EFFECT OF ANTIOXIDANTS OF THE 3-HYDROXYPYRIDINE CLASS ON CYCLIC AMP PHOSPHODIESTERASE ACTIVITY

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An extensive search for physiologically active compounds is currently in progress among the synthetic and natural antioxidants, which are being used successfully for protecting biological membrane against the action of free radicals [8]. The study of water-soluble antioxidants of biogenic type belonging to the group of 3-hydroxypyridine derivatives — structural analogs of compounds of the vitamin B_6 group — is particularly interesting in this connection [5]. It has been shown that antioxidants of the 3-hydroxypyridine class possess a broad spectrum of biological action, including radioprotective [1] and antitumor action and protection against the effects of aging [9], and that they also considerably increase the resistance of blood cells to mechanical trauma [7]. 3-Hydroxypyridine derivatives can also exert a selective action on immunocompetent cells and can reduce the degree of ischemic damage to organs and tissues [3]; in the latter case, they have also been shown to have a marked effect on the tissue levels of cyclic AMP, an important regulator of cell metabolism.

One of the factors which limits the intracellular cyclic AMP concentration is the enzyme cyclic nucleotide phosphodiesterase (PDE). In this connection, in connection with the study of the mechanism of the biological action of 3-hydroxypyridine derivatives it was considered interesting to study their effect on PDE activity and to compare it with the action of known inhibitors of the xanthine class (pentoxyphylline, theophylline, etc.) on this enzyme. Alkyl-substituted 3-hydroxypyridines, which have high antiradical activity and a broad spectrum of biological action, were chosen as test object.

In the present investigation the effect of 3-hydroxypyridines was studied on two forms of PDE: Ca-activated and Ca-insensitive (PDE-I and PDE-II), isolated from rabbit heart [9], and also on the activity of this enzyme in blood lymphocytes from healthy blood donors. In the latter case the action of 3-hydroxypyridine derivatives and of xanthine was compared.

EXPERIMENTAL METHOD

Cyclic nucleotide PDE was isolated by a modified method [12]. Calmodulin was isolated from bovine brain by the method in [13]. The incubation samples (55 µl) contained 10 mM Tris-HCl buffer (pH 8.5), 5 mM $MgCl_2$, 10^{-5} M cyclic AMP, and 0.25-0.30 µCi of cyclic [^{3}H]-AMP. The reaction was started by adding 1-2 µg protein (PDE-I or PDE-II) to the sample. Incubation was carried out for 10 min at 37°C. The reaction was stopped by applying 10 µl of the incubation mixture to a UF-254 Silufol plate (Czechoslovakia), to which 2-3 µl of a mixture of 10^{-3} M solutions of 5'-AMP and cyclic AMP had previously been applied. The plates were chromatographed in a solvent mixture of isopropanol—water—ammonia in the ratio of 7:2:1 by volume. The position of the nucleotides on the plate was revealed under UV light. The 5'-AMP spot was cut out and placed in a flask for radioactivity determination, covered with 0.5 ml of distilled water, and left to elute for 2-3 h. To each flask 10 µl of ZhS-8 scintillation mixture was then added. Radioactivity was measured on an Intertechnique SL-2000 liquid scintillation counter. The test substances — hydrochlorides of 2-tert-butyl-, 2-ethyl-, and 2-tert-butyl-6-methyl-3-hydroxypyridines (IRP-1, IRP-2, and IRP-3) — were dissolved in water

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TABLE 1. Antiradical and Lipophilic Properties of 3-Hydroxypyridine Derivatives and Their Effect on Cyclic Nucleotide PDE-I and PDE-II (M ± m)

Experimental conditions	Antiradical activity, 10^{-4} moles/liter·sec	Coefficient of lipophilicity [13]	PDE-I		Degree of inhibition, %	PDE-II	Degree of inhibition, %
			without calmodulin	10^{-6} M calmodulin			
Control	—	—	0,34±0,11	1,60±0,13	—	8,21±0,37	—
Ethyl alcohol (1,55 M)	—	—	0,20±0,02	0,83±0,25	—	5,16±0,12	—
IRP -1	4,2	245,30	0,28±0,01	1,30±0,02	18	—	—
IRP -1+1,55 M C_2H_5OH	—		—	—	—	4,38±0,16	15
IRP -2	8,5	12,35	—	—	—	4,16±0,92	50
IRP -3	8,4	448,60	0,20±0,08	1,28±0,08	36	7,65±0,64	7

Legend. PDE-I and PDE-II activity expressed in millimoles cyclic AMP/min/mg protein.

