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INHIBITION OF RAT BRAIN AND HEART MONOAMINE OXIDASE BY ATROPINE

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Abstract—Atropine sulphate was found to inhibit rat brain and heart monoamine oxidase *in vitro*. It was found to act as competitive inhibitor, the enzyme-inhibitor dissociation constant (K_i) was found to be 7.36×10^{-3} M and 5.5×10^{-3} M for brain and heart monoamine oxidase, respectively. In addition, the kinetics of inhibition of rat brain and heart monoamine oxidase by atropine sulphate was also investigated.

Atropine is the drug of choice in the treatment of organophosphate poisoning. It can antagonise the increase of cerebellar choline induced by oxotremorine or physostigmine [1] in the mouse. In rat brain it prevents the decrease in the level of iron and total flavines induced by physostigmine or amiton [2].

In continuation to our studies on rat brain cholinesterase and rat brain monoamine oxidase inhibitors [3–5], the inhibitory effect of atropine on rat brain and heart monoamine oxidase is evaluated.

MATERIALS AND METHODS

Materials. Serotonin creatinine sulphate (contains 43.5% serotonin) was obtained from May and Baker, Dagenham, England; Antemovis ampoules (each contains 5 mg of serotonin creatinine double sulphate) were obtained from Vister, Casatenovo, Italy. Atropine sulphate was obtained from E. Merck, Darmstadt.

Rat brain or heart monoamine oxidase were prepared according to the method described by Roth and Gillis [6]. Albino rat brains (30 g) or heart (50 g) were removed and placed immediately into ice-cold 250 mM sucrose, blotted dry, weighed and then each homogenized in 2 vol. of potassium phosphate buffer (w/v; pH 7.4, 0.1 M) containing sucrose (250 mM) in a Waring blender (5 sec, two times) and then in a motor driven Teflon-glass homogenizer. The resulting homogenates were centrifuged twice at 600 *g* for 10 min to remove cellular debris. The supernatant solutions from the second centrifugation were centrifuged at 10,000 *g* for 20 min and the resulting mitochondrial precipitates were resuspended by homogenization in the phosphate buffer previously described. These suspensions were again centrifuged at 10,000 *g* for 20 min and the final mitochondrial precipitates were resuspended by homogenization in potassium phosphate buffer (pH 7.4, 0.1 M) to give protein concentration of approximately 20 mg/ml. The protein content was determined by the method of Lowry [7]. These suspensions were frozen in aliquots and thawed immediately before use.

Determination of enzyme activity. Brain or heart monoamine oxidase activities were determined by the method described by Udenfriend *et al.* [8]. The protein concentrations of the enzyme suspensions were adjusted to approximately 1.5 mg/ml. In this method, the assay mixture contained serotonin as substrate, 0.124 μmole/ml; Na_2HPO_4: NaH_2PO_4, pH 7.4, 375 μmole/ml; and 0.3 ml of brain or 0.2 ml of heart monoamine oxidase preparation. Incubations were performed in a shaking water bath at 37° for 5 min. The rate of deamination of serotonin by brain monoamine oxidase was determined in absence of inhibitor and in presence of 7 mM of atropine sulphate at different intervals: 0, 3, 5, 8 and 10 min. Heart monoamine oxidase was also investigated during these time intervals.

Substrate-enzyme inhibitor response. The effect of different concentrations of atropine sulphate on brain or heart monoamine oxidase was carried out by preincubating the enzymes with different concentrations of the inhibitor for 5 min after which the substrate was added and the residual activity of the enzyme assayed after further incubation for 5 min. With brain monoamine oxidase, atropine sulphate concentrations were: 0.35, 1.1, 1.8, 3.5, 7.1, 17.8 and 35.5 mM, while in case of heart monoamine oxidase, the inhibitor concentrations were: 0.7, 1.8, 3.5, 7.1, 17.8 and 35.5 mM.

The kinetics of inactivation of brain or heart monoamine oxidase by atropine sulphate. This was carried out by preincubation of the enzyme with the inhibitor for 5, 10, 15 and 20 min in case of brain monoamine oxidase and for 5, 8, 13 and 16 min in case of heart monoamine oxidase. After the specified time intervals the substrate was added and the reaction mixture was further incubated for 5 min then the enzyme activity assayed as described above. Atropine sulphate was kept at constant concentration for each experiment (3.5 or 7.0 or 14.0 or 28.0 mM).

For the determination of the type of inhibition and the enzyme-inhibitor dissociation constants (K_i); the enzymes were instantaneously inhibited by atropine sulphate in the presence of the substrate; in this case the substrate concentration was varied (0.62, 0.94, 1.24, 1.85 and 2.47×10^{-4} M). The inhibitor was kept at constant concentration for each experiment (7 or 14 or 28 mM), then the mixture was incubated for 5 min at 37°.

