INHIBITION OF ACETYLCHOLINESTERASE BY 3-METHOXY CATECHOLAMINE DERIVATIVES

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1. Introduction

Catechol-*O*-methyltransferase is one of the two major enzymes that catalyse the metabolism of catecholamines [1]. In vivo, *O*-methylation occurs exclusively at the *meta*-position. The enzyme is found in high concentration in liver, kidney and brain, and it is also present in spleen, muscle, testes, red cells and heart. Because of this wide distribution and the relatively poor pharmacological activity of the methoxy-catecholamines, it is generally believed that the *O*-methylation process is one of the most active systems for the biological inactivation of catecholamine neurotransmitters.

We report here that the *meta O*-methylated catecholamine derivatives are able to inhibit the acetylcholinesterase from human erythrocytes and rat brain in a reversible and non-competitive manner. The $K_{0.5}$ values ranged from 0.08 mM for (±)normetanephrine to 0.56 mM for 3-methoxy dopamine.

We first observed this phenomenon during the course of experiments designed to test the molecular specificity of catecholamine analogues on the regulatory behaviour of rat erythrocyte membrane-bound acetylcholinesterase. We have shown that (−)epinephrine changed the co-operativity of the system, without affecting the specific activity of the enzyme (in the absence of allosteric effector) [2].

2. Materials and methods

2.1. *Enzyme preparations*

Human blood was centrifuged 10 min at 3000 rev./min. The plasma and the intermediate layer of white cells were discarded. The erythrocytes were washed twice with several volumes of solution containing 155 mM NaCl 2 mM sodium phosphate (buffer pH 7.4) and 10 mM glucose; finally, they were adjusted to the original hematocrit in plasma preinactivated for 30 min at 56–60°C, containing 10 mM glucose. The red cell suspension was stored at 4°C and used within 5 days.

For the determination of acetylcholinesterase activity, the membranes were prepared as in [3] while for ATPase activity, the above membrane preparations were further washed twice with imidazole–HCl buffer 20 mM, cysteine 1 mM (pH 7.5), and stored in the same buffer in liquid nitrogen. For the determination of glyceraldehyde 3-phosphate dehydrogenase the ghosts were prepared as in [4]. Synaptosomes were prepared from rat brain as in [5]. Erythrocyte membrane acetylcholinesterase was solubilized with 0.32% Triton X-100 as in [6].

2.2. *Enzyme assays*

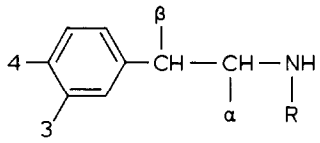
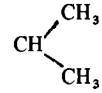
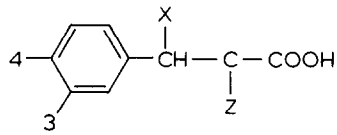
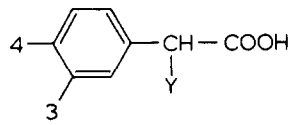
Acetylcholinesterase activity was measured as in [7]. The standard incubation mixture (2 ml) contained isotonic sodium phosphate buffer solution (pH 8.0), 0.7 mM acetylthiocholine iodide, 0.5 mM 5,5′-dithio bis-2(nitrobenzoic acid). The reaction was initiated by addition of erythrocyte, or synaptosome suspension. The ΔA_{412} was determined for 3 min every 30 s under initial velocity conditions.

The measurement of ATPase activities were performed as in [6]. Glyceraldehyde 3-phosphate dehydrogenase was assayed by a procedure recommended by Sigma.

2.3. *Others*

(±)Paranephrine, (±)norparanephrine were donated by Sterling-Winthrop Research Institute, and other catecholamines were purchased from Sigma St Louis.

Table 1
The effect of catecholamines analogues on acetylcholinesterase activity (% inhibition)

Compound (0.35 mM)	4	3	β	α	R	Whole red cells	Brain synaptosomes
							
1 (−)Epinephrine	OH	OH	OH	H	CH_3	0	0
2 (±)Metanephrine	OH	OCH_3	OH	H	CH_3	52	55
3 (±)Paranephrine	OCH_3	OH	OH	H	CH_3	0	0
4 (±)Synephrine	OH	H	OH	H	CH_3	0	0
5 (−)Neosynephrine	H	OH	OH	H	CH_3	0	0
6 (−)Norepinephrine	OH	OH	OH	H	H	0	0
7 (±)Normetanephrine	OH	OCH_3	OH	H	H	80	76
8 (±)Norparanephrine	OCH_3	OH	OH	H	H	0	0
9 Dopamine	OH	OH	H	H	H	0	0
10 3-Methoxydopamine	OH	OCH_3	H	H	H	49	51
11 (−)Isoproterenol	OH	OH	OH	H		0	0
12 L-β-3,4-Dihydroxy-phenylalanine	OH	OH	H	COOH	H	0	15
13 L-4-Hydroxy-3-methoxy-phenylalanine	OH	OCH_3	H	COOH	H	0	0
							
