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EFFECT OF ACETYLCHOLINE ON MONOAMINE OXIDASE

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Abstract—The addition of acetylcholine to rat brain monoamine oxidase *in vitro* resulted in its inhibition. This inhibition was found to be of the competitive type. Constants characterizing this inhibition, namely the binding constant K_I (5.8×10^{-2} M), and bimolecular rate constant k_a (27 M^{-1} min^{-1}) were determined.

Acetylcholine (ACh) and 5-hydroxytryptamine (5-HT; serotonin) are considered to be chemical neurohumoral transmitters (beside other functions) and are widely distributed in tissues. They are acted upon by two different enzyme systems, cholinesterase (ChE) and monoamine oxidase (MAO). On the basis of structural relationship between 5-HT and eserine (a well known inhibitor of cholinesterase) we suggested and investigated the inhibition of brain cholinesterase (ChE) by 5-HT. This inhibition was found to be of the competitive type [1]. This finding prompted us to study the reverse phenomenon, i.e. the inhibitory effect of ACh on brain MAO. Although various substituted aryl ethers of choline [2] were previously reported to possess inhibitory effect on liver MAO, yet no attempt was undertaken to evaluate the possible inhibitory effect of ACh. Our preliminary experiments showed that ACh inhibited the deamination of 5-HT by brain MAO. Accordingly, kinetic studies were conducted to determine the binding constant, the bimolecular rate constant and the type of inhibition.

MATERIALS AND METHODS

Chemicals were purchased as follows: 5-hydroxytryptamine creatinine sulphate (contains 43.5% of 5-HT) May and Baker Ltd., Dagenham, England; Antemovis ampoules (each contains 5 mg of 5-HT, creatinine double sulphate) Vister, Casatenovo, Italy; acetylcholine (ampoules each contain 200 mg S.A.F.) Hoffmann La Roche & Co. Ltd. Basle, Switzerland.

Brain MAO was prepared according to the method described by Roth and Gillis [3]. Albino rat brains (30 g), were homogenized in 2 vol of potassium phosphate buffer (w/v; pH 7.4, 0.1 M) containing sucrose (0.25 M) in a Waring blender (5 sec, two times) and then in a motor driven Teflon–glass homogenizer. The resulting homogenate was centrifuged twice at 600 *g* for 10 min to remove cellular debris. The supernatant solution from the second centrifugation was centrifuged at 10,000 *g* for 20 min and the resulting mitochondrial precipitate was resuspended by homogenization in the phosphate buffer previously described. This suspension was again centrifuged at 10,000 *g* for 20 min and the final mitochondrial precipitate was resuspended by homogenization in potassium phosphate buffer (15 ml; pH 7.4; 0.1 M). The protein content was determined by the method of Lowry [4]. The enzyme activity was assayed chemically by the method of Udenfreind *et al.* [5], using 5-HT as substrate.

In this method the assay mixture contained 5-HT, 0.124 μmole/ml; Na_2HPO_4; NaH_2PO_4, pH 7.4, 375 μmole/ml, and an amount of enzyme equivalent to 150 mg brain tissue.

The progress curve was evaluated from the data obtained by estimating the residual substrate after incubation for 2, 4, 6, 8 and 10 min at 37°.

For the determination of the binding (K_I) and bimolecular rate (k_a) constants, AChCl in different concentrations (0.68, 1.36, 2.72 and 5.45×10^{-2} M) was added to the above assay mixture and incubated at 37°. After suitable time intervals, 1, 2, 4, 6 and 8 min, samples (1 ml) were withdrawn and the remaining 5-HT assayed colorimetrically [5].

For the determination of the type of inhibition and the enzyme-inhibitor dissociation constant (K_i), the substrate concentration was varied (0.62, 0.94, 1.24, 1.85 and 2.47×10^{-4} M). The inhibitor (AChCl) was kept at constant concentration for each experiment (2.18×10^{-2} or 3.27×10^{-2} or 4.36×10^{-2} or 5.45×10^{-2} M), then the mixture was incubated for 5 min at 37°.

The enzyme (0.15 ml) with the inhibitor (10.9×10^{-2} M) was dialysed overnight against phosphate buffer at 4° with occasional change of buffer. Controls of enzyme without inhibitor dialyzed and undialysed were also taken.

