,5-biphosphate, activated by phospholipase C, does not exceed 10^{-7} M [9]. On general considerations, this mechanism of biochemical coupling between activation of the receptor and release of intracellular bound Ca ought not to depend on the Ca^{++} ion concentration in the myoplasm. Otherwise, positive feedback arises, and leads to a regenerative process of depletion of the intracellular sources of Ca. In fact, this process is dose-dependent. It has also been shown that the ability of inositol triphosphate to release Ca from the sarcoplasmic reticulum is independent of organic and inorganic Ca-channel blockers [3]. Our results may therefore be compatible with the hypothesis that inositol triphosphate participates as a chemical mediator only if hydrolysis of phosphatidylinositols which are present in the structure of the membrane and are located in its inner layer, depends on the presence of Ca channels coupled with receptors in the region where they are found, and whose conformation corresponds to their conducting state. There must also be a source of bound Ca near the outer opening of these channels, from which Ca^{++} ions can enter the cell in response to activation of the chemoreceptor.

Thus serotonin-induced tonic contraction of SMC of the rabbit pulmonary artery causes release of part (under 20%) of the bound Ca from at least two sources, which are evidently

located on different sides of the membrane. It is suggested that release of Ca^{++} ions from the intracellular source is effected by a chemical mediator, the formation of which depends on inflow of Ca^{++} ions through receptor-controlled Ca channels.

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