# EFFECT OF MEMBRANE POTENTIAL LEVEL ON SEROTONIN-INDUCED SMOOTH MUSCLE CONTRACTIONS OF THE RABBIT PULMONARY ARTERY

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

UDC 612.734.015.31:546.41:612.215.8

KEY WORDS: smooth muscles; serotonin; nifedipine; Ca-channels

Serotonin-induced contraction of vascular smooth muscles is activated chiefly by calcium entering the cell from the extracellular medium [1-4]. The entry of Ca ions into smooth-muscle cells (SMC) is effected through calcium channels controlled either by the transmembrane potential or by receptors [5, 6, 9, 12, 13]. Activity of receptor-controlled calcium channels, it is considered, is independent of membrane potential [2, 5, 6, 12, 13]. Involvement of voltage-gated calcium channels in the activation of SMC contraction induced by agonists is usually mediated through the membrane depolarization taking place under these conditions or through modulation of activity of these channels [5, 6, 12]. It has been suggested that this modulation is effected through intracellular mediators formed during interaction between agonists and receptor [10, 11]. Our investigations have shown that serotonin-induced contraction of vascular smooth muscles is voltage-dependent [3, 4].

In the investigation described below a more detailed study was made of the character of this voltage-dependence, and the sensitivity of serotonin-induced contraction to nifedipine also was determined in order to establish the role of ordinary voltage-gated calcium channels activated by membrane depolarization in this response.

## EXPERIMENTAL METHOD

Experiments were carried out on circular muscle strips of the rabbit pulmonary artery 1-1.5 mm wide and 8-10 mm long. To record electrical and contractile activity simultaneously a modified sucrose gap method was used. The experimental conditions were described previously [4]. The serotonin creatinine sulfate was obtained from Reanal (Hungary) and the nifedipine from Bayer Pharmaceutical Co. (Switzerland).

## EXPERIMENTAL RESULTS

In the initial state SMC of the rabbit pulmonary artery have a stable resting potential. Membrane hyperpolarization and mild depolarization (to 5-7 mV) induced by an electric current do not affect the muscular tension of the preparations. Stronger depolarization produces tonic contraction of SMC (Fig. 1a). Serotonin (1 $\mu$M) induced membrane depolarization of not more than 7 mV, accompanied by considerable tonic contraction of the muscle strips. It will be clear from Fig. 1a that electrical depolarization of SMC in this way does not lead to the appearance of muscle contraction. It can therefore be

*Academician of the Academy of Sciences of the Ukrainian SSR.

Department of Neuromuscular Physiology, A. A. Bogomolets Institute of Physiology, Academy of Sciences of the Ukrainian SSR, Kiev. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 1, pp. 13-16, January, 1992. Original article submitted June 10, 1991.

 0007-4888/92/0001-0016$12.50 

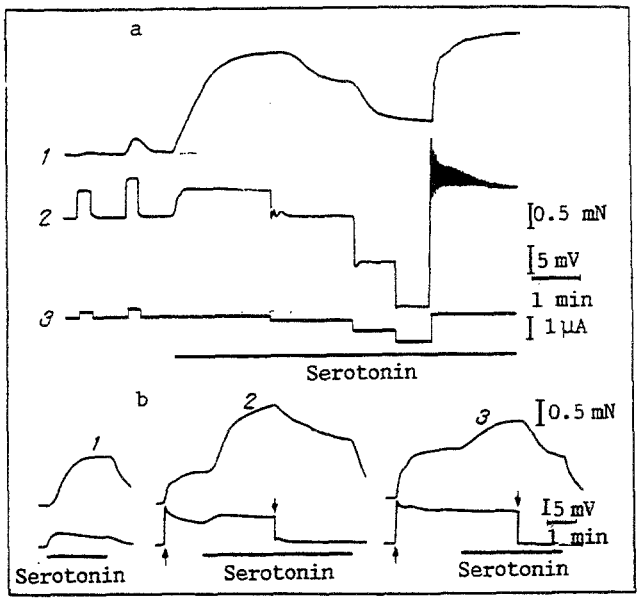


Fig. 1. Action of long-term electrotonic shifts of transmembrane potential on contraction of pulmonary arterial SMC induced by 1 $\mu$M serotonin: a) effect of anelectrotonic repolarization and hyperpolarization of SMC membrane on serotonin-induced contraction. 1, 2, 3) traces of contractile responses, electrical responses, and applied current respectively. Contractile responses of muscle strip to subthreshold and threshold depolarization of SMC by long pulses of outward current shown before beginning of serotonin action; b) effect of anelectrotonic membrane depolarization (application of outward current indicated by arrows) on serotonin-induced contraction. 1) Response to serotonin at resting potential; 2, 3) Response at different levels of depolarization.

