LITERATURE CITED

1. S. M. Bychkov and S. A. Kuz'mina, Byull. Éksp. Biol. Med., No. 6, 40 (1973).
2. S. M. Bychkov, Usp. Sovrem. Biol., 65, 323 (1968).
3. S. M. Bychkov and M. F. Kolesnikova, Biokhimiya, No. 1, 204 (1969).
4. S. M. Bychkov and V. N. Kharlamova, Biokhimiya, No. 4, 840 (1968).
5. S. M. Bychkov and V. N. Kharlamova, Byull. Éksp. Biol. Med., No. 2, 61 (1975).
6. S. M. Bychkov and V. N. Kharlamova, Byull. Éksp. Biol. Med., No. 3, 289 (1976).
7. S. M. Bychkov, Usp. Sovrem. Biol., 49, No. 1, 3 (1960).
8. S. M. Bychkov, Byull. Éksp. Biol. Med., No. 1, 7 (1951).
9. S. M. Bychkov and V. N. Kharlamova, Vopr. Med. Khim., No. 3, 269 (1972).
10. J. Trinkaus, Cells into Organs: The Forces that Shape the Embryo, Prentice-Hall, New Jersey (1968).
11. N. V. Tsvetkova, M. A. Litkind, L. Ya. Zakastel'skaya, et al., Biokhimiya, No. 5, 994 (1976).
12. C. Grobstein, Exp. Cell Res., 8, Suppl., 234 (1961).
13. S. Ito, Fed. Proc., 36, 12 (1969).
14. R. B. Kemp, Nature, 218, 1255 (1968).
15. B. A. Pethica, Exp. Cell Res., 8, Suppl., 234 (1961).

SOME KINETIC FEATURES OF MEMBRANE-BOUND MONOAMINE OXIDASE

L. G. Vasil'evykh, V. Z. Gorkin, and Z. S. Kagan

UDC 577.150.2:577.158

Initial reaction-velocity versus substrate-concentration curves for serotonin oxidation catalyzed by monoamine oxidase (MAO) from fragments of rat liver or bovine brain mitochondrial membranes have a complex, nonhyperbolic shape; this is regarded as a kinetic manifestation of substrate cooperativeness for membrane-bound MAO. The possibility of interaction between different types of MAO based on conformational changes in the membrane itself is discussed.

KEY WORDS: *monoamine oxidase; biological membranes; substrate cooperativeness; serotonin.*

In the modern view [11] membrane-bound monoamine oxidases (MAO) of type A (selectively blocked by chlorgyline) specifically oxidize serotonin; however, serotonin binds with the active sites of type B MAO which are selectively blocked by Deprenil [8] and which specifically oxidize β-phenylethylamine [5].

In this investigation the effect of substrate concentration [S] on the initial reaction velocity (v) of serotonin oxidation catalyzed by MAO of fragments of mitochondrial membranes was studied because information on the kinetics of this reaction is contradictory: The curves of v as a function of [S] were either strictly hyperbolic in shape [7, 10] or of a complex, biphasic character [14].

EXPERIMENTAL METHOD

Mitochondrial fractions were obtained from rat liver or bovine brain by differential centrifugation [13], followed by freezing and thawing and sedimentation of the fragments of the mitochondrial membranes at 40,000g (1 h). The suspension of the residue in 0.01 M phosphate

Scientific-Research Institute for Biological Trials of Chemical Compounds, Ministry of the Medical Industry of the USSR. Institute of Biological Medical Chemistry, Academy of Medical Sciences of the USSR. All-Union Vitamin Scientific-Research Institute, Ministry of the Medical Industry of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Orekhovich.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 3, pp. 288-289, March, 1977. Original article submitted July 5, 1976.

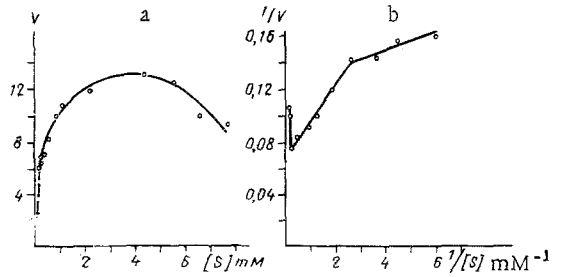

Fig. 1

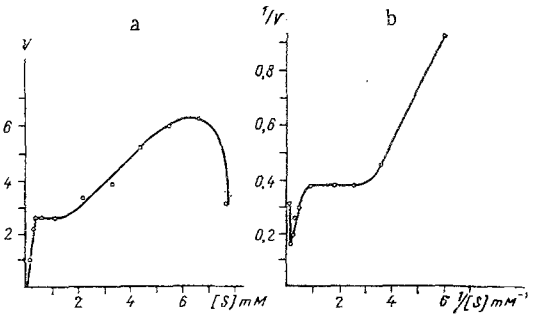

Fig. 2

Fig. 1. Initial reaction velocity (v) of deamination (μmole ammonia liberated/mg protein/min) of serotonin by fragments of rat liver mitochondrial membranes as a function of substrate concentrations [S]. Here and in Fig. 2: a) Michaelis–Menten plot; b) Lineweaver–Burke plot.

Fig. 2. Initial reaction velocity (v) of deamination (μmole ammonia liberated/mg protein/min) of serotonin by fragments of bovine brain mitochondrial membranes as a function of substrate concentrations [S].

buffer, pH 7.4 (20 mg protein/ml) was kept at −20°C. The oxidation of serotonin creatinine-sulfate (Reanal, Hungary) was judged from the amount of ammonia liberated in an atmosphere of O_2 at 37°C in 0.2 M phosphate buffer, pH 7.4, over a period of 8 min. Accumulation of the reaction products took place as a linear function of time, at least for 12 min, with all concentrations of serotonin used. The conditions of fixation of the samples and determination of ammonia with the aid of Nessler's reagent were described previously [1]. Isothermic distillation of ammonia was carried out in the vessels used for keeping penicillin [4].