RESULTS AND DISCUSSION

Figure 1 shows the rate of deamination of 5-HT by an amount of brain MAO (0.1 ml) preparation previously found to deaminate not more than 20 per cent of substrate. Under these conditions, the rate of the reaction was satisfactorily linear up to 8 min. Accordingly, the inhibition of deamination of 5-HT by AChCl was studied during this time interval. AChCl at a low concentration (0.68×10^{-2} M) produced 10 per cent inhibition of brain MAO activity after 4 min incubation. By increasing AChCl concentration up to 5.45×10^{-2} M the inhibition increased and reached 50 per cent.

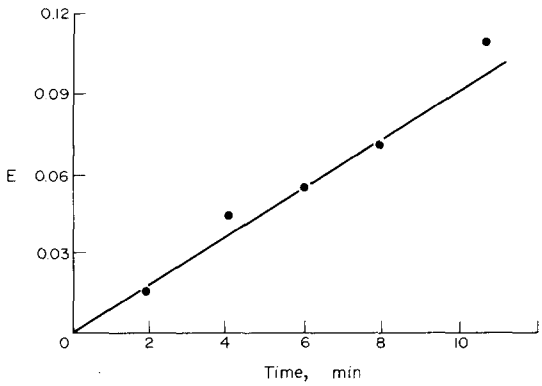

Fig. 1. Progress curve of deamination of 5-hydroxy tryptamine (5-HT, serotonin) by rat brain monoamine oxidase. Incubation was carried out at 37°.

The inhibitory effect of AChCl on MAO could be abolished by dialysis. The dialyzed enzyme recovered its original strength and hydrolyzed nearly the same amount of substrate as that of uninhibited controls (Table 1).

Graphical representation of the inhibition curves (AChCl concentrations 0.68, 1.36, 2.72, 5.45×10^{-2} M) obtained by plotting log v (where v is the velocity of hydrolysis of 5-HT by oxidase remaining uninhibited) against time (t) a straight line was obtained (Fig. 2). This indicated that the inhibition of MAO by AChCl follows first-order reaction kinetics [6]. The rate constant characterising this inhibition was calculated using the following equation [7–9].

$$k_a = \frac{2.303}{\Delta t[I]} \log \frac{v_0}{v_0 - v_i}$$

where k_a is the bimolecular rate constant ($\text{min}^{-1}\,\text{M}^{-1}$), v_0 the velocity of the uninhibited reaction, v_i the velocity of the inhibited reaction, t the time (in min) of exposure of the enzyme to the inhibitor and $[I]$ the molar concentration of the inhibitor. From Fig. 2, the slopes were computed and gave the values $2.303\,\Delta \log v/\Delta t$ as shown in Table 2. These

Table 1. The effect of dialysis on the enzyme activity before and after the addition of AChCl

Enzyme	Inhibitor (10.9×10^{-2} M)	Per cent 5-HT hydrolyzed
Without dialysis	Absent	15.8
After dialysis	Absent	15.4
Without dialysis	Present	7.7
After dialysis	Removed by dialysis	14.8

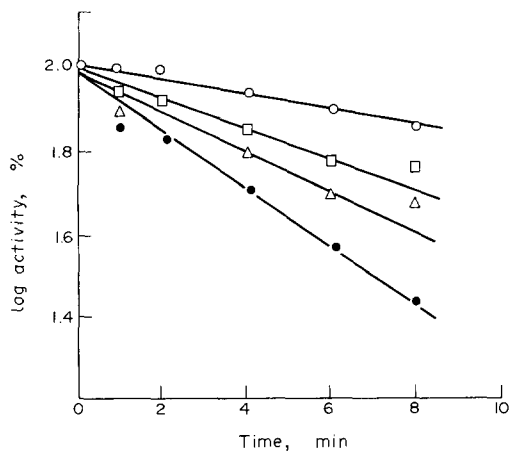

Fig. 2. The time course of inhibition of brain monoamine oxidase (MAO) by acetylcholine chloride (AChCl) *in vitro* at constant substrate concentration 5-HT, 1.24×10^{-4} M). Concentrations of AChCl $[I] \times 10^{-2}$ M; 0.68 ○; 1.36 □; 2.72 △ and 5.45 ●.