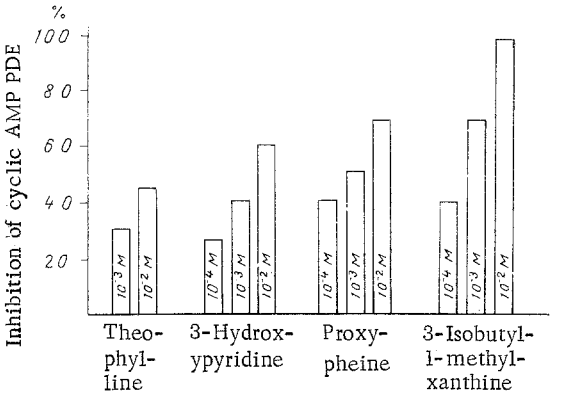

Fig. 1. Inhibition of cyclic AMP PDE activity by xanthine and 3-hydroxypyridine derivatives (IRP-2) depending on concentration of compound used (10^{-2}-10^{-4} M).

and added to the incubation medium in a final concentration of 10^{-3} M. The above-mentioned substances, as bases, were dissolved in ethyl alcohol and added to the incubation medium. The final ethanol concentration in the incubation medium was 1.55 M and of the test compound 10^{-3} M.

Lymphocytes were isolated by the method in [14] and kept at −20°C. PDE activity was determined by the method in [15]. PDE activity was measured on the basis of decrease per unit of time. The final concentrations of components of the reaction mixture were as follows: 10^{-4} M cyclic AMP, 0.15 μCi cyclic [^{3}H]-AMP, 3.3 mM $MgSO_4$, 40 mM Tris-HCl buffer (pH 7.8), protein extract of cells (enzyme) 300 μg. Incubation continued for 30 min at 30°C. Radioactivity in the supernatant was measured on a Mark II liquid scintillation counter.

EXPERIMENTAL RESULTS

Calmodulin in the presence of Ca^{++} (10^{-5} M) sharply increased the basal PDE-I activity by 4-5 times (Table 1). On the addition of 3-hydroxypyridine derivatives to the incubation medium enzyme activity was depressed both in the presence and in the absence of calmodulin. However, the degree of activation of PDE-I by calmodulin either was unchanged, in the case of IRP-1, or increased a little if IRP-3 was added to the incubation medium. The increase in the degree of PDE activation by calmodulin if compound IRP-3 was present in the experimental samples was due to a reduction of 36% in basal PDE-I activity. Depression of basal activity by the action of alkyl-substituted 3-hydroxypyridines also was observed on PDE-II, the content of which in heart tissue is much greater than that of PDE-I (relative to total PDE activity) [9]. The inhibitory effect was found to be considerable (50%) if IRP-2 was added to the incubation medium (Table 1). Other 3-hydroxypyridine derivatives lowered the activity of this form of the enzyme by a lesser degree. Comparison of data on antiradical activity, lipophilic properties, and inhibitory action of 3-hydroxypyridine derivatives on PDE activity shows absence of correlation between these parameters (Table 1). The inhibitory action of 3-hydroxypyridine derivatives on PDE is evidently determined by their structure and is reduced if the ethyl group in position 2 is replaed by a tert-butyl group.

Alkyl-substituted 3-hydroxypyridines can thus be used as PDE inhibitors, for they lower the enzyme activity of both forms of cyclic nucleotide PDE, and this may cause an increase in the cyclic nucleotide concentrations in animal tissues.

The experimental results showed that the inhibitory effect of IRP-2 and of proxypheine on lymphocyte phosphodiesterase activity was higher than that of theophylline but lower than that of 3-isobutyl-1-methylxanthine, which is one of the most powerful PDE inhibitor (Fig. 1). It can be concluded from these results that IRP-2 and proxypheine have a marked inhibitory action on lymphocyte PDE.

In the light of recent data on participation of cyclic nucleotides in the regulation of immunogenesis and immune reactions of lymphocytes [10] 3-hydroxypyridine derivatives can be classed as promising immunopharmacological agents.

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