A PHARMACOLOGICAL ANALYSIS OF THE SEROTONIN-SENSITIVE STRUCTURES IN THE AFFERENT NERVE ENDINGS OF THE CORNEA

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The difficulties arising during the study of the central effects of serotonin (5-hydroxytryptamine) and the role of serotonin-sensitive structures in these effects [5] may be partly overcome by using models representing individual elements of the nervous system: the interneuronal synapses, the pre- and postsynaptic membranes and the peripheral nervous structures. Serotonin has an effect on sensory nerve endings, including the receptors of the cornea. In particular, it lowers the intensity of the terminal anesthesia produced by cocaine and other local anesthetics [2, 10, 11].

EXPERIMENTAL

Experiments were conducted on rats. The animals were fixed to a frame and a solution of the substance for testing was introduced into the conjunctival sac of one eye. At the end of the exposure period the drug was removed with a gauze swab. The degree of anesthesia which developed was assessed by Renier's method [1], the essence of which is that tactile stimuli are applied to the rat's cornea at the rate of 100 taps per min for 1 min. This test was repeated every 5 min for 1 h. The total number of taps endured without blinking (Renier's index) was a measure of the level of anesthesia. The relative local anesthetic activity of the tested substances was determined from Valet's formula [1]. The duration of the action of serotonin and histamine solution was 2 min, and that of solutions of the other substances was 3 min. Each series of experiments included ten measurements made on ten animals.

RESULTS

The application of cocaine solutions to the rat's cornea was accompanied by the development of terminal anesthesia, the degree of which increased with an increase in the concentration of cocaine, but only up to a certain limit. Renier's index rose proportionately to the concentration during application of a 0.25-1% solution, while 2.5 and 5% solutions produced the same degree of anesthesia as a 1% solution. The values of Renier's indices for these three concentrations were practically identical (Fig. 1a). The presence of a "ceiling" of anesthesia indicates that the anesthetic interacts with certain "radicals of the protoplasmic structures" [9] or with "receptors" in the biochemical sense of the term. The study of the structural specificity of local anesthetics leads to the same conclusions [3]. Maximal anesthesia was attained only when all the receptors were occupied by anesthetic.

Instillation of serotonin solution 2 min before introduction of the cocaine solutions into the conjunctival sacs lowered their anesthetic activity. The study of the effect of a constant concentration (0.25%) of serotonin on the shape and position of the concentration—action curve of cocaine showed that serotonin did not change the proportional relationship between the concentration of cocaine and the degree of anesthesia, reduced the angle of inclination of the curve and depressed the level of the maximum and displaced it towards the higher concentrations (Fig. 1b). Such changes in the position of the concentration—effect curves are characteristic of noncompetitive antagonism. It may therefore be supposed that serotonin and cocaine intereact with different receptor substances in the tactile receptors of the cornea.

Histamine, when instilled in the form of a 0.1% solution (approximately equimolar with a 0.25% solution of serotonin), lowered the anesthesia activity of cocaine. Like serotonin, it lowered the angle of inclination of the con-

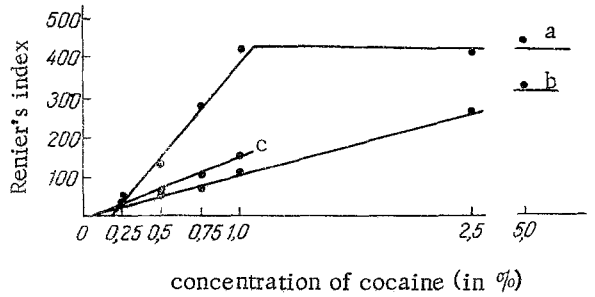


Fig. 1. Relationship between the degree of terminal anesthesia and the concentration of cocaine without exposure (a) and after preliminary exposure to a 0.25% solution of serotonin (b) and a 0.1% solution of histamine (c). Vertical lines) confidence limits corresponding to $p < 0.05$.

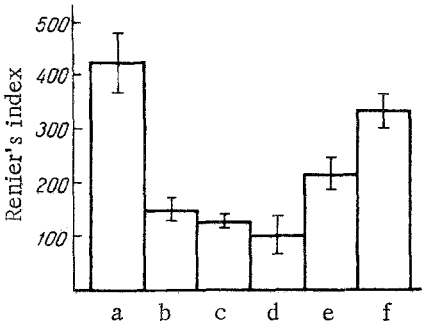


Fig. 2. Effect of morphine on anesthetic effect of a combination of cocaine with histamine and of cocaine with serotonin. a) 1% cocaine solution; b) 0.1% histamine solution + 1% cocaine solution; c) 5% morphine solution 0.1% histamine solution + 1% cocaine solution; d) 0.25% serotonin solution + 1% cocaine solution; e) 5% morphine solution + 0.25% serotonin solution + 1% cocaine solution; f) 5% morphine solution + 1% cocaine solution.

