EFFECT OF IMIDAZOLE COMPOUNDS ON 3',5'-AMP PHOSPHODIESTYERASE ACTIVITY

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The effect of 24 imidazole compounds on activity on the phosphodiesterase (3',5'-AMP phosphohydrolase; EC 3.1.4.1) from rat brain and skeletal muscles of Rana temporaria was investigated. Imidazole compounds were shown to have both an activating and an inhibitory action on the enzyme. Imidazole itself and seven of its alkyl substitution products activated phosphodiesterase. Of the inhibitors, tetrachloro-2-trifluoromethylbenzimidazole had the strongest action on the enzyme.

KEY WORDS: 3',5'-AMP phosphodiesterase of brain and skeletal muscles; imidazole and its derivatives.

Phosphodiesterase (PDE) (EC 3.1.4.1) is the only known enzyme which can hydrolyze cyclic adenosine-3',5'-monophosphate (cyclic AMP) [15]. It has been shown that cyclic AMP participates as "secondary mediator" in hormonal actions and also plays an important role in the regulation of membrane permeability [13, 16]. Imidazole has been shown to activate, and methylaxanthine to inhibit PDE activity of bovine heart [2]. It was shown later that imidazole stimulates PDE of rat brain [6, 10, 12], of rabbit skeletal muscles [7], and of pig coronary arteries [17]. However, the mechanism of the activating action of imidazole is unknown.

The effect of several imidazole derivatives on PDE activity was assessed in this investigation. Preparations of PDE from rat brain and from skeletal muscles of the frog Rana temporaria were used as the test objects.

EXPERIMENTAL METHOD

Rat brain was homogenized in 0.9% NaCl (1 part of brain to 10 parts of solution, w/w). PDE activity was determined by a modified Cheung's method [4], in which a preparation of venom from the Central Asiatic viper Echis carinatus [1] was used as the source of the 5'-nucleotidase. The incubation medium (volume 0.5 ml) contained the following components: Tris–HCl (pH 7.8) 50 mM, $MgCl_2$ 5 mM, cyclic AMP 1 mM, homogenate 0.7-0.8 mg protein, venom 100 μg. The imidazole derivatives for testing were added in a concentration of 20 mM, except where specially stated to the contrary. The reaction was initiated by the addition of substrate. After incubation for 15 min at 37°C the reaction was stopped by the addition of 0.05 ml 55% TCA. After centrifugation at 1200g for 20 min the content of inorganic phosphate in the supernatant was measured by the method of Chen et al. Samples to which cyclic AMP was added after fixation with TCA acted as the control. The protein concentration was determined by Lowry's method [9]. PDE activity was expressed in nanomoles cyclic AMP hydrolyzed per milligram protein per minute (activity units).

The effect of some compounds on PDE of rat brain homogenate was compared with their action on partially purified PDE from frog skeletal muscles. Partial purification of the supernatants after centrifugation of muscle homogenate for 40 min at 10,000g and subsequent dialysis [7] was undertaken by salting out with ammonium sulfate. Activity of the muscle PDE was determined as described above in samples of 2-3 mg incubated for 60 min.

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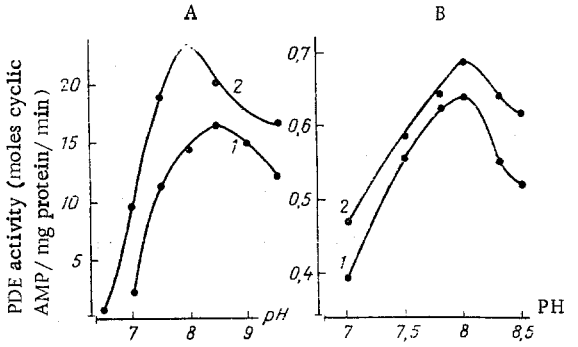


Fig. 1. PDE of rat brain (A) and frog muscles (B) as a function of pH: 1) 0.1 M Tris; 2) 0.08 M Tris + 0.02 M imidazole.

