NATURE OF INCREASED SENSITIVITY OF SMOOTH MUSCLES TO ACETYLCHOLINE CAUSED BY CATECHOLAMINES AND SEROTONIN

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Preliminary administration of adrenalin, noradrenalin, isoprenaline, and serotonin increases the contraction of smooth-muscle strips from rat stomachs evoked by acetylcholine. The sensitizing effects of adrenalin and serotonin were abolished by sodium fluoride, monoiodoacetate, sodium cyanide, and 2,4-dinitrophenol, which in the concentrations used significantly reduced the absolute magnitude of the contractions of the smooth muscle also. Adrenalin and serotonin did not change the contractions of the smooth-muscle preparation produced by pilocarpine.

The results obtained indicate that the increase in sensitivity of the smooth muscles to acetylcholine is effected at the level of the intramural ganglia and is independent on intensification of metabolism or on cholinesterase activity in the muscle.

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In the presence of serotonin, the action of acetylcholine on the smooth muscles of mollusks is accompanied by the appearance of spike discharges which are not found under normal conditions [15, 22]. Serotonin also produces similar changes in the smooth muscles of the intestine, and preliminary treatment of these muscles by serotonin causes subsequent sensitization to histamine or acetylcholine [9, 10]. The nature of this phenomenon is uncertain.

The object of the present investigation was to study the sensitizing effect of catecholamines and serotonin on contractions of smooth muscles produced by acetylcholine and to investigate the mechanism of these effects.

EXPERIMENTAL METHOD

Experiments were carried out on 168 smooth-muscle strips taken from the fundal part of the rat stomach [23], which were kept in a bath for isolated organs containing Tyrode solution at 38°C with constant aeration. Contractions of the strips due to acetylcholine or pilocarpine were recorded under isotonic conditions. To study the relationship between the magnitude of the contraction and the concentration of the cholinomimetics, the method of cumulative curves was used [19]. The following concentrations of acetylcholine were used: $1 \cdot 10^{-8}$, $3 \cdot 10^{-8}$, $1 \cdot 10^{-7}$, $3 \cdot 10^{-7}$, $1 \cdot 10^{-6}$, $3 \cdot 10^{-6}$, $1 \cdot 10^{-5}$, $3 \cdot 10^{-5}$, and $1 \cdot 10^{-4}$. The magnitudes of the contractions obtained by the action of each acetylcholine concentration were expressed as percentages of the contraction observed by the action of acetylcholine in a concentration of $1 \cdot 10^{-4}$. Pilocarpine was used in concentrations from $1 \cdot 10^{-8}$ to $1 \cdot 10^{-5}$ (the last produced the maximal contraction). The effect of catecholamines ($1 \cdot 10^{-6}$) and serotonin ($2.2 \cdot 10^{-6}$) was investigated as follows: a strip placed in the bath of an ultrathermostat was kept in Tyrode solution for 10 min, after which the catecholamines or serotonin were added to the bath for 8 min. The amines were then washed out for 8 min, the bath was filled with fresh Tyrode solution, and 10 min later acetylcholine was added to it in the concentrations specified. The specific effects of the catecholamines and serotonin were analyzed by the use of dihydroergotamine ($1 \cdot 10^{-6}$), a specific antagonist of the α-effects of catecholamines [17] and the D-effects of serotonin [13], and of morphine ($1 \cdot 10^{-6}$), suppressing effects due to the action of serotonin on muscarine-sensitive serotonin receptors [13], and also of the inhibitors of cell metabolism: sodium fluoride (0.02 M), monoiodoacetate ($1 \cdot 10^{-4}$), sodium cyanide ($1 \cdot 10^{-4}$), or 2,4-dinitrophenol ($3 \cdot 10^{-4}$).

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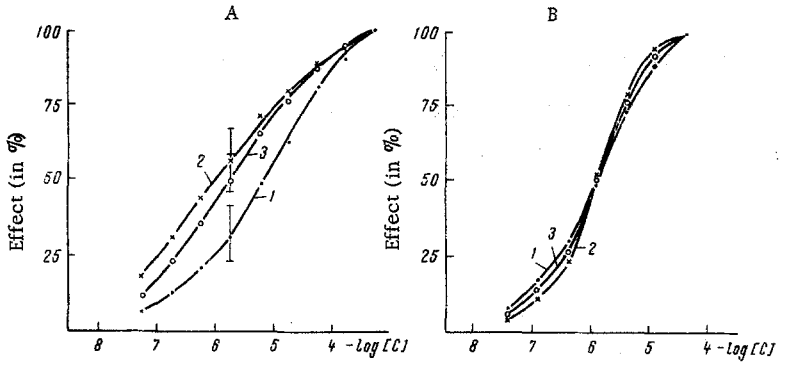

Fig. 1. Effect of adrenalin and serotonin on contractions of strips of rat stomach muscle produced by acetylcholine (A) and pilocarpine (B). 1) "Logarithm of concentration — effect" curve of acetylcholine or pilocarpine; 2) the same for strips preliminarily treated with adrenalin; 3) the same for strips preliminarily treated with serotonin.

