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Comparative study of the sensitivity of acetylcholinesterases and cholinesterases from animal and bacterial sources to inhibition by serotonin and its derivatives

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Summary. Serotonin was found to inhibit human erythrocyte and electric-eel acetylcholinesterase activities. The serotonin amino group, free of negative charges in its vicinity and its hydroxyl group, were important for the inhibition. Serotonin precursors and several related compounds had little or no effect. Human plasma cholinesterase was also inhibited by serotonin and tryptamine. In contrast to these animal enzymes, the cholinesterase of *Pseudomonas aeruginosa* was refractory to serotonin and its derivatives under the same experimental conditions.

Serotonin and acetylcholine (ACh) are closely related in their diverse important biological and pharmacological activities, such as neurohumoral transmission, constrictory and dilatory effects on muscle and blood vessels, etc.[1-3]. Both of them are of wide occurrence in nature, frequently present in the same tissues as they are in brain. The observation that serotonin inhibits the activity of brain and erythrocyte acetylcholinesterases[4] was therefore of interest.

In the present investigation, the effect of serotonin derivatives and related compounds on acetylcholinesterases (AChE) and cholinesterases (ChE) from different sources was compared. The aim of this comparison was to evaluate the contribution of the serotonin structural components to its effect and to determine the enzymes sensitivity to inhibition by it. The enzymes employed were: human erythrocyte[5] and electric-eel[6] acetylcholinesterases and human plasma and *Pseudomonas aeruginosa*[7,8] cholinesterases.

Materials and methods. Human erythrocytes were separated from heparinized venous blood from healthy donors, by centrifugation at 1000×g for 10 min (4 °C) and washed 3 times in 0.9% NaCl solution. 1 vol. of the thrice washed cells was suspended in 9 vol. of either 0.9% NaCl solution ('intact erythrocytes') or distilled water with sonication (80 sec in MSE 150 W ultrasonic disintegrator Mk2) and after centrifugation at 30,000×g for 10 min, the supernatant fluid was separated ('hemolysate'). Both the 'intact erythrocytes' suspension and the 'hemolysate' were diluted 1:10 before use for AChE determination.

Electric-eel *(Electrophorus electricus)* AChE purified preparation was purchased from Sigma and used at a concentration of 0.003 ng/ml for the enzyme activity determination. Human plasma was separated from heparinized venous

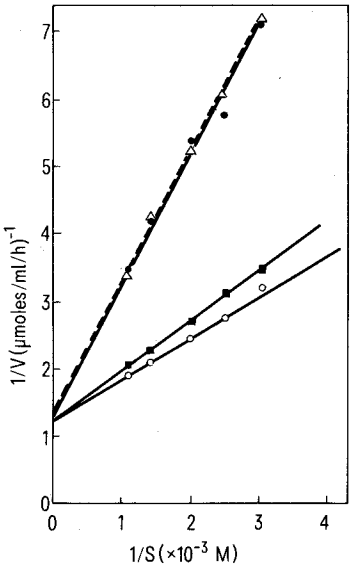

Fig. 1. Double reciprocal plot indicating the kinetics of the reaction of human erythrocyte AChE with varying concentrations of ATCh ○——○ and in the presence of serotonin-creatinine sulfate ●——●, serotonin oxalate △– – –△ and creatinine sulfate ■——■ employing intact erythrocytes or hemolysate and Ellman's reaction[9]. The plots represent mean values of 6 experimental results.

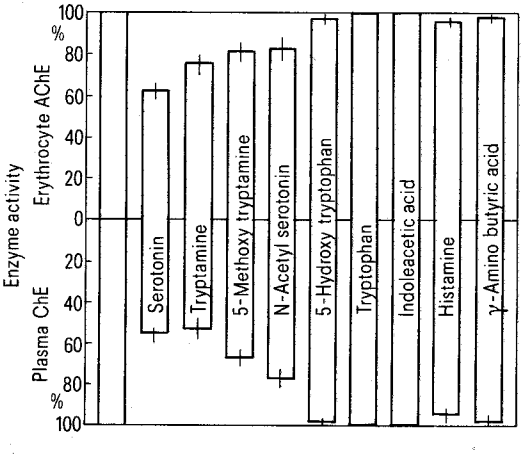

Fig. 2. Effect of serotonin, its derivatives and related compounds on the activities of human erythrocyte AChE and plasma ChE. Each compound was included at a final concentration of 2×10^{-3} M. Acetylcholine concentration was 7 μmoles/ml and 100% enzyme activity catalyzed decomposition of 3 μmoles of it during 30 min at 37 °C (determined by the hydroxamate reaction according to Hestrin[10] and confirmed by Ellman's reaction). The columns represent mean values of 6 experimental results. SEM are represented by vertical bars.

blood after the centrifugation as described above. It was diluted 1:3 in water before use for ChE determination. *Pseudomonas aeruginosa* preparation was produced as previously described[7].

