ACTION OF SEROTONIN ON D-RECEPTORS OF SMOOTH MUSCLES

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Experiments on strips of smooth muscle from the rat stomach showed that the cumulative "concentration–effect" curve of serotonin is bell-shaped. The relaxation observed with high concentrations of serotonin, and only against a background of an existing serotonin contraction, is the result of bimolecular interaction between serotonin and D-receptors of the smooth muscles. A study of "concentration–effect" curves of serotonin at different pH values showed that ionized amino groups and the hydroxyl group of serotonin take part in the interaction between serotonin and the D-receptors. With an increase in the degree of ionization of the hydroxyl group, the possibility of bimolecular interaction between serotonin and receptor (formation of an inactive PA_2 complex) and development of noncompetitive autoantagonism are facilitated.

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According to Gaddum and Picarelli [3], the effect of serotonin on smooth muscles is mediated through D-receptors. The amino group of serotonin, which is in the ionized form at physiological pH values [11], takes part in the interaction with these receptors. This view assumes the presence of an anionic center in the structures of the D-receptors. Other functional centers of these receptors are unknown.

In the present investigation results were obtained clarifying the existing views on the nature of interaction between serotonin and the D-receptors of smooth muscles.

EXPERIMENTAL METHOD

Experiments were carried out on strips of smooth muscle from the rat stomach. The preparation was placed at 37° in Tyrode solution (pH 7.9-8.0) aerated with oxygen. The method of cumulative curves [7] was used to study the relationship between effect and concentration of serotonin. Points on the concentration–effect curve represent mean values of results obtained in experiments on 6-8 strips. Concentration–effect curves of serotonin were found for different pH values of the Tyrode solution (from 6.0 to 10.0). The change in pH was achieved by adding NaOH or HCl. Fluctuations of pH from the assigned values did not exceed ±0.1.

Chlorpromazine, promethazine, diphenhydramine, cocaine, 1-benzyl-2,5-dimethylserotonin (BAS), and dihydroergotamine were used as serotonin antagonists [4]. The character of their antagonism with serotonin was studied by Schild's method [8] by determining the pA_2-pA_{10} difference in normal (pH 8.0) Tyrode solution.

EXPERIMENTAL RESULTS

The values of the pA_2-pA_{10} differences (Table 1) suggest the possibility of competitive antagonism with serotonin only in the case of BAS and dihydroergotamine. This is in agreement with reports of the competitive nature of antagonism between ergot alkaloids and serotonin [4]. The considerable scatter of the data in the experiments with dihydroergotamine and BAS is explained by the fact that the two substances, being partial serotonin antagonists, exhibit serotonin-like effects over the same range of concentrations as serotonin. The discovery of possible competitive relationships between dihydroergotamine and serotonin (Table 1) and published data [4] indicate that this amine produces its effects through the D-receptors of the gastric smooth muscles; the presence of D-receptors in the rat stomach was also demonstrated by the work of Vane [11, 12].

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TABLE 1. Antagonism of Serotonin With Certain Substances

Substance	pA_2	pA_{10}	pA_2-pA_{10}	Confidence limits of pA_2-pA_{10} difference
Chlorpromazine	5.56	5.05	0.51	0.27-0.75
Promethazine	5.98	5.39	0.63	0.39-0.87
Diphenhydramine	4.56	4.64	−0.13	−0.65-0.39
Cocaine	3.98	3.89	0.09	−0.09-0.27
BAS	4.88	4.47	0.41	−0.42-1.24
Dihydroergotamine	6.34	5.72	0.62	−0.24-1.48

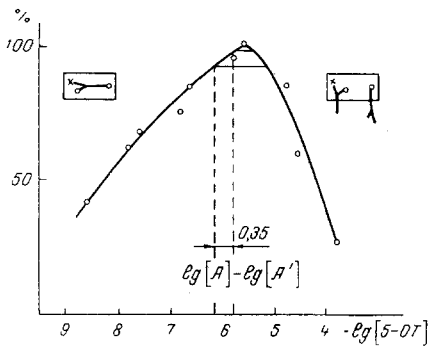

Fig. 1. Cumulative concentration–effect curve of serotonin obtained in normal Tyrode solution (pH 8.0). Ordinate, effect in percent of maximum; abscissa, negative logarithms of molar concentrations. Schemes illustrating mono- (left) and bimolecular (right) interaction between serotonin and receptor shown in rectangles.

In normal (pH 8.0) Tyrode solution the curve of logarithm of concentration against effect of serotonin is bell-shaped (Fig. 1). Contraction reached a maximum with a concentration of $10^{-5.6}$, and serotonin in higher concentrations caused relaxation. This was not due to liberation of catecholamines, because the shape of the curve remained unchanged when reproduced in the presence of cocaine ($3 \cdot 10^{-6}$ M) or morphine ($2.6 \cdot 10^{-8}$ M) and with strips of stomach from rats preliminarily treated with reserpine. This relaxation, like the contraction, was due to the direct action of serotonin on the smooth muscles, because the shape of the curve was unchanged if reproduced at a lower temperature (18°), when nerve cells in smooth muscles are paralyzed [5].

Like the contraction, the relaxation caused by serotonin in supermaximal (above $10^{-5.6}$ M) concentrations was due to its action on D-receptors. In fact, serotonin caused relaxation of the gastric smooth muscles only if they were in a state of maximal contraction produced by serotonin; during the action of serotonin on muscles in a state of maximal acetylcholine contraction no such relaxation took place.