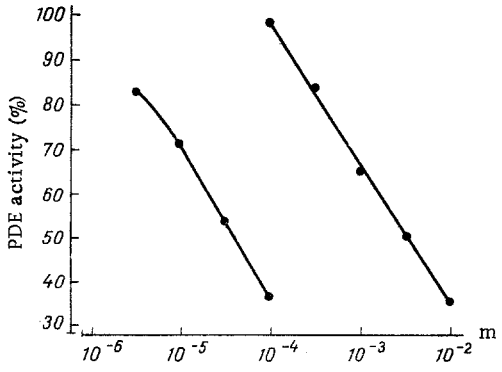


Fig. 2. Effect of various concentrations of TTFB (left) and theophylline (right) on activity of rat brain PDE.

The substrate used when testing the effect of imidazole compounds on 5'-nucleotidase activity was 5'-AMP-Na (1 mM). The composition of the incubation mixture (15 min, 37°C) was similar to that described above.

Commercial preparations of histamine, histidine, imidazole, pilocarpine, theophylline, 2-methylimidazole, benzimidazole, 2-methylbenzimidazole, tetrachloro-2-trifluoromethylbenzimidazole (TTFB), and m-chlorocarbonyl-cyanide phenylhydrazone (CCCP) were used. The remaining 15 compounds were synthesized by methods described in the literature. The constants of these products agreed with values given in the literature. The purity of the substances was verified in a thin layer of silica-gel (Merck) and by paper chromatography in a system of n-butanol–acetic acid–water (4 : 1 : 5).

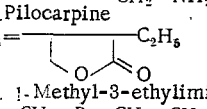
EXPERIMENTAL RESULTS

PDE of rat brain homogenate had a specific activity of 16.3 ± 0.7 units. In the presence of EDTA (1 mM), which selectively binds Ca^{++} ions, activity of the enzyme was reduced by 30–40%. EDTA (1 mM) completely suppressed PDE activity. The pH optimum for PDE of brain homogenate was 8.5. Imidazole (20 mM) shifted the pH optimum only negligibly (Fig. 1A). Partially purified PDE from frog skeletal muscles had a specific activity of 0.72 ± 0.08 unit and pH optimum of 8,0, which was not changed by the addition of imidazole (Fig. 1B).

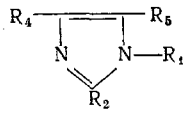
Preliminary tests showed that the imidazole derivatives had no effect on the snake venom nucleotidase.

Of the 24 imidazole compounds tested, imidazole and 7 of its alkyl substitution products, namely 1-methylimidazole, 1-ethylimidazole, 1-propylimidazole, 1-isopropylimidazole, 4-methylimidazole, 4-ethylimidazole, and 2-methylimidazole, activated PDE (Table 1); 2-methylimidazole and 1-isopropylimidazole had a weaker action than imidazole itself on brain PDE. The difference between the action of the remaining alkyl imidazoles and imidazole was not significant. On the introduction of electron-acceptor substituents (the nitro

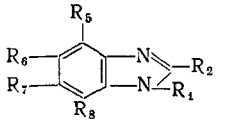
TABLE 1. Effect of Imidazole Compounds on Rat Brain and Frog Muscle PDE (M ± m)

