THE INHIBITORY EFFECT OF ACETYLCHOLINE ON MONOAMINE OXIDASE A AND B ACTIVITY IN DIFFERENT PARTS OF RAT BRAIN

By

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Abstract

On the basis of the resemblance of the chemical structure between acetylcholine (CAS 60-31-1) (ACh) and the carbamate aldicarb which inhibited brain monoamine oxidases (MAO A and B). The effect of ACh on MAO types A and B extracted from whole and five different parts of rat brain, namely: frontal cortex, basal ganglia, cerebellum, pons and medulla oblongata was studied.

MAO was extracted from five different parts of male albino rat brains. The enzyme activities were measured by the methods reported in the literature.

The results indicated that ACh inhibited the MAO-A of cerebellum extract and MAO-B of basal ganglia extract more than MAO's extracted from the other brain parts. The inhibition was of the competitive type. The enzyme inhibitor dissociation constants (Ki) as well as the affinity constants (Ki/Km) in case of MAO-A were higher than those in case of MAO-B.

ACh inhibited both MAO-A and B of the parts responsible for balance (cerebellum) and the parts responsible for coordination and automatic reflex movement (basal ganglia), respectively, more than other parts.

Key words: acetylcholine, serotonin, monoamine oxidase, benzylamine.

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1. INTRODUCTION

Acetylcholine (ACh) (CAS 60-31-1) is one of the most extensively studied neurotransmitters. Its synthesis is catalyzed by the enzyme choline acetyltransferase (ChAT) and is hydrolyzed by acetycholinesterase (AChE). It is distributed throughout the CNS with highest concentration in the motor cortex and the thalamus [1].

There is much evidence for the existence of two types and functional forms of monoamine oxidase E.C. 1.4.3.4 (MAO). The two forms are called A and B according to their preferential sensitivity to inhibition by clorgyline and deprinyl[2]. Serotonin is the active substrate of MAO-A, while benzylamine[3] is the active substrate of MAO-B. MAO-inhibitors are slow-acting drugs producing mood elevation and having beneficial effect in parkinsonism [4].

On the basis of the resemblance of chemical structure between ACh and the carbamate aldicarb which inhibited brain MAO A and B [5], the present work was conducted to study the effect of ACh on MAO A and B extracted from whole and five different parts of rat brain, namely; frontal cortex, basal ganglia, cerebellum, pons and medulla oblongata.

Enzyme kinetic studies were done to determine the type of inhibition and enzyme-inhibitor dissociation constants of MAO (A and B) by Ach, and to know which of these parts was affected by ACh more than the other

2. MATERIAL AND METHODS

2.1. Chemicals: 5-hydroxytryptamine creatinine sulphate (contains 43.5% of 5HT, serotonin) from May and Baker LH, Dagenham, England. Benzylamine (BA) from Aldrich chemical co. Ltd. Gillingham, England. Acetylcholine (ampoules each contain 200 mg) from Hoffmann La Roche & Co. Ltd. Basle, Switzerland.

2.2. Animals

Fifty male albino rats (weighing 100-150 g) aged 2 months were used in the experiments. Rats were supplied from the Medical Research Institute animal house, Alexandria University (Egypt). Rats were housed in group cages (5 in each cage) and given free access of food and tap water. The rats were killed by decapitation after subjected to an overnight fast of food, with free access of water. Each brain weighed 1.6 gm.

2.3. Preparation of the enzyme from whole and different parts of the brain.

The enzymes were prepared form whole and different parts of rat brain as described before [6-8]. Ten whole brains out of 50 brains were used (15 g) for whole brain enzyme preparation. From the other forty brains the following parts were isolated, washed with ice cold saline and weighed each separately; frontal cortex (15 g), basal ganglia (8 g), pons (7 g), medulla oblongata (10 g) and cerebellum (15 g).

Whole brain as well as the different parts were homogenized in ice cold phosphate buffer Na_2HPO_4: NaH_2PO_4, pH 7.4, 0.1 mole/L containing 0.25 mole/L sucrose. The homogenates were centrifuged at 600×g for 10 min, pellets were discarded and supernatants were recentrifuged at 12000×g for 20 minutes. The resulting supernatants from second centrifugation were discarded, and the mitochondrial precipitates were resuspended in the same phosphate buffer to give a final protein

concentration of 10 mg/ml. The protein content was determined by Lowry method [9].