AChE activity was measured in a final volume of 1 ml containing 100 µmoles of Tris-HCl at pH 7.5, 300 µmoles of NaCl, 50 µmoles of $MgCl_2$, 0.1 ml of the examined enzyme preparation (each of them prepared and diluted as described above), substrate (7 µmoles acetylcholine or 1 µmole acetylthiocholine) and distilled water. The compounds examined (serotonin and its derivatives and related compounds, purchased from Sigma) were included in 0.1 ml volume instead of water. Each of them was examined at a final concentration of 2×10^{-3} M. They were incubated with the enzyme in the reaction mixture for 15 min before the addition of the substrate. The substrate decomposition during 30 min was followed by the determination of thiocholine released from acetylthiocholine (ATCh) employing Ellman's method[9] and by the determination of residual ACh according to Hestrin[10].

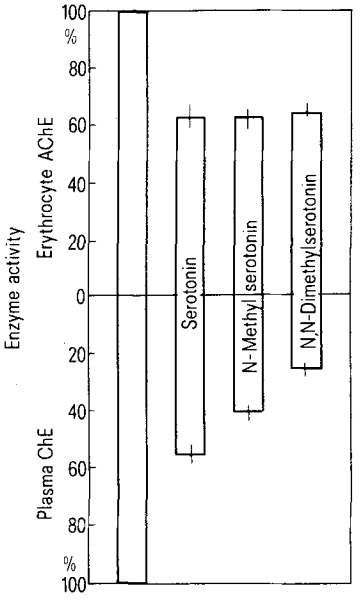

Fig. 3. Effect of serotonin and its N-methylated derivatives on the erythrocyte AChE and plasma ChE activities. The columns represent mean values of 5 experiments. The experimental conditions were as described for figure 2.

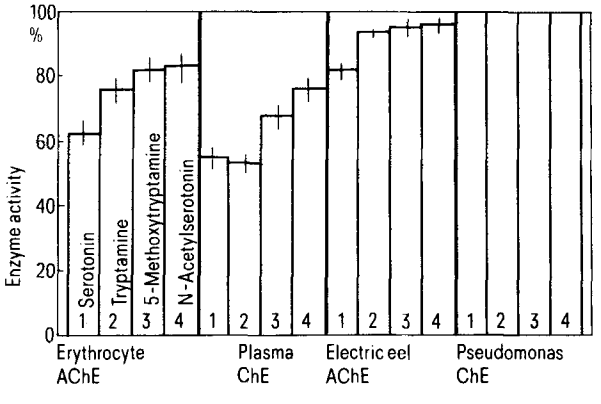

Fig. 4. Effect of serotonin (1), tryptamine (2), 5-methoxytryptamine (3) and N-acetylserotonin (4) on erythrocyte, plasma, electric-eel and *Pseudomonas aeruginosa* ACh decomposing activities. The columns represent mean values of 4–10 experimental results. The experimental conditions were as described for figure 2.

Results and discussion. Serotonin at a concentration of 2×10^{-3} M was found to inhibit reversibly AChE of human erythrocytes (figure 1). Similar results were obtained whether the enzyme was bound to the intact erythrocytes or detached from them. Serotonin in complex with creatinine-sulfate exerted the same inhibition as serotonin oxalate. In the presence of creatinine sulfate alone, a slight reversible inhibition of the enzyme was also observed. However, this creatinine sulfate effect was not additive to that of serotonin oxalate due to competition (figure 1).

Tryptamine, which lacks the serotonin hydroxyl group, also inhibited the erythrocyte AChE activity (figure 2) but not so strongly as serotonin, indicating that the hydroxyl group increases the binding of serotonin to the enzyme. No such contribution of the hydroxyl group was observed when the effect of tryptamine and serotonin on plasma ChE was examined. This enzyme was inhibited by the 2 compounds to almost the same extent (figure 2). Addition of methoxy group to tryptamine, instead of the serotonin hydroxyl, interferred with its inhibitory effect on both the erythrocyte and the plasma enzymes (figure 2). N-acetyl serotonin was even less inhibitory than the methoxy derivative and 5-hydroxytryptophan (carboxylated serotonin) affected them only very slightly (figure 2), indicating that the serotonin amino group must be free of negative charges near it. The erythrocyte and the plasma enzymes were both relatively insensitive to inhibition by histamine and γ-aminobutyric acid and resistant to tryptophan and indoleacetic acid (figure 2). N-methyl serotonin, in contrast to the N-acetyl derivative, was similar to serotonin in inhibition of the erythrocyte AChE and even considerably more effective in inhibition of the plasma ChE (figure 3). This trend was even more pronounced with N,N-dimethylserotonin which inhibited the erythrocyte enzyme to the same extent as serotonin but was significantly stronger than it as inhibitor of the plasma cholinesterase (figure 3). The stronger binding of the N-methylated serotonin to the plasma ChE, but not to the erythrocyte AChE, may be in agreement with the variation in hydrophobic areas of the 2 enzymes[11]. The electric-eel AChE, like the erythrocyte enzyme, was also inhibited more strongly by serotonin than by tryptamine (figure 4). Kitz and Ginsburg[12] described a similar phenomenon in the inhibition of the electric-eel AChE by a series of quaternary hydroxy-aminophenols. They found that the addition of OH group to these compounds increased their binding to free AChE.

The *Pseudomonas* cholinesterase differed from the animal enzymes in being refractory to serotonin and its derivatives (figure 4). Since bacterial production of acetylcholinesterase is rare[8], contrary to the wide occurrence of the animal enzyme in nature, there is no possibility to conclude as to the significance of this difference.

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