postulated that the development of contraction in this case was connected with activation of receptor-controlled voltage-insensitive calcium channels only. However, anelectrotonic membrane repolarization to the original level led to partial relaxation of serotonin-induced contraction. Membrane hyperpolarization led to additional relaxation of the muscle strip. Stronger hyperpolarization no longer caused any further relaxation. These experiments show that only about half of the contractile response induced by serotonin can be connected with activation of receptor-operated voltage-insensitive calcium channels. The other half of the contraction is voltage-sensitive, for it is abolished by membrane hyperpolarization. It is natural to suggest, therefore, that this part of contraction is activated by calcium ions entering the cells through calcium channels operated simultaneously by serotonin receptors and membrane potential.

An alternative explanation is the possible modulating effect of serotonin on activity of ordinary voltage-operated calcium channels, during which their activation curve is shifted toward more negative voltages. This kind of mechanism has been found for the action of noradrenalin on cardiomyocytes [11] and is suggested for SMC of the mesenteric artery [10].

Further experiments were aimed at studying the character of voltage-dependence of serotonin-induced contraction in response to shifts of membrane potential toward depolarization. The experiments showed that during electrical membrane depolarization to a certain level a proportional increase in the contractile response to serotonin was observed. However, elevation of this level of depolarization led to a significant reduction of serotonin-induced contraction (Fig. 1b). It will be clear from this illustration that saturation of the contractile mechanism of the cell is not the cause of the weakening of serotonin contraction during strong depolarization, for the total contraction induced by both depolarization and serotonin was greater in the case of weak depolarization. The strength of depolarization at which reduction of the response to serotonin is observed varied widely in different experiments. This is evidently associated with nonhomogeneity of the voltage distribution along the test segment of muscle strip. This nonhomogeneity may be considerably increased during strong depolarization because of an increase in membrane permeability. We therefore carried out a series of similar