Compound, substituents (20 mM)	Rat brain PDE		Frog muscle PDE	
	Number of experiments	Change in activity, in % of control	Number of experiments	Change in activity, in % of control
1. Imidazole	21	143,8±4,1*	6	132,9±4,2*
2. 1-Methylbenzimidazole $R_1=CH_3$	7	137,4±4,2*	4	121,4±2,4*
3. 1-Ethylimidazole $R_1=CH_2-CH_3$	10	135,6±4,1*	3	127,6±3,3*
4. 1-Propylimidazole $R_1=CH_2-CH_2-CH_3$	7	140,7±5,7*	2	126,6±0,7*
5. 1-Isopropylimidazole $R_1=CH(CH_3)-CH_3$	4	123±3,2*	—	—
6. 2-methylimidazole $R_2=CH_3$	11	122,3±3,5*	—	—
7. 4-Methylimidazole $R_4=CH_3$	8	149,0±7,7*	—	—
8. 4-Ethylimidazole $R_4=CH_2-CH_3$	4	130,2±5,5*	—	—
9. 4-Hydroxymethylimidazole $R_4=CH_2-OH$	4	99,1±1,7	3	109,3±4,8
10. 4-Bromoimidazole $R_4=Br$	4	84,6±3,4*	—	—
11. 1-Methyl-4-nitroimidazole $R_1=CH_3$; $R_4=NO_2$	6	86,0±4,0*	—	—
12. 1-Methyl-5-nitroimidazole $R_1=CH_3$; $R_5=NO_2$	4	80,8±3,1*	1	90
13. 4-Imidazolecarboxylic acid $R_4=COOH$	3	98,0±3,2	1	100
14. Histamine $R_4=CH_2-CH_2-NH_2$	5	96,4±2,9	2	104,1±1,9
15. Histidine $R_4=CH_2-CH(NH_2)-COOH$	7	103,3±2,5	4	103,5±7,4
16. Carnosine $R_4=CH_2-CH(COOH)-NH-CO-CH_2-CH_2-NH_2$	11	104,6±2,8	4	108,2±2,1* $P<0,05$
17. Homocarnosine $R_4=CH_2-CH(COOH)-NH-CO-CH_2-CH_2-CH_2-NH_2$	1	100	1	94
18. Pilocarpine $R_4=$ 	5	99,2±3,9	2	107,1±2,9
19. 1-Methyl-3-ethylimidazoliumiodide $R_1=CH_3$; $R_3=CH_2-CH_3$	7	89,3±3,7*	2	103,0±3,0
20. Benzimidazole	6	85,4±3,6*	—	—
21. 1- $R_1=CH_3$	1	62,8	—	—
22. 2-methylbenzimidazole $R_2=CH_3$	1	75,0	—	—
23. TTFB ** $R_2=CF_3$; $R_5=R_6=R_7=R_8=Cl$	6	43,3±8,7*	2	38,9±2,2*
24. Theophylline **	4	41,8±2,9*	3	56,1±7,2*
25. CCCP **	2	94,4±1,7	—	—

Legend. 1) Substances 1-19 are derivatives of the structure:



Substances 20-23 are derivatives of the structure:



2) Radicals differing from hydrogen for the structures given above are shown in Table 1.

One asterisk denotes $P < 0.05$ (compared with the control without addition of the test substances); two asterisks denote TTFB and CCCP in a concentration of 0.1 mM and theophylline in a concentration of 10 mM.

group and bromine) into the imidazole molecule its activating power was lost and the resulting compounds became weak PDE inhibitors (1-methyl-4-nitroimidazole, 1-methyl-5-nitroimidazole, and 4-bromoimidazole) (Table 1). The positively charged 1-methyl-3-ethylimidazolium ion also had a slight inhibitory action.

TABLE 2. Effect of Imidazole Derivatives (20 mM) on Brain PDE Activity in Conjunction with Theophylline (1 mM)

Compound	Change in PDE activity, % of control	
	in absence of theophylline	in presence of theophylline
2-Methylimidazole	119,1	123,7
1-Ethylimidazole	120,2	159,7
4-Ethylimidazole	125,8	160,9

Activity in presence of theophylline taken as 100%.

Benzimidazole and its derivatives 1-methylbenzimidazole and 2-methylbenzimidazole had an inhibitory action on PDE in a concentration of 20 mM, whereas TTFB, also a substituted benzimidazole, inhibited the enzyme in a concentration of 0.1 mM (Table 1). The effect of theophylline, the most commonly used PDE inhibitor, and of TTFB on PDE activity is compared in Fig. 2. To obtain the same degree of inhibition of the enzyme the theophylline concentration had to be almost two orders of magnitude higher than the TTFB concentration. This applied to both muscle and brain PDE. An increase in the Ca^{++} ion concentration (1 mM) did not weaken the inhibitory action of theophylline and TTFB. TTFB is known to be a powerful uncoupler of oxidative phosphorylation. The question arose whether the inhibition of this enzyme is a quality of other uncouplers also. To shed light on this problem an even more powerful uncoupler of oxidative phosphorylation, namely CCCP, was used. However, in a concentration of 0.1 mM it had virtually no effect on the rate of uncoupling of cyclic AMP (Table 1). Inhibition of PDE was thus evidently not connected with the uncoupling action of TTFB.

