EFFECT OF SOME ANTIHYPOXIC AGENTS ON CYCLIC NUCLEOTIDE CONCENTRATIONS IN BRAIN STRUCTURES UNDER NORMAL AND HYPOXIC CONDITIONS

P. P. Denisenko and E. Yu. Poltavchenko

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Hypoxia, as the cause or result of many pathological processes, and adaptation to it with the aid of various antihypoxic agents (in particular, the highly effective agent gutimin* and the new preparation isothiobarbamine) have been studied on a sufficiently wide scale.

We know that cyclic nucleotides take part in the regulation of every biologically important process [1]. Accordingly, in the investigation described below, changes in the cAMP and cGMP concentrations were studied in certain brain structures (cerebral cortex, hippocampus), both under the influence of hypoxia itself and during administration of the antihypoxic agent isothiobarbamine and gutimin, used under normal conditions and in the presence of hypoxia.

EXPERIMENTAL METHOD

Experiments were carried out on 66 male Wistar rats weighing 180-220 g. The animals were divided into two groups depending on the experimental conditions (group 1 — normoxia, group 2 — hypoxia). The control level of the cyclic nucleotides was determined separately for animals of each group. Hypoxic conditions were created in an airtight chamber (without absorption of carbon dioxide). Isothiobarbamine and gutimin were injected intraperitoneally into the rats 60 min before sacrifice (group 1) or before the animals were placed in the airtight chamber (group 2), in doses of 10 and 50 mg/kg respectively. The animals were decapitated, the brain removed, and the required structures were isolated. Weighed samples of tissue were homogenized in the cold. The resulting homogenate was deproteinized with perchloric acid, then neutralized to pH 7.4. Precipitated proteins were removed by centrifugation (3000 rpm, 10 min). The cAMP and cGMP concentrations in the supernatant were determined by means of kits of reagents from Amersham Corporation (England). The results were subjected to statistical analysis [3].

EXPERIMENTAL RESULTS

Under normoxic conditions isothiobarbamine did not affect the quantity of cAMP, whereas gutimin significantly raised the cAMP level in the cortex, irrespective of its dose, without changing its level in the hippocampus (Table 1).

A state of hypoxic hypoxia caused a significant fall in the cAMP level both in the cortex and in the hippocampus. Isothiobarbamine in a dose of 10 mg/kg, under hypoxic conditions, lowered the cAMP level in the cortex almost by half, but in the hippocampus (50 mg/kg), on the contrary, it increased it virtually to the level of the intact control. Gutimin (10 mg/kg) had a similar action in the hippocampus, but did not change the cAMP level in the cortex.

The trend of changes in the cGMP level repeated that for cAMP on the whole, although cGMP was more stable under the influence of hypoxia and antihypoxic agents. Under normoxic conditions isothiobarbamine did not affect the cGMP level in either of the two structures tested. Gutimin, while not changing the cGMP level in the hippocampus, increased it almost threefold in the cortex in a dose of 10 mg/kg.

The cGMP content was unchanged in the cortex and hippocampus during hypoxia, but the action of the compounds under these conditions was different from that during normoxia. In the

*Guanylthiourea.

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TABLE 1. Effect of Isothiobarbamine and Gutimin on Cyclic Nucleotide Concentration in Cortex and Hippocampus of Rats under Normoxic and Hypoxic Conditions (M ± m)

Experimental conditions	Preparation injected	Dose, mg/kg	cAMP, picomoles/mg tissue		cGMP, picomoles·10^3/mg tissue	
			cortex	hippocampus	cortex	hippocampus
Normoxia	Control	—	0,66±0,19	1,67±0,26	10,5±1,40	37,8±6,98
	Isothiobarbamine	10	0,92±0,10	1,4±0,17	12,0±3,80	35,3±4.48
		50	0,88±0,10	1,52±0,12	15,0±?,90	42,5±4,02
	Gutimin	10	1,48±0,19*	1,57±0,10	29,2±1,80*	38,8±1,74
		50	1,41±0,23*	1,89±0,16	20,2±5,40	42,3±5,47
Hypoxia	Control 1 (normoxia)	—	1,22±0,13	1,51±0,095	39,0±4,50	66,4±7,47
	Control 2 (hypoxia)	—	0,82±0,08*	0,83±0,068*	40,2±3,61	58,5±5,25
	Isothiobarbamine	10	0,48±0,07*	1,06±0,19	37,5±1,49	67,2±5,16
		50	0,89±0,20	1,51±0,11*	24,8±4,16*	81,0±14,50
	Control	10	1,05±0,20	1,49±0,16	34,3±6,35	52,3±6,01
		50	0,66±0,11	1,15±0,18	25,3±1,27*	38,6±7,72

Legend. *P < 0.05 compared with corresponding control: for hypoxia, control 2 compared with control 1, all the rest — compared with control 2.

cortex both substances, in a dose of 50 mg/kg, depressed the cGMP level equally whereas in the hippocampus isothiobarbamine caused a tendency for the cGMP level to rise, whereas gutimin caused a distinct tendency for it to fall (P < 0.07).

