MEMBRANE MECHANISMS OF THE EXCITATORY ACTION OF SEROTONIN ON SMOOTH MUSCLES OF THE RABBIT PULMONARY ARTERY

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UDC 612.731:612.133].014.46:612.018:577.175.823

KEY WORDS: serotonin, Ca^{++}, pulmonary artery

In most cases serotonin (5-HT) is an effective vasoconstrictor and exhibits its action through activation of two types (5-HT_1 and 5-HT_2) of receptors, which are represented unequally in different blood vessels [12]. Activation of 5-HT-induced contraction takes place on account both of the arrival of Ca^{++} ions from the extracellular medium and of their release from intracellular sources. These sources are involved by agonists in different proportions in different blood vessel tissues [13].

This paper describes an attempt to analyze the pathways whereby Ca^{++} ions enter the cytoplasm from the extracellular medium during activation of smooth muscle cells (SMC) of the rabbit pulmonary artery by 5-HT.

EXPERIMENTAL METHOD

Circular strips of rabbit pulmonary artery about 0.5 mm wide were used in the experiments. Electrical and contractile activity of SMC was recorded simultaneously by means of a modified single sucrose gap method [1]. The solutions for application and the experimental conditions were the same as in previous investigations [2-4]. Electrical and contractile activity of the muscle strips was recorded simultaneously by means of a KSP-4 automatic potentiometer. Numerical values are shown as the mean $\pm\sigma$ (σ denotes the standard deviation; n the number of observations).

EXPERIMENTAL RESULTS

In a concentration of 10^{-8}-10^{-5} M 5-HT caused dose-dependent contraction of muscle strips of the pulmonary artery (Fig. 1a). The mean effective dose ($K_{ED_{50}}$) of 5-HT was $2.7 \cdot 10^{-7}$ M, in agreement with $K_{ED_{50}}$ for 5-HT_2-receptors identified in the CNS [12]. Prozasin, a specific blocker of α_1-adreno-receptors, through which noradrenalin mainly exerts its excitatory influence on these SMC [4], did not affect 5-HT-induced contraction. Consequently, the action of 5-HT cannot be mediated either through its presynaptic effect on adrenergic nerve terminals [7, 14] or through nonspecific activation of α_1-adrenoreceptors in the SMC membrane. The results of an experiment to study dependence of the contractile response to 5-HT on the transmembrane potential level, which can be modified by application of an inward current, are shown in Fig. 1b. At the same time, changes in SMC membrane conductance were monitored by recording the anelectrotonic potentials induced by pulses of inward current, and changes in excitability of SMC were monitored in turn by recording the anode-breaking response and the amplitude of the action potentials arising upon the catelectrotonic potentials. 5-HT induced depolarization of SMC of the pulmonary artery, amounting to about 5 mV, and reduced almost by half the resistance of their membrane. Excitability of the muscle cells was increased under these circumstances.

The decrease in membrane resistance during 5-HT-induced depolarization is evidence of its increased conductance for Na^+ and (or) Cl^- ions, whose equilibrium potential is more positive than the resting potential. However, whereas in the presence of 5-HT the membrane was repolarized to its initial level, its conductance was restored or actually reduced (Fig. 1b). Consequently, at the initial moment 5-HT either did not increase membrane conductance,

Department of Neuromuscular Physiology, A. A. Bogomolets Institute of Physiology, Academy of Sciences of the Ukrainian SSR, Kiev. (Presented by Academician of the Academy of Medical Sciences of the USSR G. N. Kryzhanovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 105, No. 2, pp. 131-133, February, 1988. Original article submitted June 4, 1987.

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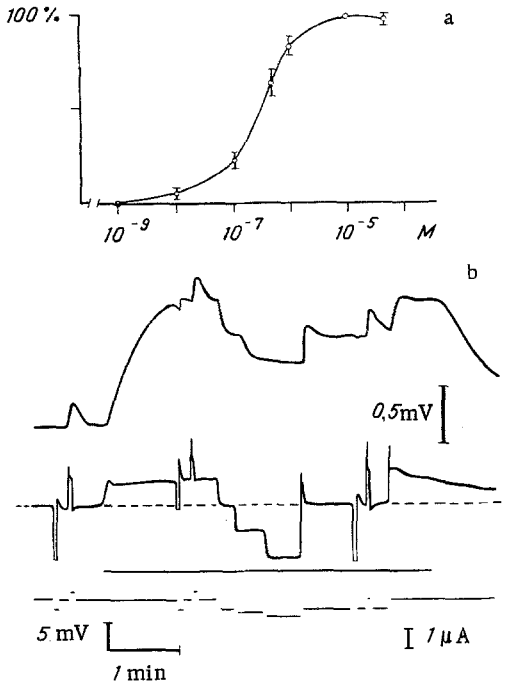

