SIMPLE METHODS OF IN VIVO TESTING FOR PERIPHERAL M- AND D-ANTAGONISTS OF SEROTONIN

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A study of the effect of peripheral serotonin antogonists on exophthalmos and diarrhea induced by serotonin showed that the universal antagonist (preparation PVM*) inhibits both the exophthalamos and the diarrhea, whereas D-antagonists (5-methoxytryptamine, 6-methoxytryptamine, dihydroergotamine) inhibit only the exophthalmos and M-antagonists (morphine, trimeperidine) inhibit only the diarrhea.

Laborious methods of investigation have to be used at the present time for the primary screening of substances inhibiting peripheral M- and D-serotoninergic structures by in vivo tests [4, 7, 9].

In the investigation described below the possibility of identifying peripheral M- and D-antagonists of serotonin by means of simple experimental models was studied.

EXPERIMENTAL METHODS

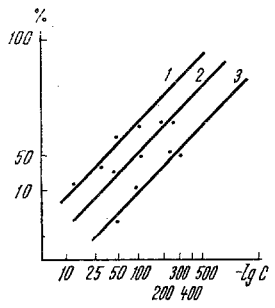

Fig. 1. Logartihm of dose–effect curves of serotonin before (1) and after intravenous injection of morphine in doses of 2 (2) and 5 (3) mg/kg. Abscissa, negative logarithm of serotonin dose (in μg/kg); ordinate, effect (in % of maximal).

Tests were carried out on mice of both sexes weighing 18-22 g. The effect of peripheral serotonin antagonists were studied on the exophthalmos and diarrhea developing after injection of serotonin.

Exophthalmos was induced by rapid injection of serotonin in a dose of 10 mg/kg into the caudal vein. The effect appeared immediately after the injection and lasted for 30-60 sec. From 2 to 3 min after the injection of serotonin the animal developed severe diarrhea, which continued for 20-30 min.

The substances to be tested were injected intraperitoneally in aqueous solution 20 min before the injection of serotonin. To determine the specificity of the antiserotonin properties of the compounds tested, their effect was studied on the exophthalmos induced by adrenalin, injected intravenously in a dose of 200 μg/kg. In addition, the specificity of the serotonin-negative action of some serotonin antagonists (in particular, morphine) was studied by the diarrhea test. The relationship between the effect and dose of serotonin with and without previous injection of morphine was expressed as a straight line on the logarithm of dose–effect graph. Both the exophthalmos and diarrhea were assessed in alternative form. Statistical analysis of the results was carried out by the method of Litchfield and Wilcoxon.

*An analog of dixyrazine.

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TABLE 1. Effect of Various Substances on Serotonin and Adrenalin Exophthalmos and on Serotonin-Induced Diarrhea

Preparation	ED_{50} (in mg/kg, intraperitoneally)		
	serotonin exophthalmos	adrenalin exophthalmos	diarrhea
Morphine	>5	>5	2,9 (1,5÷5,6)
Trimeperidine	>40	>40	21,0 (10,0÷44,0)
5-Methoxytryptamine	5,2 (3,3÷8,0)	>80	causes diarrhea
6-Methoxytryptamine	4,3 (3,2÷6,2)	>50	>50
Dihydroergotamine	2,5 (1,6÷3,9)	3,3 (2,2÷4,9)	causes diarrhea
PVM	10,5 (5,8÷18,9)	>50	12,0 (7,9÷21,6)

EXPERIMENTAL RESULTS AND DISCUSSION

The results given in Table 1 show that the exophthalmos induced by serotonin is inhibited by the D-antagonists 5-methoxytryptamine, 6-methoxytryptamine, and dihydroergotamine [1, 6] and by the universal antagonist preparation PVM [3].

By contrast, the M-antagonists morphine and trimeperidine [2, 8, 10] did not affect this action of serotonin.

Investigation of the effect of serotonin antagonists on adrenalin-induced exophthalmos showed that 5-methoxytryptamine, 6-methoxytryptamine, and preparation PVM, in doses exceeding ED_{84} in relation to serotonin-induced exophthalmos, did not alter the effect of adrenalin. By contrast with these components, dihydroergotamine also prevented the adrenalin exophthalmos (Table 1). This fact can be explained by the adrenolytic properties of dihydroergotamine [5].

Serotonin-induced exophthalmos in mice is thus blockedby D-antagonists (5-methoxytryptamine, 6-methoxytryptamine, dihydroergotamine) and by the universal antagonist (preparation PVM). All these compounds, except dihydroergotamine, inhibit the action of serotonin specifically, for even in much larger doses they have no effect on adrenalin-induced exophthalmos.

In the next series of experiments the propulsive action of serotonin was found to be suppressed by M-antagonists (morphine, trimeperidine) and by the universal antagonist PVM, whereas the D-antagonist 6-methoxytryptamine had no effect on the action of serotonin. Both 5-methoxytryptamine and dihydroergotamine themselves produced diarrhea (Table 1).

Further experiments showed that in the presence of morphine (2 and 5 mg/kg) the serotonin "logarithm of dose–effect" straight lines are shifted parallel to each other to the right (Fig. 1). The parallel nature of the straight lines and the significant difference between the ED_{50} values of serotonin before and after administration of various doses of morphine were confirmed by statistical analysis of the results. The parallel shift of the straight lines indicates the competitive character of the antagonism of morphine with serotonin, and confirms the specificity of action of the antagonist. The diarrhea induced by serotonin is thus suppressed by M-antagonists (morphine, trimeperidine) and the universal antagonist (preparation PVM), with well-marked M-serotoninolytic properties.

It can be concluded from these results that serotoninergic structures responsible for the development of exophthalmos in mice are receptors of the D-type, and that the propulsive action of serotonin is effected through excitation of M-serotoninergic structures.

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