2.4. Enzyme assay system

The activity of MAO'A was assayed chemically by the method of Udenfriend et al [10]. In this method the assay mixture contained: 166 µ mole 5HT/ml and 1 ml phosphate buffer containing 10 mg protein was added in a glass-stoppered centrifuged tube. Incubation was performed in a shaking water bath at 37°C for 1h. After incubation 1 ml of each of 1-nitroso, 2-naphthol (0.1% in 95% ethyl alcohol) and acid nitrite (freshly prepared) reagents were added to react with the unhydrolyzed 5HT. Measurements were carried out at 540 nm.

The activity of MAO-B was assayed chemically by the method of Tabor et al [11]. In this method the reaction mixture (2 ml) contained 1ml of the enzyme (10 mg protein in phosphate buffer ph 7.2, 0.2 mole/L) and 1 ml of 1.2 mmol/L benzylamine (BA) in the same buffer. The control tubes were identical except that BA was added after the end of incubation (2 hours). Following incubation to all the tubes; 1.5 ml of 60% perchloric acid and 1.5 ml cyclohexane were added. The absorbance was measured at 250 nm.

To determine the type of inhibition and the enzyme-inhibitor dissociation constant (Ki) of MAO-A, the enzyme activity was measured at different concentrations of ACh (4 or 7 or 10 mmol/L) with various substrate concentrations: 111, 166, 222 and 333 µmol/L simultaneously added to each concentration of the inhibitor. Then the enzyme was assayed at 540 nm.

For MAO-B the same concentrations of ACh were used and assayed as described above with different benzylamine concentrations: 0.3, 0.6. 0.9

and 1.2 mmol/L and the amount of benzaldehyde produced was measured at 250 nm.

The inhibitory effect of ACh on MAO A and B was abolished by dialysis. Both enzymes (MAO A and B) with ACh were dialyzed over night against phosphate buffer at 4°C. The dialyzed enzymes recovered their original strength and hydrolyzed nearly the same amount of substrate; indicating that the inhibition was reversible.

3. RESULTS

The activity of MAO towards 5HT and BA in whole and five different parts of rat brain extracts is represented in Table 1. The results showed that basal ganglia possessed the highest specific activity of MAO-A and B. The values of Km for MAO-A and B indicated that Km of the different parts varied between 0.23 to 0.37 mmol/L for type A and between 0.36 to 0.66 mmol/L for type B, indicating that the affinity for the substrates 5HT and BA was of the same order of magnitude.

Double reciprocal (Lineweaver Burk)[12] plot of 1/V versus 1/S in the absence and presence of ACh gave curves of the competitive type of inhibition in case of MAO.A and B (Figs. 1 and 2). This was confirmed by Cleland replot[13] of the slopes of the lines of Figs. 1 and 2 versus inhibitor concentrations (taking basal ganglia for types A and B as examples) (Figs. 1 and 2).

Ki/Km indicated that ACh had higher affinity to the enzymes of the pons and cerebellum in case of MAO-A and for the basal ganglia in case of MAO-B. The values of Ki with all the parts of the brain are indicated in table 1.

Table (1): The rate of hydrolysis of 5-hydroxy tryptamine and benzylamine by monoamine oxidase extracted from whole and five different parts of rat brain. under the effect of ACh. Values represent mean ± S.D. of 3 repeated experiments.

Part of the brain	MAO-A				MAO-B			
	µmol/L 5HT hydrolyzed/mg protein / hr	a) Km mmol/L	b) Ki mmol/L	c) Ki/Km	BA hydrolyzed in unit/mg protein/min	a) Km mmol/L	b) Ki mmol/L	c) Ki/Km
Whole brain	135±6.7	0.27	3.7	13.70	2.4±0.06	0.45	2.2	4.89
Frontal cortex	138±6.4	0.34	4.8	14.00	1.8±0.04	0.66	2.2	3.03
Basal ganglia	142±8.9	0.35	1.7	4.85	3.6±0.09	0.50	4.0	8.00
Pons	128±5.7	0.33	5.2	15.70	2.7±0.04	0.50	2.0	4.00
Medulla oblongata	101±7.5	0.23	1.9	8.20	2.8±0.07	0.50	3.0	6.00
Cerebellum	128±4.2	0.37	7.1	19.00	1.7±0.07	0.36	2.0	5.50

a) Km, Michaelis constant.

b) Ki, enzyme inhibitor (ACh) dissociation constant.

c) Ki/Km is the affinity of (Ach) to the enzyme.