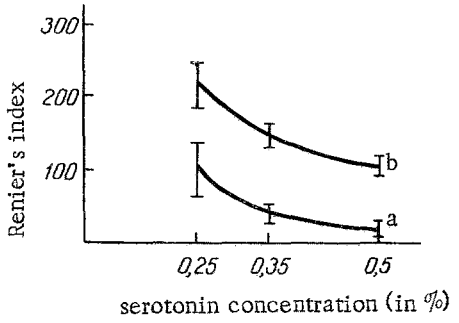


Fig. 3. Relationship between anesthetic effect of 1% cocaine solution and serotonin concentration without the action (a) and after the action (b) of 5% morphine solution.

centration–action curve of cocaine (Fig. 1c). Hence histamine, like serotonin, is a noncompetitive antagonist of cocaine.

To determine whether the serotonin-receptive structures in the sensory nerve endings of the cornea are identical or different, morphine was used. According to data in the literature, morphine is a specific antagonist of serotonin in its action on nerve structures [7, 8, 12, 14]. Even in high concentrations (5%), morphine had no anesthetic action of its own. In fact, a 5% solution of morphine, instilled into the eye before a 1% cocaine solution, actually lowered the level of the developing anesthesia slightly. Meanwhile, morphine differed in its action on the anesthesia produced by combinations of cocaine with histamine and with serotonin. Whereas morphine did not change the anesthetic effect of the first combination (cocaine–histamine) (the differences observed were not statistically significant), it largely abolished the antagonism effect of serotonin on cocaine (Fig. 2). That this effect was due to antagonism between serotonin and morphine and not to an increase in the anesthetic power of cocaine under the influence of morphine is proved by the following facts. First, morphine in 5% solution lowers the level of anesthesia produced by cocaine; and second, morphine does not change the anesthetic effect of a combination of cocaine with histamine. The results of these experiments show that histamine and serotonin, despite the similarity between their effects, differ in the point of application of their action on the sensory nerve endings of the cornea.

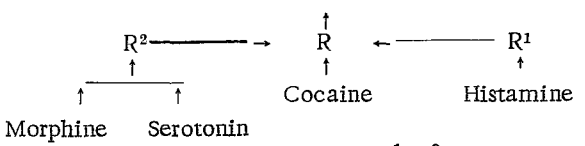
The specificity of the antagonism between serotonin and morphine suggests that it is competitive in nature. This suggestion was confirmed by the study of the effect of morphine on the curve showing the relationship between the anesthetic effect of cocaine and the serotonin concentration. It is clear from Fig. 3 that morphine shifted the curve parallel to itself, a characteristic sign of competitive antagonism [4, 6].

In the light of the classification suggested by Gaddum and Picarelli [7], it may be postulated that the serotonin-sensitive structures (serotonin receptors) of the sensory nerve endings of the cornea are of the muscarine type. This agrees with other findings [8, 14] indicating the presence of muscarine receptors in other nerve formations (autonomic ganglia).

It may be concluded from the results described above that the sensory nerve endings of the cornea contain several functionally related receptive (in the biochemical sense) structures. The following scheme demonstrates their relationship:

Relative Local Anesthetic Activity of Tested Substances (in molar concentration)

Preparation	Relative activity
Cocaine	1.0
Promethazine	14.5
Diphenhydramine	1.04
Dihydroergotamine	3.1
BAS	9.6



These receptive structures (R, R^1, R^2) capable of interacting only with certain substances, belong to the same tactile receptors of the cornea. However, they differ biochemically and, evidently, functionally. The action of cocaine is probably exerted directly on the excitable membrane of the receptor, and it is essentially an action directed towards the mechanism of formation of the receptor potential. Serotonin and histamine have different points of application in the tactile receptor, and they sensitize the excitable membrane, increasing its sensitivity to the adequate (mechanical) stimulus. This suggests that it is possible, in principle, for local anesthesia to develop not only as a result of the direct suppression of the excitability of the sensory nerve endings, but also as a result of their desensitization by factors acting on the serotonin- and histamine-sensitive structures of the receptors. To test this hypothesis the degree of terminal anesthesia caused by a number of substances known to be serotonin and histamine antagonists was investigated: promethazine, diphenhydramine, dihydroergotamine, and 1-benzyl-2,5-dimethylserotonin (BAS). With the exception of dimedrol, all these compounds exhibited a more powerful anesthetic activity than cocaine (see table). These results can be understood if it is assumed that, besides their direct action on the excitable membrane of the receptor, these compounds also depress the sensitivity of the receptor to mechanical stimulation through their action on its serotonin- and histamine-sensitive structures.

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