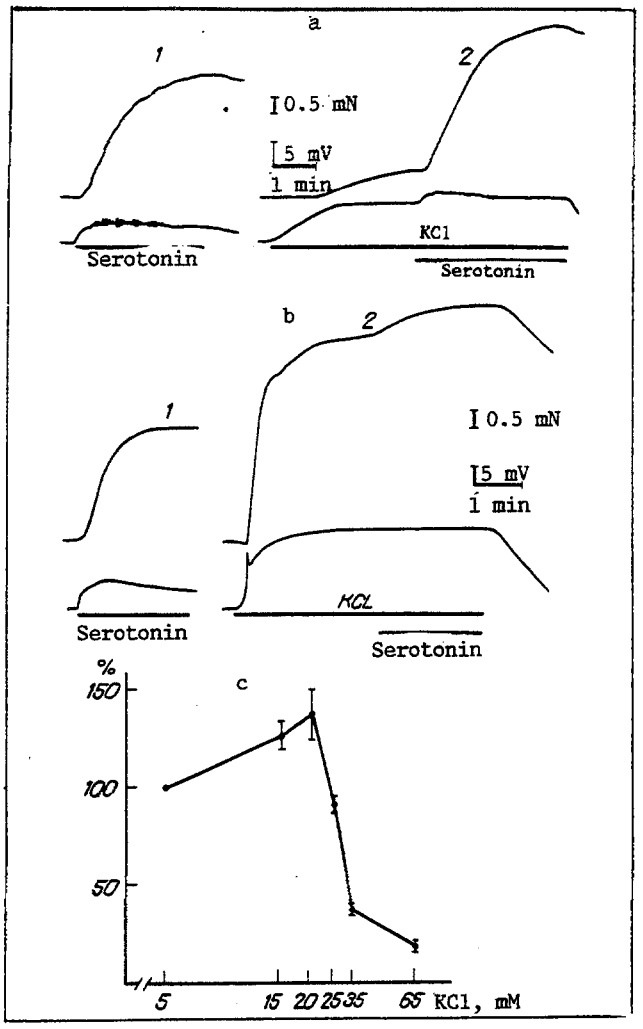


Fig. 2. Effect of hyperpotassium membrane depolarization of pulmonary arterial SMC on contractile and electrical responses induced by 1 $\mu$M serotonin. On left – responses before, on right – during action of hyperpotassium solution. Traces a and b obtained on different muscle strips during exposure to potassium ions in concentrations of 15 and 65 mM respectively. c) Averaged dependence of magnitude of serotonin-induced contractile response on extracellular potassium ion concentration.

experiments in which depolarization was induced by raising the extracellular potassium ion concentration from 5 to 15, 20, 25, 45, and 65 mM. The experiments showed that weak depolarization induced by potassium ions in concentrations of 15 and 20 mM increased contraction of the muscle strip in response to serotonin by 30 and 40% respectively compared with the initial value (Fig. 2a). However, a further rise of potassium ion concentration led to a sharp decrease in serotonin contraction. Against the background of depolarization induced by potassium ions in a concentration of 65 mM it was only 25% of the initial value (Fig. 2b). The averaged result of 8 such experiments are shown in Fig. 2c. Considering that only half of the contractile response is voltage-dependent, it can be concluded that depolarization acts on a certain component in the membrane that is common to both voltage-sensitive and voltage-insensitive serotonin-activated calcium inflow.

Since contraction induced by hyperpotassium depolarization is effectively suppressed by nifedipine, a selective blocker of voltage-gated potassium channels of L type, these channels are the principal voltage-operated carriers of calcium ions in the cells under investigation. It may therefore be expected that if channels of the L type are the target for the modulating influence of secondary messengers during the action of serotonin and, if, as a result of this effect, the affinity of the channel for dihydropyridines is not impaired, the voltage-sensitive part of serotonin contraction will be blocked by ni-

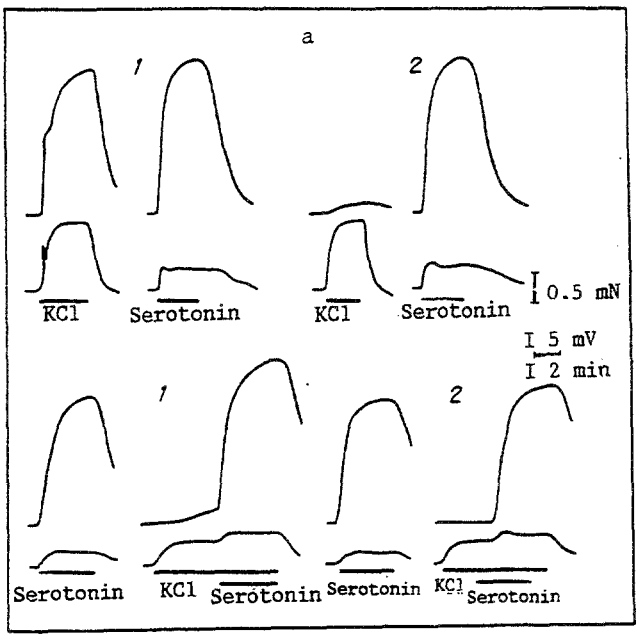


Fig. 3. Effect of nifedipine on electrical and contractile responses of pulmonary artery SMC induced by hyperpotassium solution and serotonin: a) responses to hyperpotassium solution and serotonin before (1) and during (2) action of nifedipine; b) responses induced by serotonin in normal Krebs' solution and associated with hyperpotassium depolarization before (1) and during action of nifedipine (2).

