ACTION OF SEROTONIN ON SMOOTH MUSCLES

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Serotonin causes contraction of the smooth muscles of the vas deferens and of a strip of stomach from rats by its action on D-serotonin receptors. Only a small part (about 10-14%) of its contractile effect is due to liberation of endogenous catecholamines. The action of serotonin on a strip of rat stomach is accompanied by increased entry of isotopes ^{45}Ca and ^{22}Na into its cell. The change of direction of the curve of concentration versus effect of serotonin on the strip of stomach is due to accumulation of sodium ions in the cells.

KEY WORDS: *serotonin*; *smooth muscles*; *resection*; *contraction processes.*

Contraction of smooth muscles caused by serotonin is mediated through the D receptors of the muscle cells or M receptors of the nervous structures of the smooth-muscle organs [10]. The muscle of the stomach [7] and vas deferens of rats [1, 10] contract as a result of the direct action of serotonin on D receptors. However, serotonin also processes the properties of an indirect adrenomimetic, liberating noradrenalin from the endings of adrenergic nerves in the stomach [7] and vas deferens [4]. Although in relatively low concentrations serotonin increases the tone of strips of stomach, in higher concentrations it relaxes them. The phenomenon of autoantagonism has been explained by the independent effect of serotonin α-adrenoreceptors of the smooth muscle of the stomach [11] or by the change from its monomolecular interaction with the active site of the D receptors into bimolecular [2].

The relative importance of the direct and indirect components in the action of serotonin on the smooth muscles of the stomach and vas deferens of rats was assessed in this investigation and the ionic mechanisms of the contractile and autoinhibitory effects of serotonin were analyzed.

EXPERIMENTAL METHOD

Isolated vasa deferentia and strips of stomach of rats were placed in a bath containing oxygenated Krebs solution at 37°C. Changes in tone of the organs were recorded under isotonic conditions by means of a mechanical-electrical transducer with a load of 0.5 g on the vas deferens and of 1 g on the strip of stomach. Contractions were recorded by means of a PSRT-1.03 ink-writing potentiometer. The effect of noradrenalin, serotonin, and the indirect adrenomimetic tyramine was investigated in control experiments and after disturbance of the liberation of noradrenalin from sympathetic nerve endings (through the action of the sympatholytic ornid (bretylium) in a concentration of $5 \cdot 10^{-4}$ g/ml for 15 min). To assess the indirect component of the action of these agonists, the method of cold degeneration of axons of adrenergic neurons [9] in the vas also was used. In the experiments on strips of rat stomach the reserves of endogenous catecholamines were reduced by giving the animals two injections (24 and 48 h before the experiments) of reserpine in a dose of 5 mg/kg. To determine the nature of the receptors through which noradrenalin, serotonin, and tyramine cause contractions of the smooth muscles of the rat vas deferens, the effect of phentolamine, deseryl (methysergide), and mexamine (5-methoxytryptamine) was analyzed. In experiments on the strips of rat stomach, the entry of the isotopes ^{45}Ca and ^{22}Na into the cells was determined radiometrically [3]; the effect of replacement of Na^+ ions in the Krebs solution by NH_4^+ ions and of the preliminary

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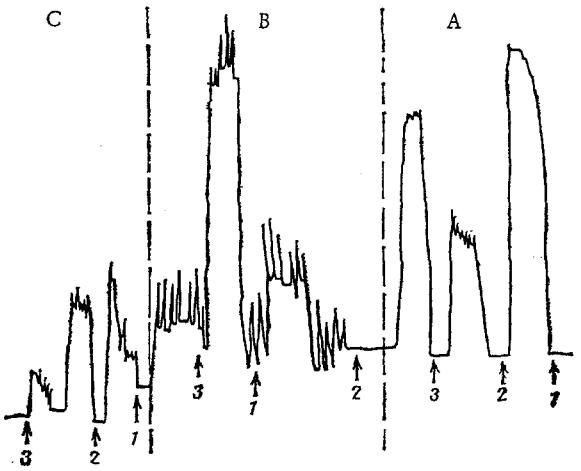

Fig. 1. Effect of noradrenalin $1 \cdot 10^{-5}$ g/ml (1), serotonin $2 \cdot 10^{-4}$ g/ml (2), and tyramine $4 \cdot 10^{-5}$ g/ml (3) on tone of the isolated rat vas deferens. A) Control; B) after action of ornid, $5 \cdot 10^{-4}$ g/ml, for 15 min; C) after phentolamine $1 \cdot 10^{-6}$ g/ml.

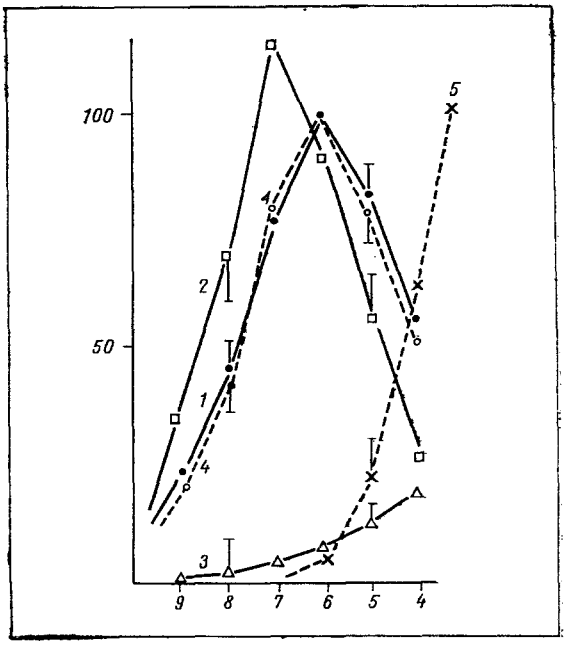

Fig. 2. Effect of serotonin on tone of rat stomach and on transmembrane movements of isotopes ^{45}Ca and ^{22}Na. 1) Change in tone of strip of stomach under influence of serotonin; 2) ditto after preliminary action of strophanthin $5 \cdot 10^{-6}$ g/ml; 3) ditto after replacement of Na^+ ions in Krebs's solution by NH_4^+ ions; 4) change in entry of ^{45}Ca into cells of strip of stomach after action of serotonin; 5) ditto for ^{22}Na. Ordinate, effect (in % of maximum). Abscissa, negative logarithms of serotonin concentration.

action of strophanthin (5×10^{-6} g/ml, 30 min) on the concentration–effect curves of serotonin also was studied.