Figure

Captions of figures (1) and (2)

Figure (1) : Competitive inhibition, obtained from Lineweaver-Burk plot of 1/V versus 1/S, under the effect of acetylcholine on the rate of deamination of 5-*hydroxytryptamine* by basal ganglia extract *(MAO-A)*. (●) Control, (□) 4.0, (△) 7.0 & (◦) 10 mmol/L. Inset: Cleland replot of the slopes obtained from figure (1) against the inhibitor concentration [I]

Figure (2): Competitive inhibition, obtained from Lineweaver-Burk plot of 1/V versus 1/S, under the effect of acetylcholine on the rate of deamination of *benzylamine* by basal ganglia extract *(MAO-B)*. (●) Control, (□) 4.0, (△) 7.0 & (◦) 10 mmol/L. Inset: Cleland replot of the slopes obtained from figure (2) against the inhibitor concentration [I]

4. DISCUSSION

The lowest Km values of MAO-A and B were obtained with the extracts of medulla oblongata and cerebellum respectively, showing that the substrates had more affinity to the enzymes of these extracts more than others. Comparing the value of Ki obtained in the reaction of ACh with MAO-A (3.7 mmol/L) with that previously obtained in the reaction of aldicarb[5] with MAO-A (0.18 mmol/L); indicates that ACh is 20 times less potent than aldicarb in its inhibitory effect on MAO-A. The type of inhibition (competitive) and the reversibility of the inhibition of ACh was also similar to that of aldicarb towards MAO-A and B. This could be due to the similarity in the structure between aldicarb and ACh.

Previous work indicated that 5-HT (the substrate of MAO) inhibited whole brain[14] as well as erythrocyte and plasma AChE[15]. In the present work the addition of ACh (the substrate of AChE) on the activity of MAO A as well as B manifested an inhibitory effect on both enzymes in different parts of rat brain. This indicated that MAO and AChE are competitively inhibited by each other's own substrate, which is of biological importance specially in the field of neurochemistry (cross-inhibition)[7].

The values of Ki/Km for MAO-A were higher than those of MAO-B, indicating that ACh had a relatively higher affinity to the substrate binding site in the presence of 5-HT than in the presence of BA. Moreover, ACh showed higher affinity to the substrate binding site of the cerebellum MAO-A extract (the part responsible for balance of the body), while in type B the highest affinity of ACh occurred with the basal ganglia more than the extracts of other parts (the part responsible for coordination of movements and the control of automatic reflex movements).

In the present work ACh was found to be more potent to MAO-A than MAO-B (about 2.5-3 times higher), except in case of basal ganglia where its affinity to MAO-B is higher than type A. In connection with the present results; Naoi *et al*.,[16] found that the inhibition of MAO's (A and

B) isolated from the brain by 2-N-methyl 6,7-dihdroxy isoquinolinium ions was of the competitive type and more potent to MAO-A. They also added that 2-N-methyl isoquinolinium ions and 1,2 dimethyl isoquinolinium ion inhibited both type A and B with almost the same values of the enzyme-inhibitor dissociation constants (Ki). When comparing the effect of inhibition by ACh with the above mentioned MAO inhibitors, the results of the present work indicated that ACh inhibited both MAO types A and B and the inhibition was of the competitive type, with almost the same order of magnitude of the inhibitor constants. However, the difference in the values of Ki with the different parts of the brain suggests the existence of different substrate-binding sites of MAO-A and MAO-B. All these discrepancies indicate that one amino acid can be responsible for binding of some (but not all) substrates and inhibitors[17]. Shih[18] attributed the difference between type A and B to the distinctly different gene loci. So; the inhibition of MAO's by ACh will save the biogenic amines important for the patients suffering from Alzheimer & dementia.

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