Fig. 1. Action of 5-HT on electrical and contractile activity of muscle strip of pulmonary artery. a) Dependence of contractile response of muscle strip on 5-HT concentration in external Kreb's solution (maximal response taken as 100%); b) effect of transmembrane potential level on 5-HT-induced contraction and conductance of muscle cell membrane (from top to bottom: contractile responses, transmembrane potential); change in transmembrane potential level induced by application of an inward or outward current (bottom trace).

or if it did, the increase was small and was compensated by a decrease of potassium conductance, which is the primary cause of depolarization. The increase in excitability of SMC during the action of 5-HT, which was preserved during anelectrotonic membrane repolarization also, was evidently connected with the decrease in potassium conductance. A similar mechanism of depolarization and of increased excitability of SMC of the pulmonary artery was found by the writers also under the influence of noradrenalin [2]. In both cases a primary decrease of potassium and increase of sodium conductance triggered membrane depolarization, and in the course of its development, voltage-dependent potassium conductance was activated, preventing any further depolarization.

5-HT-induced depolarization did not exceed the threshold of activation of voltage-dependent calcium channels, which was 5-7 mV and was estimated by the appearance of contractile responses to catelectrotonic depolarization in normal solution. Consequently, an increase in the intracellular concentration of Ca^{++} ions, activating the contractile response to 5-HT, could take place through the inflow of Ca^{++} ions through calcium channels, gated by serotonin receptors.

As a rule, receptor-gated channels are independent of transmembrane potential, but it has been shown on Aplysia neurons that 5-HT-activated inflow of Ca^{++} ions is voltage-dependent [11]. In the present experiments anelectronic repolarization and subsequent hyperpolarization of the SMC membrane also lead to an appreciable (about 50%) decrease in 5-HT-induced contraction. With the achievement of a certain level, subsequent membrane hyperpolarization no longer reduced muscle contraction (Fig. 1b).

It can be postulated on the basis of the results of these experiments that about half of the serotonin-gated calcium channels are additionally controlled by membrane potential. The threshold of activation of voltage-sensitive gates of these channels is more negative than the resting potential, and during membrane hyperpolarization they change into a non-conducting state, even though the activation gates controlled by the receptor remain open.

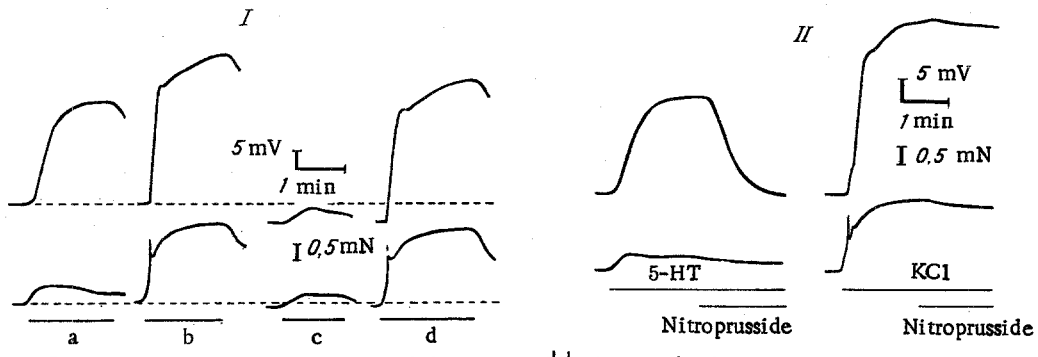

Fig. 2. Effect of verapamil (I) and Cd^{++} ions (II) on electrical (bottom traces) and contractile (top traces) responses to hyperpotassium solution (60 mM) and 5-HT (10^{-6} M). a, b) Responses to hyperpotassium solution and 5-HT in normal solution, respectively; c, d) the same responses superposed on the action of verapamil (10^{-6} M) and Cd^{++} ions (10^{-4} M), respectively. Action of 5-HT and of hyperpotassium solution indicated by horizontal line.