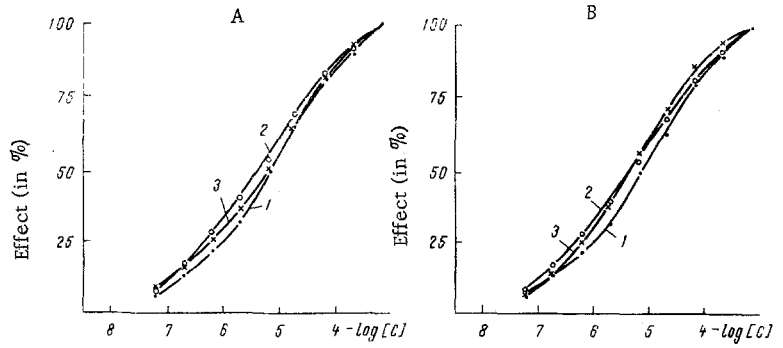

Fig. 2. Changes in acetylcholine-induced contractions of rat stomach strips under the influence of adrenalin (A) and serotonin (B) in the presence of 2,4-dinitrophenol. 1) "Logarithm of concentration — effect" curve for acetylcholine; 2) the same in the presence of 2,4-dinitrophenol; 3) the same on strips preliminarily treated with amines in the presence of 2,4-dinitrophenol.

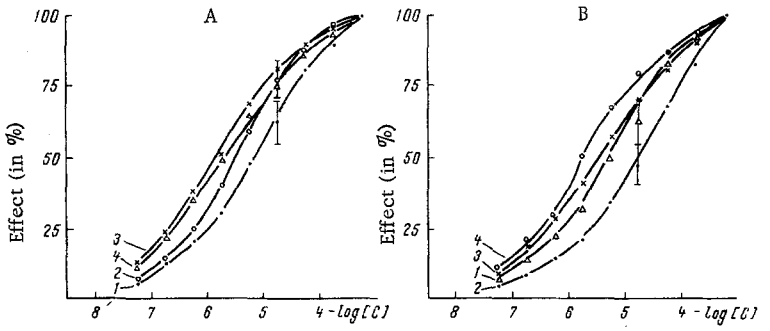

Fig. 3. Changes in acetylcholine-induced contractions of rat stomach strips under the influence of amines in the presence of morphine (A) and dihydroergotamine (B). 1) "Logarithm of concentration — effect" curve of acetylcholine; 2) the same in the presence of morphine or dihydroergotamine; 3) "logarithm of concentration — effect" curve of acetylcholine obtained on strips preliminarily treated with serotonin (A) or adrenalin in the presence of dihydroergotamine (B); 4) the same on strips preliminarily treated with serotonin in the presence of morphine or dihydroergotamine.

EXPERIMENTAL RESULTS AND DISCUSSION

Preliminary exposure of the smooth-muscle strip to adrenalin, noradrenalin, and isoprenaline in concentrations of $1 \cdot 10^{-6}$ or to serotonin creatinine-sulfate in an equimolar concentration ($2.2 \cdot 10^{-6}$) produced an increase in the contractions of the smooth-muscle strips produced by addition of acetylcholine to the bath in all experiments. The cumulative "logarithm of concentration — effect" curves for acetylcholine thus obtained were shifted to the left along the concentration scale, indicating an increase in the sensitivity of the smooth muscles to acetylcholine (Fig. 1A).

The sensitizing effect of catecholamines and serotonin on the smooth-muscle strips may be due either to changes in cholinesterase activity or to metabolic effects of the bioamines. The first suggestion must be ruled out, because monoamines increasing the sensitivity of smooth muscle to acetylcholine (Fig. 1A) differ in their effects on cholinesterase activity: serotonin increases it [21], while adrenalin decreases it [4, 11]. The second possibility is based on experiments showing that adrenalin and serotonin cause intensification of carbohydrate metabolism, accumulation of high-energy compounds, and an increase in the contractile power of muscles [8, 16, 20]. This conclusion was apparently confirmed by a series of experiments which showed that in the presence of inhibitors of glycolysis (sodium fluoride, monoiodoacetate) or of tissue respiration (sodium cyanide), or of agents dissociating coupled reactions of oxidative phosphorylation (2,4-dinitrophenol), i.e., of substances blocking enzymo-chemical reactions in the cell leading to ATP formation, no sensitization developed (Fig. 2). Nor is it contradicted by the fact that, in the concentrations used, the inhibitors significantly reduced the absolute magnitude of the acetylcholine-induced contractions of the smooth-muscle preparation. If the increase produced by bioamines in the acetylcholine-induced contractions of the strips was a metabolic muscular effect, adrenalin and serotonin must increase the sensitivity of the smooth-muscle preparation to all agents producing spasm. However, contractions of the smooth-muscle strip produced by pilocarpine were found to be virtually unchanged by adrenalin and serotonin (Fig. 1B). The fact that bioamines increased acetylcholine-induced but not pilocarpine-induced contractions of the smooth-muscle preparation from the stomach indicates that the sensitizing effect of the catecholamines and serotonin is independent of intensification of metabolism in the muscle, and is effected at the level of the intramural ganglia, the sensitivity of whose nicotine-sensitive cholinergic structures to acetylcholine is significantly increased by the action of bioamines. This conclusion does not conflict with the well-known fact that sensitivity of nicotine-like cholinergic structures of skeletal muscles [2, 3, 5], the superior cervical ganglion [6, 7, 12, 14], and the carotid body [1] to acetylcholine is increased by catecholamines and serotonin. Other workers [18] also have concluded that adrenergic inhibition of intestinal motor activity is an indirect effect on the muscle due to the direct action of sympathetic mediators on cholinergic structures of autonomic ganglia.

Pharmacological analysis of the sensitizing effect of adrenalin and serotonin on smooth-muscle strips by the use of bioamine antagonists did not permit identification of the specific structures of the intramural ganglia through which the ability of bioamines to increase contractions of smooth muscles is effected. The sensitizing activity of morphine itself and the cholinolytic effect of dihydroergotamine were largely instrumental in preventing this possibility (Fig. 3).

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