Dialysis. The enzyme (0.3 ml of brain or 0.2 ml of heart monoamine oxidase preparations) with the inhibitor (28 mM) were dialysed overnight against phos-

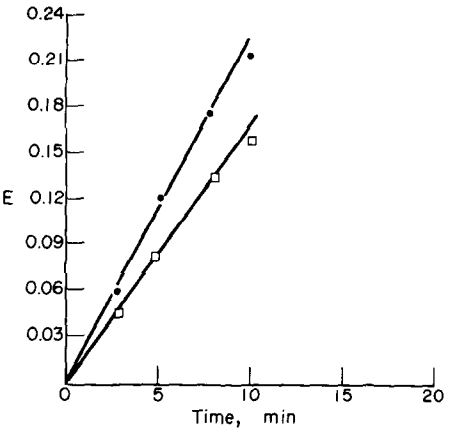

Fig. 1.

phate buffer at 4° with occasional change of buffer. Controls of enzyme without inhibitor dialysed and undialysed were also taken.

RESULTS AND DISCUSSION

In all experiments carried out in this work the amount of brain or heart monoamine oxidase preparations used were adjusted to deaminate not more than 20% of the substrate. Under these conditions, the rate of the reaction was satisfactorily linear during the first 8 min in absence or presence of atropine sulphate (Fig. 1).

Behaviour of brain and heart monoamine oxidase under the influence of different concentrations of atropine sulphate. Figure 2 shows the effect of preincubating increasing concentrations of atropine sulphate with brain or heart monoamine oxidase prior to assaying their activities towards serotonin as substrate. It can be seen that inhibition of serotonin deamination in case of heart monoamine oxidase shows the biphasic response ($pI_{50} = 1.6$) which is in agreement with the results previously described by Mantle *et al.* [9] in their studies on the inhibition of tryptamine deamination by rat liver monoamine oxidase using 5-phenyl-3-(cyclopropyl)ethyl-amine 1, 2, 4-oxadiazole (PCO) as inhibitor. In case of the inhibition of serotonin deamination by brain monoamine oxidase using atropine sulphate as inhibitor, the inhibition followed a classical sigmoidal dose–response curve ($pI_{50} = 1.65$) which is similar to that described by Mantle *et al.* [9] for the inhibition of serotonin deamination by rat liver monoamine oxidase using PCO as inhibitor.

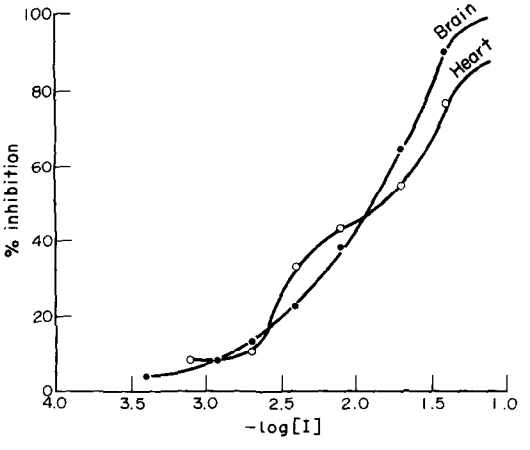

Fig. 2.

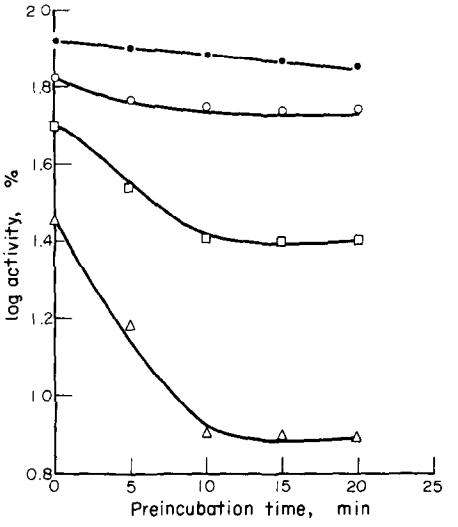

Fig. 3.