EXPERIMENTAL RESULTS AND DISCUSSION

During oxidation of serotonin by fragments of mitochondrial membranes of liver or brain, substrate inhibition was observed (Figs. 1a, 2a). The ascending limbs of the corresponding kinetic curves were not hyperbolic, as was particularly clear between double-reciprocal coordinates (1b and 2b); in the experiments with brain mitochondria the curve of v versus [S] had an intermediate plateau at low concentrations of serotonin (Fig. 2a). These kinetic curves are evidence of the cooperativeness during substrate binding that is characteristic of allosteric enzymes.

It is considered that these results can be explained on the basis of the following four models.

First Model. MAO is an oligomer consisting of several identical monomers (polypeptide chains), each of which has two active sites A and B (for a discussion of "two-headed" enzymes see the survey [2]). On binding with site A serotonin is oxidized, whereas on interaction with site B it behaves as an allosteric effector (negative heterotropic cooperativeness, substrate inhibition) unable to induce reversible association–dissociation of the membrane-bound enzyme, in which cooperative interactions between the active sites take place only in accordance with Koshland's scheme [9].

Second Model. MAO is an oligomer of the A_nB_n type in which each of the monomers contains one active site. The B monomers, which bind with the substrate but do not oxidize it, are regulatory subunits for the A monomers, on interaction with which serotonin is specifically oxidized.

Third Model. Spatially separate MAO oligomers of types A and B are present in biological membranes. Serotonin, on binding with type B MAO, is not oxidized [10], but induces a change in the conformation of the adjacent membrane [3], and later a change in the conformation of the A oligomer, in accordance with Koshland's scheme [9]. In fact, as would be expected from this model, the properties of the membrane-bound and solubilized MAO are different [12].

Fourth Model. MAOs of types A and B are monomers separated spatially in the biomembrane. Binding of the ligand (serotonin) with the polypeptide chain carrying a type B active site leads to a change in the conformation of the A monomer, an effect brought about through reconstruction of the membrane protein–lipid complex [3].

The third model comes the closest to explaining the kinetic properties of MAO and, in particular, the presence of the intermediate plateau on the v versus [S] plots (Fig. 2) and also the observed possibility of separation of mitochondrial MAOs under mild conditions without the use of detergents [6].

LITERATURE CITED

1. V. Z. Gorkin et al., in: Modern Methods in Biochemistry [in Russian], Vol. 2, Moscow (1968), p. 155.
2. Z. S. Kagan, in: Allosteric Enzymes (Progress in Science and Technology, Vol. 8) [in Russian], Moscow (1975), p. 164.
3. S. V. Konev, S. L. Aksentsev, and E. A. Chernitskii, Intracellular Cooperative Transitions of Proteins [in Russian], Minsk (1970), p. 33.
4. N. P. L'vov, in: Modern Methods in Biochemistry [in Russian], Moscow (1975), p. 58.
5. B. Ekstedt and L. Oreland, Biochem. Pharmacol., 25, 119 (1976).
6. V. Z. Gorkin, Experimentia, 25, 1142 (1969).
7. M. D. Houslay and K. F. Tipton, Biochem. J., 139, 645 (1976).
8. J. Knoll and K. Magyar, Adv. Biochem. Psychopharmacol., 5, 393 (1972).
9. D. E. Koshland, G. Nemethy, and D. Filmer, Biochemistry (Washington), 5, 365 (1966).
10. T. J. Mantle, K. Wilson, and R. F. Long, Biochem. Pharmacol., 24, 2031 (1975).
11. N. H. Neff and H.-Y. Yang, Life Sci., 14, 2061 (1974).
12. L. Oreland and B. Ekstedt, Biochem. Pharmacol., 21, 2479 (1972).
13. W. C. Schneider and G. H. Hogeboom, J. Biol. Chem., 183, 123 (1950).
14. L. Sierens and A. D'Iorio, Can. J. Biochem., 48, 659 (1970).

EFFECT OF CHRONIC STIMULATION OF RATS WITH INTACT AND DEAFFERENTED HYPLTHALAMI ON LIPID METABOLISM AND THE ADRENAL CORTEX

V. E. Ryzhenkov, N. S. Sapronov, G. G. Khechinashvili, I. V. Mosina, and T. A. Oletskaya

UDC 612.397.4+612.453/-06:612.826.4

Chronic stimulation of rats after a mock operation and rats with deafferented hypothalami leads to a decrease in the total cholesterol concentration in the blood and to the accumulation of triglycerides in the liver. Injection of dexamethasone into rats is accompanied by an increase in the blood cholesterol and triglyceride concentrations and accumulation of triglycerides in the liver. The action of dexamethasone is stronger in animals with a deafferented hypothalamus. The rate of secretion of 11-hydroxycorticosteroids into the adrenal vein was reduced in the latter.

KEY WORDS: *deafferentation of the hypothalamus; cholesterol; triglycerides; 11-hydroxycorticosteroids; dexamethasone.*

Previous investigations have shown that hypophysectomy in rats prevents the increase in the nonesterified fatty acid level in the blood plasma in response to administration of certain drugs with central action or to short-term immobilization of animals [4, 6].

The object of this investigation was to study the effect of prolonged stimulation of rats and deafferentation of the hypothalamus on indices of lipid metabolism and on the state of the adrenal cortex.

N. N. Anichkov Department of Atherosclerosis and Department of Pharmacology, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. N. Klimov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 3, pp. 289-291, March, 1977. Original article submitted August 9, 1976.