Table 2. Inhibition of brain MAO by AChCl. Calculation of binding constant (K_I) and the rate of inhibition (k_i) from measurements of ($2.303\,\Delta \log v/\Delta t$) at different concentrations of AChCl[I]: k_a is the bimolecular rate constant of inhibition of MAO by AChCl

$[I]$ (10^{-2} M)	$2.303\,\Delta \log v/\Delta t$ (10^{-2} min)
0.68	4.00
1.36	4.73
2.72	5.90
5.45	7.10

$K_I = 5.8 \times 10^{-2}$ M
$k_i = 1.55\ \text{min}^{-1}$
$k_a = 27\ \text{M}^{-1}\ \text{min}^{-1}$

Table 3. The apparent dissociation constant (K_p) and the enzyme-inhibitor dissociation constant (K_i) for brain MAO in the presence of AChCl: K_m is the Michaelis constant

Constant	Value
K_m	2.65×10^{-4} M
K_p	0.66×10^{-3} M
*K_p/K_m	2.5
†K_i	$3.7 \times 10^{-2} \pm 0.3$ M

* These values calculated at an inhibitor concentration of 5.45×10^{-2}M.

† The mean value of K_i at different inhibitor concentrations: 2.18, 3.27, 4.36 and 5.45×10^{-2} M.

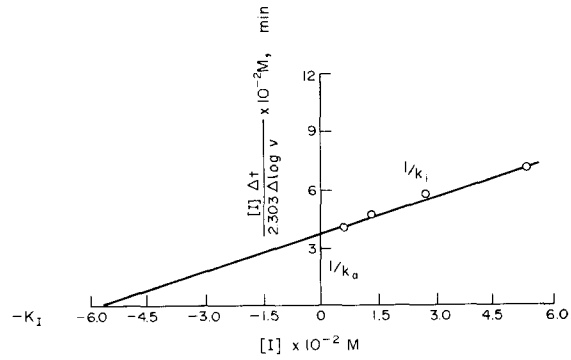

Fig. 3. Main plot of the data of the inhibition of rat brain monoamine oxidase by acetylcholine chloride. The slope of the straight line gave $1/k_i$ (where k_i is the rate of inhibition); the intercept on $[I]$-axis gave K_I (K_I, binding constant) and the intercept on the ordinate gave $1/k_a$ (k_a, bimolecular rate constant.

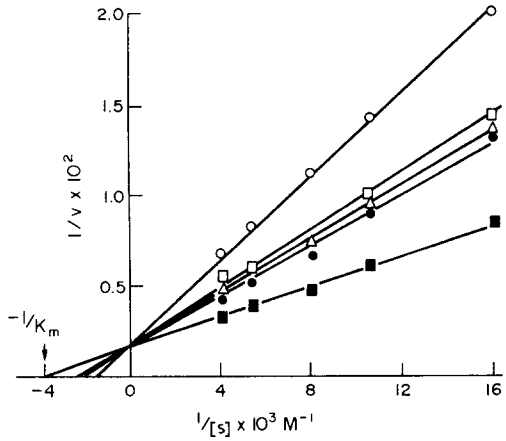

Fig. 4. Lineweaver–Burk plot of the inhibitory effect of acetylcholine chloride on the rate of deamination of 5-hydroxytryptamine (concentrations; 0.62, 0.94, 1.24, 1.85 and 2.47×10^{-4} M). Concentrations of acetylcholine chloride; 2.18 ■; 3.27 △; 4.36 □ and 5.45×10^{-2} ○ M.

values were used to construct the graph of $[I]$ $\Delta t/2.303\ \Delta \log v$ plotted against $[I]$. The slope of the straight line obtained in Fig. 3 gave $1/k_i$ (k_i being the rate of inhibition), the intercept on the $[I]$-axis gave K_I (K_I being the binding constant), and the intercept on the ordinate gave $1k_a$ ($k_a = k_i/K_I$). The values of K_I, k_i and k_a are given in Table 2.

Regarding the type of inhibition, Fig. 4 shows that the double reciprocal curves of $1/v$ plotted against $1/[s]$, keeping the inhibitor at constant concentration in each experiment and changing the substrate concentration, were in accordance with those mentioned by Dixon [10] for the competitive type of inhibition. The values of the enzyme-inhibitor-dissociation constant (K_i) and Michaelis constant (K_m) are given in Table 3.

The results indicated that monoamine oxidase and choline esterase are competitively inhibited by each other substrate, namely 5-hydroxytryptamine and acetylcholine, (cross inhibition) and, in both cases, the inhibition followed first order kinetics.

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