Kinetics of inhibition of rat brain and heart monoamine oxidase by atropine sulphate. Varying the period of preincubation of the enzymes with atropine sulphate showed that in all cases, the inhibition was time dependent in the first part of the curves. Figures 3 and 4 show the time course of the inhibition of brain and heart monoamine oxidase by increasing concentrations of atropine sulphate. By increasing the preincubation time up to 10 min in case of brain monoamine oxidase or up to 8 min in the case of heart monoamine oxidase, inhibition of the enzymes increased progressively. On further increase in the preincubation time no more inhibition could be observed. In the case of both brain or heart monoamine oxidase the curves were nearly similar in outline and also in accordance with those given by

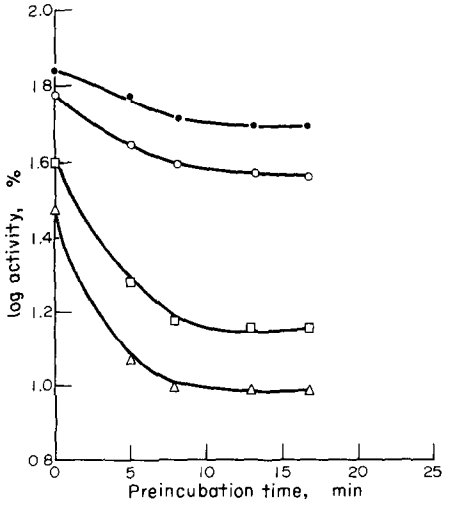

Fig. 4.

Table 1. First order and bimolecular rate constants for the inactivation of rat brain or heart monoamine oxidase by atropine sulphate

Atropine sulphate concn. (mM)	Rate constant 10^3 k_i (min^{-1})	Bimolecular rate constant k_a (M^{-1} min^{-1})	Rate constant 10^3 k_i (min^{-1})	Bimolecular rate constant k_a (M^{-1} min^{-1})
3.5	10.3 ± 1.5	2.9 ± 0.42	27.6 ± 0.0	7.9 ± 0.0
7.0	23.0 ± 0.0	3.3 ± 0.00	55.3 ± 0.0	7.9 ± 0.0
14.0	56.1 ± 2.7	4.0 ± 0.20	123.2 ± 3.2	8.8 ± 0.2
28.0	120.4 ± 9.5	4.3 ± 0.35	234.7 ± 6.7	8.4 ± 0.3

First order rate constants (k_i) were calculated from the first part of the curves (Figs. 3 and 4), where the slope is: $-k_i/2.303$. The bimolecular rate constant (k_a) was calculated from the relationship: $k_i = k_a$ [I] [9].

Mantle *et al.* [9] in their study on liver monoamine oxidase inhibition by PCO.

In case of heart monoamine oxidase, in presence of a concentration of 3.5 mM of atropine sulphate, serotonin deamination showed 30 per cent inhibition at zero time preincubation. By increasing the concentration of atropine sulphate to 7, 14 and 28 mM and also at zero time preincubation, the percent inhibition was increased and gave 40, 60 and 70, respectively. A similar behaviour of brain monoamine oxidase was also obtained. At zero time preincubation, increasing concentrations of atropine sulphate, 3.5, 7.0, 14.0 and 28.0 mM, serotonin deamination was inhibited by 16, 33, 50 and 71 per cent, respectively (Fig. 4). At low inhibitor concentrations, 3.5 and 7.0 mM, heart monoamine oxidase showed higher degree of inhibition than brain monoamine oxidase, at higher concentrations both enzymes were similarly inhibited.

The kinetic behaviour of atropine sulphate towards brain or heart monoamine oxidase could be explained on the basis of "fast" and "slow" reaction centres on the enzyme surface [9]. The inhibition values obtained at zero time preincubation represent the fast inhibition while the inhibition values obtained during the first part of the curves (Figs. 3 and 4) represent the slow inhibition. The rate constant and bimolecular rate constant (Table 1) for the inactivation of rat brain and heart monoamine oxidase by atropine sulphate were calculated from the first parts of the curves, Figs. 3 and 4. It seems that the slight variations (Table 1) in the apparent bimolecular rate constants (k_a) caused by using different concentrations of atropine on brain or heart monoamine oxidase is insignificant within each system. However, there is significant difference between those values of apparent bimolecular rate constants (k_a) obtained for brain or heart monoamine oxidase, under the influence of the same concentration of atropine sulphate.

