EFFECT OF SEROTONIN AND HISTAMINE ON CELL CONTRACTILITY IN THE INTERNAL CAROTID ARTERY

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UDC 612.731.15:[615.218.1+615.357:577.175.823

Experiments on isolated segments of the dog internal carotid artery showed that serotonin (5-HT), in a concentration of $5 \cdot 10^{-11}$ to $5 \cdot 10^{-5}$ g/ml, activates contractions. Histamine in a concentration of $5 \cdot 10^{-9}$ g/ml causes relaxation of the segments, but higher concentrations activate contractile responses. 5-HT and histamine were found to evoke contractile responses of depolarized vascular smooth muscle. It is suggested that 5-HT activates the inward calcium ion current through the membrane whereas histamine activates both the inflow of extracellular calcium and the outflow of calcium ions from the intracellular depots.

KEY WORDS: vascular smooth muscles; serotonin; histamine; calcium ions.

Interpretation of the results of experiments to study the effect of serotonin (5-HT) and histamine on the cerebral blood flow is difficult because of indirect effects of these substances on the systemic blood pressure, the blood flow in the extracerebral vessels, and vascular permeability [3, 4].

To study the role of 5-HT and histamine in the regulation of the cerebral blood flow it is essential to investigate their action on contractility of smooth muscles of isolated cerebral vessles.

EXPERIMENTAL METHOD

Experiments were carried out on blood vessels from 25 dogs weighing 15-20 kg. The animals were killed by exsanguination under pentobarbital anesthesia (40 mg/kg). After removal of the brain, segments 3 mm wide were excised from the cavernous portion of the internal carotid artery. The segments were placed between the fixed plate and the shaft of a 6MKhlS mechanotrom. The temperature of the Krebs–Ringer solution [1] in all the experiments was 37±0.5°C. The solutions were oxygenated with a mixture of 95% O_2 and 5% CO_2; the pH of the solution was maintained within 7.40±0.05.

The serotonin creatine sulfate used for testing was from Sigma, histamine dihydrochloride from Sigma, and lysergic acid diethylamide (LSD) from Ciba.

EXPERIMENTAL RESULTS

5-HT and histamine were used on concentrations from $5 \cdot 10^{-12}$ to $5 \cdot 10^{-5}$ g/ml. The appearance of contractions was recorded to 5-HT in a concentration of $5 \cdot 10^{-11}$ g/ml. An increase in concentration caused a gradual rise in the amplitude of contraction. Maximal contractile responses were recorded to 5-HT in a concentration of $5 \cdot 10^{-6}$ g/ml (Fig. 1). Keeping the vascular segments for 10 min in solution containing LSD in a concentration of $2 \cdot 10^{-8}$ g/ml shifted the dose–response curve to the right and caused a significant decrease in amplitudes of the contractile responses (Fig. 1).

Recording the contractions of the smooth-muscle cells of the internal carotid artery in response to histamine showed that concentrations of $5 \cdot 10^{-12}$ to $5 \cdot 10^{-10}$ g/ml were ineffective. In a concentration of $5 \cdot 10^{-9}$ g/ml histamine caused relaxation of the vascular smooth muscle. A further increase in concentration led to a gradual, dose-dependent rise in the level of evoked contractions (Fig. 1). Repeated application of an active concentration of histamine led to the development of histamine tachyphylaxis.

Department of Normal Physiology, Leningrad San.-Gig. Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Chernigovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 88, No. 7, pp. 3-5, July, 1979. Original article submitted June 20, 1978.

0007-4888/79/8807-0673$07.50

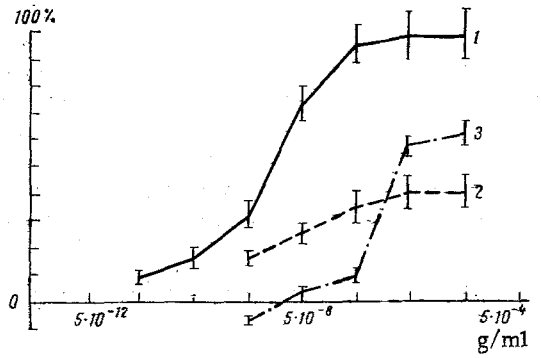

Fig. 1

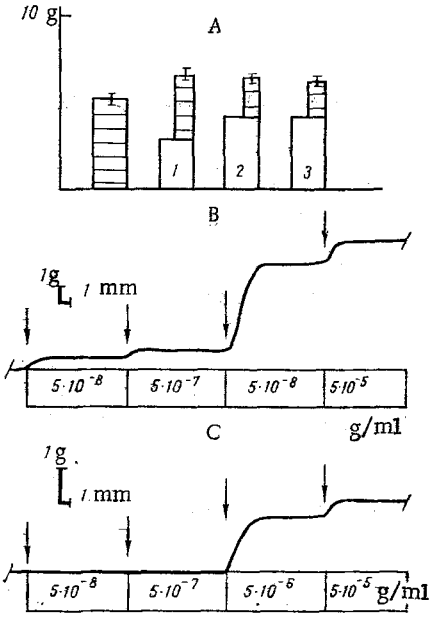

Fig. 2

Fig. 1. Effect of 5-HT and histamine on contractions of internal carotid artery cells. Level of contractile responses to 5-HT in a concentration of $5 \cdot 10^{-6}$ g/ml taken as 100%. 1) 5-HT; 2) 5-HT + LSD; 3) histamine.