	4	3	X	Z			
14 4-Hydroxy-3 methoxy-phenylpiruvic acid	OH	OCH_3	H	=O		0	0
15 4-Hydroxy 3-methoxy-phenyllactic acid	OH	OCH_3	H	OH,H		0	0
							
	4	3	Y				
16 D,L-3,4 Dihydroxy-mandelic acid	OH	OH	OH			0	0
17 D,L-4 Hydroxy-3 methoxy-mandelic acid	OH	OCH_3	OH			0	0
18 3,4-Dihydroxyphenyl-acetic acid	OH	OH	H			0	0
19 4-Hydroxy-3 methoxy-phenylacetic acid	OH	OCH_3	H			0	0

3. Results and discussion

3.1. *Acetylcholinesterase from erythrocyte*

Table 1 summarizes the actions of catecholamine analogues (0.35 mM) on the activity of acetylcholinesterase from human whole red cells. (−)Epinephrine, (−)norepinephrine and dopamine (rows 1, 6 and 9) did not inhibit the enzyme whereas those compounds with a methoxy-group in position 3 were active (rows 2, 7 and 10). (±)Paranephrine and (±)norparanephrine with the methoxy-group in position 4 had no effect (rows 3 and 8). Inhibitory activity did not appear to be influenced by the hydroxyl group on the β-carbon (see 3-methoxy dopamine row 10). The presence of a carboxyl group on the α-carbon inhibited the action of a 3-methoxy catecholamine derivative (row 13). Catecholamines with a methoxy-group in position 3, but lacking amino function, were inactive (rows 14, 15, 17 and 19).

Similar results to those presented in table 1 were obtained with acetylcholinesterase from erythrocyte membrane or Triton X-100 membrane soluble preparation.

Normetanephrine-inhibited acetylcholinesterase from human membrane erythrocyte in a simple noncompetitive manner (not illustrated). The inhibition is reversible after removing the inhibitor by washing the membrane-bound enzyme. Preincubation with the inhibitors did not produce any more inhibition than that observed when each inhibitor was added just prior to the assay. The concentration of inhibitors required to produce 50% inhibition is shown in table 2. The $K_{0.5}$-values ranged from 0.08 mM for (±)normethanephrine to 0.50 mM for 3-methoxy dopamine. These $K_{0.5}$-values were not modified by the simultaneous presence of catecholamines (1.4 mM) lacking inhibitory activity such as (−)epinephrine, (−)norepinephrine and (−)isoproterenol. Pyridine 2-aldoxime methiodide (PAM) which inhibits acetylcholinesterase by interacting with its active site did not change the values of $K_{0.5}$ for *meta O*-methyl catecholamines.

3.2. *Other enzymes from membrane erythrocytes*

The enzymatic activities of the erythrocyte membrane-bound enzymes $(Na^+ + K^+)$ATPase, (Mg^{2+})ATPase and glyceraldehyde 3-phosphate dehydrogenase were unaffected by the presence of 0.5 mM *meta O*-methylcatecholamines.

Table 2
Values of $K_{0.5}$ (mM) for acetylcholinesterase

Compound	Erythrocyte membrane	Brain synaptosomes
(±)Normetanephrine	0.08	0.09
(±)Metanephrine	0.40	0.56
3-Methoxy dopamine	0.50	0.60

The values of h and $K_{0.5}$ were determined graphically using the equation:

$$\text{Log}\,(v/(V_0 - v)) = \text{Log}\,K - h\,\text{log}\,I$$

since the inhibition by 3 methoxy derivatives approached 100%; V_0, reaction rate in absence of effector; v, reaction rate in the presence of a inhibitor concentration; $K_{0.5}$, concentration that produces 50% inhibition of the reaction rate; A value of $h = 1$ was obtained for all the different inhibitors

3.3. *Acetylcholinesterase from brain synaptosomes*

Acetylcholinesterase is present in acetylcholine and catecholamine neurons [1] and is associated with the interactions between cholinergic and monoaminergic systems [8,9]. Table 1 shows that the *meta O*-methylcatecholamines also inhibit the acetylcholinesterase from synaptosomes isolated from rat brain homogenates. The $K_{0.5}$-values for each inhibitor are similar to those shown for erythrocyte enzymes (table 2).

3.4. *Physiological significance*

The concentrations of the methoxycatecholamines able to inhibit the acetylcholinesterase (table 2) exceed those that could be expected to occur in vivo. Thus, the physiological relevance of the observations presented here is questionable. However, the selectivity for the 3-methoxy catecholamine serviatives action shown in table 1 is of interest, and one is tempted to point out the importance of the changes in the acetylcholinesterase activity by 3-methoxy neurotransmitter derivatives in the process of neuronal function. This idea is attractive because in Parkinsonism, for example, the loss of dopamine alters the cholinergic–dopaminergic balance, and an anticholinergic drug is of benefit along with L-dopa (precursor of dopamine) in the treatment of the disease [10]. Thus, the possibility exists that the above balance is related to the inhibitory action of *meta O*-methyl catecholamine derivative on the acetylcholinesterase activity.

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