With respect to the type of inhibition, Figs. 5 and 6 showed that the double reciprocal curves of 1/v plotted against 1/[s], keeping the inhibitor (atropine sulphate) at constant concentration for each experiment (7, 14 and 28 mM) and changing the substrate concentrations (serotonin) in case of both brain or heart monoamine oxidase, were in accordance with those mentioned by Dixon [10] for the competitive type of

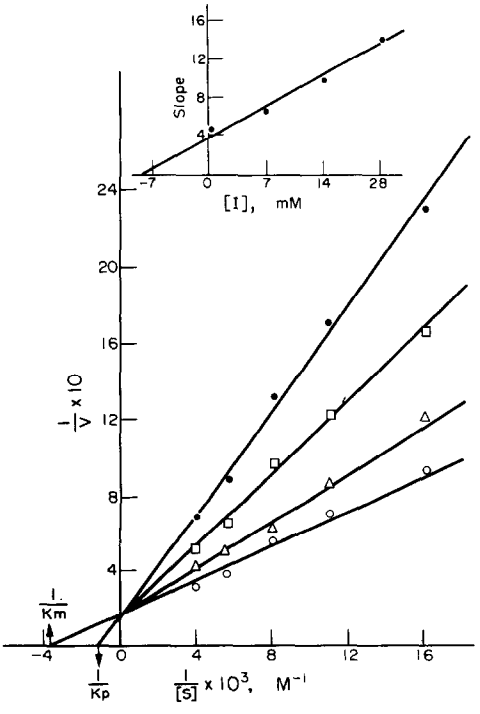

Fig. 5.

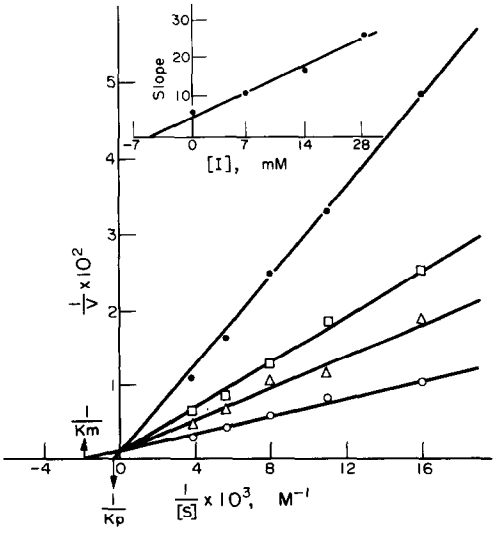

Fig. 6.

Table 2. The enzyme inhibitor dissociation constant (K_i) for brain and heart monoamine oxidase in the presence of atropine sulphate. K_m is Michaelis constant

Constant	Brain	Heart
*K_i (calculated)	$7.36 \times 10^{-3} \pm 0.9$ M	$5.4 \times 10^{-3} \pm 0.8$ M
K_i (slope replot)	7.70×10^{-3} M	5.2×10^{-3} M
K_m	2.65×10^{-4} M	6.25×10^{-4} M

* The mean value of K_i at different inhibitor concentrations: 7, 14 and 28×10^{-3} M of atropine sulphate.

inhibition. The slope replots (inset Figs. 5 and 6) showing that atropine sulphate was a linear competitive inhibitor (after the Cleland nomenclature, Mantle *et al.* [9]) of brain and heart monoamine oxidase with a K_i of 7.7 and 5.2 mM, respectively. The calculated values of K_i (Table 2) (the enzyme-inhibitor dissociation constant) and those found from the slope replots are in satisfactory agreement with each other. Michaelis constant (K_m) for both brain and heart monoamine oxidase are also given in Table 2.

The inhibitory effect of atropine sulphate on brain or heart monoamine oxidase could be abolished by dialysis. The dialysed enzymes recovered their original strength and hydrolyzed nearly the same amount of substrate as that of uninhibited controls. These results suggest that the action of atropine sulphate as inhibitor on brain or heart monoamine oxidase is reversible.

REFERENCES

1. J. Bajgar, A. Jakl and V. Hrdina, *Biochem. Pharmac.* **20,** 3230 (1971).
2. B. B. Mrsulja and L. M. Rakic, *Biochem. Pharmac.* **21,** 1209 (1972).
3. Y. S. Mohammed, M. Y. Osman and Y. Gabr, *Arzneimittel-Forsch.* **24,** 121 (1974).
4. Y. S. Mohammed, M. Y. Osman and Y. Gabr, *Arzneimittel-Forsch.* **25,** 1714 (1975).
5. Y. S. Mohammed, M. Y. Osman and M. M. Mahfouz, *Biochem. Pharmac.* **25,** 1665 (1976).
6. J. Roth and C. Gillis, *Biochem. Pharmac.* **23,** 2537 (1974).
7. O. H. Rosebrough, H. Lowry, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193,** 265 (1951).
8. S. Udenfriend, H. Weissbach and B. B. Brodie, in *Methods of Biochemical Analysis*, vol. VI. (Ed. David Glick) p. 96. Interscience Publishers, New York, London (1958).
9. T. J. Mantle, K. Wilson and F. Long Robert, *Biochem. Pharmac.* **24,** 2031 (1975).
10. M. Dixon, *Biochem. J.* **55,** 170 (1953).