The experimental results were subjected to statistical analysis [5].

EXPERIMENTAL RESULTS AND DISCUSSION

Noradrenalin ($1 \cdot 10^{-5}$ g/ml), serotonin ($2 \cdot 10^{-4}$ g/ml), and tyramine ($4 \cdot 10^{-5}$ g/ml) caused contraction of the vas of different amplitudes (Fig. 1). Contraction due to serotonin developed after a latent period of 1.5 min and was accompanied by stimulation of peristalsis. The action of ornid ($5 \cdot 10^{-4}$ g/ml) for 15 min on the vas caused frequent peristaltic contractions of its wall of high amplitude, which made it difficult to record the effect of the agonists. Nevertheless, ornid perceptibly depressed the contractions of the preparation produced by tyramine and did not significantly reduce the effect of serotonin (by only 14.2 ± 3.7%). Under conditions of cold denervation of the vas the effects of tyramine were completely suppressed, but serotonin continued to cause contractions of the vas (Table 1). Keeping the vas at 2°C for 4 days reduced by half the amplitude of its contractions, probably on account of a disturbance of the processes coupling the receptor stimulus and the change in muscle tone. Cooling blocked the effect of serotonin more than those of noradrenalin.

After treatment with serotonin in low concentrations ($1 \cdot 10^{-9}$-$1 \cdot 10^{-7}$ g/ml) relaxation of the strip of stomach was observed in almost 25% of cases, but with an increase in the concentration of the amine this was replaced by contraction. This relaxation was due to liberation of endogenous catecholamines and was abolished by cooling the smooth-muscle preparation to 18°C and also by preliminary treatment with reserpine. Under these conditions the amplitudes of the contractions caused by low concentrations of serotonin also were increased.

Since the effects of tyramine were completely abolished and those of serotonin were only reduced following treatment with sympatholytics or by cold denervation it can be concluded that serotonin has mainly a direct effect on the smooth muscles of the rat stomach and vas deferens. The indirect adrenergic component is responsible for not more than 15% of the effect of serotonin on the vas and for a small decrease in tone of the stomach muscles following exposure to low concentration of the amine.

Investigation of the affinity of antagonists of noradrenalin and serotonin for chemoreceptors [12] showed that phentolamine, a typical adrenolytic, exhibits greater affinity for α-adrenergic receptors, whereas the serotonin antagonists deseryl and mexamine possessed greater affinity for the D-serotoninergic receptors of the smooth muscles (Table 2). These facts are evidence of the independent existence of α-adrenergic and D-serotoninergic receptors in the smooth muscles of the rat vas deferens and also that the direct effect of serotonin on smooth muscles is produced through its influence on D-serotoninergic receptors.

The degree of contraction of the strip of stomach under the influence of serotonin coincided with the degree of increase in the inflow of the isotope ^{45}Ca into the cells (Fig. 2).

TABLE 1. Effect of Cold Denervation of Vas Deferens on Contractions Produced by Agonists (M ± 2.5 m)

Agonist	Concentration, g/ml	Contractions of vas deferens, mm	
		initially	after keeping at 2°C
Noradrenalin	$1 \cdot 10^{-5}$	103,2±31,1	52,7±20,5
Serotonin	$2 \cdot 10^{-4}$	45,0±13,1	18,3±5,1
Tyramine	$4 \cdot 10^{-5}$	93,5±18,8	0

TABLE 2. Affinity (pA_2) of Noradrenalin and Serotonin Antagonists for α-Adrenergic and D-Serotoninergic Receptors of Smooth Muscles of Rat Vas Deferens (M ± 2.5 m)

Agonist	pA_2 of antagonists		
	phentolamine	deseryl	mexamine
Noradrenalin	8,53±0,08	5,38±0,08	<4
Serotonin	6,37±0,10	9,47±0,50	4,33±0,06

The inflow of Ca^{2+} ions evidently activates the mechanisms of electrogenesis, for in a depolarizing solution of potassium chloride the serotonin contractions of the strip of stomach were virtually absent. An increase in the intracellular concentration of Ca^{2+} ions probably activates Ca–Na exchange diffusion [8], as a result of which if the serotonin concentrations are high enough (over 10^{-6} g/ml), the inflow of Na^{+} ions into the cell is intensified. Because of their possible competition with Ca^{2+} ions [6], the accumulation of Na^{+} ions inside the cell causes the change in direction of the curve of concentration versus effect of serotonin. The fact that the autoantagonism revealed during the action of high concentrations of serotonin is of this nature was confirmed by the following evidence: replacement of the Na^{+} ions in the Krebs solution by NH_4^{+} ions prevented any such change in direction of the concentration–effect curve, whereas the presence of strophanthin, which blocks the mechanisms of outward pumping of the intracellular Na^{+} [13], in the solution, on the other hand, facilitated the development of the autoantagonism phenomenon.

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