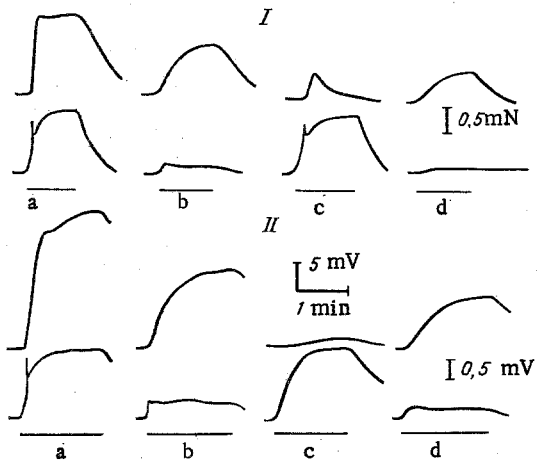

Fig. 3. Effect of sodium nitroprusside (10^{-6} M) on responses of muscle strips evoked by hyperpotassium solution and 5-HT. I: a, b) Responses to 5-HT (10^{-6} M) and to hyperpotassium solution (60 mM), respectively; c, d) the same, at 5th minute of action of nitroprusside. Horizontal line indicates action of hyperpotassium solution and 5-HT, broken line shows initial level of muscle contraction and transmembrane potential; II) effect of nitroprusside superposed on responses to 5-HT and hyperpotassium solution (top traces — contractile, bottom — electrical responses).

Additional arguments in support of this hypothesis were obtained in experiments with verapamil (10^{-6} M) and Cd^{++} ions (10^{-4} M), blockers of calcium channels. In the chosen concentrations these blockers effectively (by 85.1 ± 2.4 and 98.1 ± 0.9% respectively; n = 8) depressed the tonic component of contraction in response to the action of the hyperpotassium solution, which is activated by inflow of Ca^{++} ions through voltage-dependent inactivated calcium channels (Fig. 2). Meanwhile the 5-HT-induced contraction was depressed by verapamil and cadmium by about the same degree (by 53.8 ± 2.6%, n = 14) and 48.9 ± 4.7%, n = 4) as during anelectrotonic membrane hyperpolarization.

5-HT-induced depolarization was virtually not reduced at all by the action of Cd^{++} ions. This shows that membrane depolarization is unconnected with an increase in membrane permeability for Ca^{++} ions. Reduction of depolarization by verapamil is evidently due to nonspecific blockadge of sodium channels [10].

Part of the 5-HT-induced contraction which was not inhibited by blockers of voltage-dependent calcium channels and not abolished by membrane hyperpolarization could be activated both by the arrival of Ca^{++} ions through serotonin-gated voltage-dependent calcium channels and also by the release of intracellularly bound calcium. To estimate these possibilities, the action of sodium nitroprusside on 5-HT-induced contraction was investigated.

This compound is used as a selective blocker of receptor-gated calcium channels, although in addition it causes a marked increase in the intracellular cGMP concentration [5, 6, 8, 9]. To rule out the possibility of an indirect action of nitroprusside through its effect on intracellular mechanisms involved in the contraction process, we compared its action on contractile responses induced by 5-HT and by the hyperpotassium solution (Fig. 3). Nitroprusside in a concentration of 10^{-6} M, applied before (Fig. 3, I) or against the background of (Fig. 3, II) the action of the hyperpotassium solution, caused virtually no change in the contractile response to entry of Ca^{++} ions through voltage-dependent calcium channels. Meanwhile the contractile response to 5-HT was inhibited by nitroprusside virtually completely (by 94.2 ± 1.3%; n = 14).

5-HT-induced contraction of SMC of the pulmonary artery is therefore activated by Ca^{++} ions which enter the cell from the extracellular medium through serotonin receptor-gated calcium channels. About half of these channels are additionally controlled by the transmembrane potential.

The present experiments, however, do not rule out the possibility of partial involvement of Ca^{++} ions, released from juxtamembranous regions or intracellular reserves, in 5-HT-induced contraction.

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