The relaxation observed with high concentrations of serotonin, and only against the background of an existing serotonin contraction, could be the result of bimolecular interaction between serotonin and the D-receptors of smooth muscles, in the nature of noncompetitive autoantagonism [1]. Analysis of data in the literature shows that interaction of this character is probably observed not only in the smooth muscles of the stomach, but also in other smooth-muscle objects [6, 9].

Serotonin produces a contraction when it forms an active complex with the D-receptor: $R + A = RA$ (R, receptor; A, agonist). In the presence of very high serotonin concentrations, a second molecule of the amine may be attached to the receptor: $RA + A = RA_2$, the formation of this inactive complex being accompanied by diminution of the effect. In the case of bimolecular interaction, the relationship between effect and concentration of serotonin may be expressed by the equation:

$$V = \frac{100}{1 + \frac{K}{[A]} + \frac{[A]}{K_1}},$$

where V represents effect as a percentage of maximal; [A] the molar concentration of serotonin; and K and K_1 the dissociation constants of the RA and RA_2 complexes.

Under the conditions when serotonin causes contraction followed by relaxation, an effect of the same magnitude is possible in concentrations differing by 2 and 10 times (on the logarithmic scale by 0.3 and 1). It follows from the equality of the effects that:

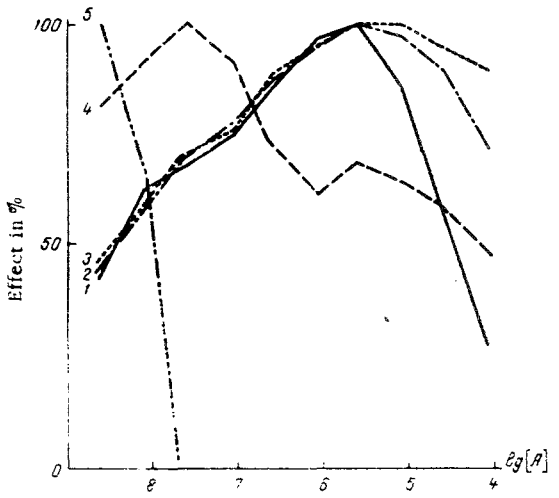

Fig. 2. Cumulative concentration–effect curves of serotonin in Tyrode solution with different pH values. pH values shown for each curve by numbers: 1) pH 8.0; 2) pH 7.0; 3) pH 6.0; 4) pH 9.0; 5) pH 10.0.

$$\frac{100}{1+\frac{K}{[A]}+\frac{[A]}{K_1}}=\frac{100}{1+\frac{K}{2[A]}+\frac{2[A]}{K_1}},\ \text{whence}\ KK_1=2[A]^2$$

$$\frac{100}{1+\frac{K}{[A']}+\frac{A'}{K_1}}=\frac{100}{1+\frac{K}{10[A']}+\frac{10[A']}{K_1}},\ \text{whence}\ KK_1=10[A']^2$$

or

$$2[A]^2=10[A']^2.$$

Transforming and taking logarithms of the last equation, we have:

$$\log[A]-\log[A']=\frac{\log 10-\log 2}{2}=0.35.$$

The graphic method of determination of the difference log [A]−log[A'], with a value of 0.35, is shown in Fig. 1. The good measure of agreement between the experimental and theoretical values confirms the existence of bimolecular interaction between serotonin and D-receptors. This suggests that these receptors contain at least two points of importance for the formation of the active serotonin–receptor complex.

Knowing that the ionization constants (pKa) of the phenolic hydroxyl group and the amino group of serotonin are 7.8 and 9.9 respectively [2], the role of these groups in the mechanism of interaction between serotonin and D-receptors during the study of effect of the amine at different pH values can be estimated.

At pH 6.0-9.0 the mean maximal serotonin contraction was 46.8-28.6 mm, whereas at pH 10.0 it did not exceed 7 mm. Consequently, a decrease in the degree of ionization of the amino group reduced the contractile effect of serotonin. It is clear from Fig. 2 that the maxima of the concentration–effect curves at pH 8.0 were shifted toward lower concentrations, indicating that ionization of the hydroxyl group is of some importance for the contractile action of serotonin, because at pH 8.0 ionization of the hydroxyl group increases (pKa = 7.8). On the other hand, it is easy to see that relaxation of the smooth-muscle preparation arises more easily and in lower concentrations the higher the pH of the Tyrode solution. Consequently, the greater the degree of ionization of the phenolic hydroxyl group, the more easily the second serotonin molecule is attached to the D-receptor and the inactive RA_2 complex formed. The results obtained were due to changes in interaction between serotonin and D-receptors and not the results of the nonspecific effect of pH because under analogous experimental conditions changes in pH had no effect on the position and shape of the "logarithm of concentration–effect" curve of acetylcholine and produced changes opposite to those of serotonin in the curve for noradrenalin.

The data described above suggest the existence of an anionic (for interaction with the ionized amino group) and a cationic center (for interaction with the ionized hydroxyl group of serotonin) in the structure

of D-receptors. They do not rule out the possible significance of certain functional groups (OH, SH, NH), capable of forming a hydrogen bond with the indole nitrogen atom of serotonin.

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