fedipine. It was found that nifedipine, in a concentration sufficient to suppress hyperpotassium contraction had virtually no effect on serotonin contraction (Fig. 3a). To determine whether this result is connected or not with the voltage-dependence of the blocking action of nifedipine, the effect of nifedipine on the contractile response to serotonin was investigated on the contractile response to serotonin against the background of hyperpotassium depolarization. If the channels activated by depolarization are the same as the channels activated by serotonin, sensitivity to nifedipine of these channels ought to be equal. However, as Fig. 3b shows, nifedipine abolished only that part of the contractile response that can be connected with hyperpotassium membrane depolarization, and it reduced serotonin-induced contraction only a very little. This is evidence that the channels activated by serotonin are not the same as the channels activated by hyperpotassium depolarization. Moreover, serotonin evidently does not reduce the affinity of voltage-operated calcium channels for nifedipine. Otherwise an increase would have been observed in serotonin contraction against the background of hyperpotassium depolarization, and an effect of nifedipine on the magnitude of hyperpotassium contraction were present. The decrease observed in serotonin-induced contraction under these conditions, however, is evidence that nifedipine blocks those voltage-operated calcium channels activated by serotonin depolarization.

Thus these experiments revealed a complex dependence of seroton-in-induced contraction of pulmonary arterial SMC on the membrane voltage. The character of this dependence, with small changes of resting potential toward hyper- and depolarization, is in agreement with the view that serotonin receptors control both voltage-insensitive and voltage-sensitive calcium channels. The latter differ from ordinary voltage-operated calcium channels in possessing a more negative activation threshold and resistance to the blocking action of nifedipine. Since the activation threshold of these channels is more negative than the resting potential, serotonin-induced contraction may be potentiated by depolarization or inhibited by hyperpolarization, independently of the causes leading to these changes of membrane potential. An example of this potentiation may be the increase in sensitivity to serotonin of the smooth muscles of the cat cerebral arteries when depolarized by high arterial pressure [7]. In the absence of additional depolarization the ordinary voltage-controlled calcium channels are evidently not involved in calcium transfer under the action of serotonin, for serotonin depolarization itself does not raise their activation threshold. However, in the presence of strong depolarization, causing activation of these channels also, we found inhibition of serotonin contraction. Reduction of serotonin-induced contraction against the

background of membrane depolarization by hyperpotassium solution also has been described in muscle cells of the cat basilar artery [8]. This effect cannot be explained in terms of the known properties of voltage-operated calcium channels, or of certain specific properties of hypothetical calcium channels with dual (by both voltage and receptor) control, for in the presence of strong depolarization serotonin-induced contraction becomes less than that part of the contraction which is independent of voltage. The common stage affecting voltage-sensitive and voltage-insensitive calcium inflow into the cell is the serotonin receptor. This suggests that strong membrane depolarization may influence the efficacy of interaction of the serotonin molecule with its receptor.

## LITERATURE CITED


1. V. A. Buryi, A. V. Gurkovskaya, N. I. Gokina, and M. F. Shuba, Byull. Éksp. Biol. Med., 105, 261 (1988).
2. N. I. Gokina, I. G. Butenko, A. V. Gurkovskaya, and M. F. Shuba, Fiziol. Zh. (Kiev), **34**, 74 (1988).
3. A. V. Gurkovskaya, V. A. Buryi, N. I. Gokina, and M. F. Shuba, Byull. Éksp. Biol. Med., **105**, 131 (1988).
4. A. V. Gurkovskaya, V. A. Buryi, N. I. Gokina, and M. F. Shuba, Fiziol. Zh. (Kiev), **34**, 66 (1988).
5. M. F. Shuba, Fiziol. Zh. (Kiev), No. 6, 533 (1981).
6. T. B. Bolton, Physiol. Rev., **59**, 607 (1979).
7. D. R. Harder, Pflügers Arch., **411**, 698 (1988).
8. D. R. Harder and A. Waters, Eur. J. Pharmacol., **93**, 95 (1983).
9. N. G. Kochemasova and M. F. Shuba, Second International Symposium on Physiology and Pharmacology of Smooth Muscle, Abstracts, Varna, Bulgaria (1979), p. 44.
10. M. T. Nelson, N. B. Standen, J. E. Baryden, and J. F. Worley, Nature, **336**, 382 (1988).
11. W. Osterrieder, G. Brum, J. Hescheler, et al., Nature, **298**, 576 (1982).
12. M. F. Shuba, 28th International Congress of Physiological Sciences, Budapest (1980), p. 83.
13. C. van Breeman, P. Aaronsen, and R. Loutzenhiser, Pharmacol. Rev., **30**, 167 (1979).