Several other imidazole derivatives also were investigated: 4-Imidazolecarboxylic acid, pilocarpine, histidine, histamine, 4-hydroxymethylimidazole, carnosine, and homocarnosine also were tested. These compounds had no appreciable effect on the enzyme (Table 1). It was shown previously that the last three compounds did not affect PDE of mouse brain homogenate, whereas histidine, 4-imidazolecarboxylic acid, and histamine were activators [14]. Histamine is known to block PDE in isolated rat fat cells [11].

It is interesting to note that imidazole derivatives with similar chemical structures (Table 1) had opposite effects on PDE activity. The most common difference between activators and inhibitors, to judge from these findings, was perhaps the more basic character of the former ($pK_{a_1} > 6.9$) than of the latter ($pK_{a_1} < 6.2$).

The effect of activators could be demonstrated in the presence of PDE inhibitors; for example, the action of imidazole activators was manifested in the presence of theophylline (1 mM) (Table 2).

It will be recalled that a natural protein activator of PDE exists [5, 8]. Since either tissue homogenate or the partially purified preparation was used as the source of the enzyme it is possible that a natural activator was present in these systems. The values obtained for activation of PDE by the imidazole compounds may therefore be too low.

Imidazole compounds can thus be useful during the investigation of PDE, for they include both activators and inhibitors of enzymic hydrolysis of cyclic AMP.

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RESPIRATION OF MITOCHONDRIA AND STATE OF PHOSPHORYLATION OF RAT LIVER ADENINE NUCLEOTIDES AFTER REPEATED ADMINISTRATION OF 3-METHYLCHOLANTHRENE AND PHENOBARBITAL

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After four injections of phenobarbital (PB) and 3-methylcholanthrene (MC) in olive oil and a single injection of olive oil into rats the acyl-CoA content in the liver (in % of the control) was 73, 167, and 230 respectively. The liver mitochondria of rats receiving injections of oil and MC were characterized by a decrease in the respiration rate in Chance's 3rd metabolic state, but this was abolished by preincubation with carnitine. The blood ketone body level after injection of PB, MC, and oil was 31, 136, and 342% respectively. The phosphate potential was lowered only after injection of oil, when the ATP concentration in the liver was considerably reduced. The AMP concentration in the liver was doubled after injections of PB and oil. Comparison of the data for induction of microsomal monooxygenases of PB and MC leads to the conclusion that acyl-CoA metabolism proceeds in different directions in the two cases.

KEY WORDS: induction; injection of oil; adenine nucleotides of the liver; respiration of mitochondria; liver acyl-CoA.

Mitochondrial adenine-nucleotide translocase (ANT) controls both the kinetics of the phosphorylating respiration of the mitochondria [1] and the level of the cytoplasmic phosphate potential [8]. It has accordingly been postulated that inhibition of the mitochondrial carrier of ATP and ADP by acyl-derivatives of coenzyme A, especially by palmitoyl-CoA, plays an important role in the mechanisms of regulation of energy metabolism during adaptation to altered metabolic conditions [11].

The object of this investigation was to study the connection between changes in the acyl-CoA concentration and respiration of the mitochondria and the state of phosphorylation of cytoplasmic adenine nucleotides after injection of 3-methylcholanthrene (MC) and phenobarbital (PB) into rats.

EXPERIMENTAL METHOD

Male Wistar rats were given daily injections of PB (10 mg/100 g body weight in 0.9% NaCl solution) and MC (2 mg/100 g body weight in 0.5 ml olive oil) for 4 days. Some animals received injections of olive oil only. Before the experiments the rats were deprived of food for 24 h. Mitochondrial respiration was determined polarographically as described earlier [10]. The concentrations of ATP, ADP, and AMP in the liver were de-

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