Fig. 2. Effect of 5-HT and histamine on contractile activity of internal carotid artery cells after preliminary potassium depolarization (A), blocking of the inflow of extracellular calcium (B), and in calcium-free solution (C). For the sake of clarity, mean values of responses of different preparations are matched. A) 5-HT ($5 \cdot 10^{-6}$ g/ml); shaded columns) normal Krebs–Ringer solution; unshaded columns) potassium depolarization. 1) 25 mM KCl; 2) 100 mM KCl; 3) 200 mM KCl; B, C) histamine.

Testing the action of 5-HT and histamine after preliminary depolarization of the excitable surface membranes of the smooth-muscle cells of the internal carotid artery by potassium ions gave the following results. 5-HT increased the amplitude of contractile responses after both incomplete (25 mM KCl) and more intensive depolarization (200 mM KCl) of the cell membranes. However, during depolarization of the cell membrane the amplitude of the increase in contractions was significantly reduced compared with the amplitudes of the responses to the same concentration of 5-HT in normal Krebs–Ringer solution (Fig. 2A).

Since the use of histamine led to the rapid development of tachyphylaxis, the experiments were carried out only after intensive depolarization of the plasma membranes (200 mM KCl). Under these experimental conditions (Table 1) histamine, in a concentration of $5 \cdot 10^{-8}$ g/ml, caused no response of the vessel, but in higher concentrations histamine caused it to contract. However, the amplitude of the contractions was significantly lower than the amplitude of responses to the corresponding concentration of histamine in normal Krebs–Ringer solution (except histamine in a concentration of $5 \cdot 10^{-5}$ g/ml).

The role of extracellular calcium in the mechanism of action of 5-HT and histamine was studied by means of manganese ions (20 mM $MnCl_2$), which inhibit passive transport of calcium ions.

Exposure of the segments for 20 min in solutions containing manganese ions completely suppressed responses to 5-HT in concentrations of $5 \cdot 10^{-11}$ to $5 \cdot 10^{-5}$ g/ml. Under similar experimental conditions contractile responses to histamine were preserved (Fig. 2B). Their amplitude, however, was significantly ($P<0.05$) reduced compared with those to the corresponding concentrations of histamine in normal Krebs–Ringer solution. To assess the contribution of intracellular calcium to contractions caused by histamine after preliminary blocking of the flow of extracellular calcium into the smooth-muscle cells, the vascular segments were

TABLE 1. Mean Values (M±m) of Contractile Responses (in mg) of Isolated Segments of Dog Internal Carotid Artery in Normal Krebs–Ringer Solution and After Potassium Contracture

Histamine concen., g/ml	Magnitude of responses, mg		P
	normal Krebs–Ringer solution	Krebs–Ringer solution + KCl (200 mM)	
5.10^{-8}	3067±184	0	<0,001
5.10^{-7}	3457±243	1617±136	<0,01
5.10^{-6}	3935±307	2477±198	<0,01
5.10^{-5}	4217±380	3545±330	>0,05

placed in calcium-free solution. To prevent leakage of intracellular calcium, the test arterial segments were kept for 1 h in solution containing 20 mM $MnCl_2$, after which the external solution was replaced by calcium-free solution with the same concentration of manganese chloride. In that case, just as in the experiments in which the inflow of extracellular calcium was blocked, histamine had an activating effect. However, the threshold concentration was higher and the amplitude of the responses lower ($P<0,05$; Fig. 2C).

It can be concluded from the results of these experiments that 5-HT and histamine play an essential role in the regulation of contractility of the smooth-muscle cells of the internal carotid artery. Experiments with LSD revealed D-serotonin receptors in the muscular coat of the artery. Relaxation of the vascular segemtnts under the influence of low concentrations of histamine can evidently be explained by the higher sensitivity of the N_2-histamine receptors. It has been shown [2] that receptors of H_2 type are five times more sensitive to histamine than those of the H_1 type. Predominance of contractile responses to histamine in concentrations of over 10^{-9} g/ml evidently points to predominance of H_1-histamine receptors in the wall of the internal carotid artery.

It can be tentatively suggested that *in vivo* the final results of the response of the smooth muscles of the cerebral vessels to histamine depends, other conditions being the same, on the existing histamine concentration and the relative numbers of H_1- and H_2-histamine receptors.

The ability of 5-HT and histamine to induce contractions after preliminary depolarization of the membrane indicates a pharmacomechanical method of activation of the smooth-muscle cells of the internal carotid artery. The marked decrease in amplitude of the responses to 5-HT and histamine under conditions of intensive depolarization of the cell membranes, as is confirmed by experiments in which the transmembrane potential was recorded [5, 6].

The results of experiments in which the calcium permeability of the cell membranes was blocked by manganese ions suggest that the trigger mechanism of contraction of the smooth-muscle cells of the internal carotid artery for 5-HT is based on an increase in permeability to extracellular calcium. Histamine is able to activate contractions both by increasing permeability for extracellular calcium and also through the liberation of intracellular calcium.

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