Differences discovered in the changes in the cyclic nucleotide system under the influence of hypoxia and antihypoxic agents are convincing evidence of the complexity and dissimilarity of the mechanisms of the protective action of these agents. However, there is no doubt that cyclic nucleotides play a definite role in the mechanisms of action of these compounds.

Data in the literature on the functional role of cAMP and cGMP in the CNS and their interaction are extremely contradictory. It has been shown that cGMP excites, whereas cAMP inhibits unit activity in different parts of the brain [10]. Meanwhile the opposite views also are held [2, 11]. Taken as a whole, data in the literature support an independent role for cGMP, but there are plenty of examples of the existence of both antagonistic and of reciprocal relations between cAMP and cGMP [4], and also of synchronized changes in them under the influence of various factors [8].

In the present experiments changes in the concentrations of the two cyclic nucleotides under the influence of isothiobarbamine were synchronized: In normoxia there were no changes, and in hypoxia their level fell in the cortex and rose in the hippocampus. It can accordingly be postulated that under hypoxic conditions the fall in the cyclic nucleotide level in the cortex, as the structure most sensitive to hypoxia, enables the cortex to "survive" conditions of hypoxia, evidently by lowering the oxygen demand of the tissues, on a basis of more economical utilization of oxygen. Meanwhile, under the influence of isothiobarbamine neurochemical processes were activated in deeper parts of the brain (hippocampus). The reduction in the cAMP concentration in the cortex may perhaps take place through stimulation of postsynaptic α_2-adrenoreceptors and inhibition of adenylate cyclase [6]. Barbiturates are considered to have a stabilizing action on cell membranes [5, 9] and also to be able to inhibit the Ca^{++} inflow into brain synaptosomes [7]. Changes in the cyclic nucleotide concentrations under the influence of isothiobarbamine may perhaps be linked with its effect not only on the adenylate cyclase and guanylate cyclase systems, but also on the biological accessibility of Ca^{++}.

Unlike isothiobarbamine, gutimin caused synchronized changes in the cyclic nucleotide level only in mormoxia: It increased their concentration in the cortex (thus preparing it for subsequent possible exposures to stress), but did not change it in the hippocampus. Under hypoxic conditions relations between the cyclic nucleotides became reciprocal: The cAMP level rose (hippocampus) or was unchanged (cortex), whereas the cGMP level fell in both structures.

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ACTION OF RETINOIC ACID ON LIPID PEROXIDATION IN RAT LIVER MICROSOMES *in Vitro*

I. Ya. Kon' and L. Sh. Gorgoshidze

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Despite much research, the biochemical mechanisms of the systemic function of retinol, which plays a role in growth, reproduction, cell proliferation and differentiation, and maintenance of an adequate immunologic status of the body, are insufficiently understood [4, 9, 15]. It can be postulated that these mechanisms are "multivalent" in character and are determined by the effect of retinol on several metabolic reactions, including microsomal oxidation and lipid peroxidation (LPO) [4]. It was shown previously that retinol *in vitro*, and also if given in large doses to experimental animals *in vivo*, causes marked depression of LPO in rat liver microsomes [6, 12]. Later these results were confirmed [10, 11]. However, many aspects of the antioxidant action of vitamin A have not been adequately studied. This is true in particular of the presence of antioxidant properties in other representatives of the vitamin A group and, in particular, of trans-retinoic acid, which can perform many of the functions of retinol (except its visual and reproductive functions), and which in some cases exhibits higher physiological and pharmacological (including antitumor) activity than retinol itself [7, 8, 13, 15].

The aim of this investigation was to compare the effects of retinol and trans-retinoic acid *in vitro* on enzymic and nonenzymic LPO by rat liver microsomes.

EXPERIMENTAL METHOD

Experiments were carried out on adult male Wistar rats weighing 250-300 g, kept on a natural balanced diet. The microsomal fraction was isolated by differential centrifugation, as described previously [6, 12], from liver homogenates prepared in the ratio of 1:3 (w/v) in 25 mM Tris-HCl buffer, pH 7.4, containing 0.175 M HCl. The microsomes were resuspended in isolation medium, so that 1 ml of suspension contained microsomes isolated from 0.2 g of liver. To 10 ml of the suspension 0.2 ml of a solution of retinol or trans-retinoic acid (both from Serva, West Germany) in ethanol was added to a final concentration of 7×10^{-5} M for retinol and from 3.5×10^{-5} to 2.8×10^{-4} M for retinoic acid. The samples were thoroughly mixed and preincubated for 30 min at 37°C on a water bath with constant stirring. After the end of preincubation the samples were quickly transferred to an ice bath and materials were taken for investigation of the LPO capacity of the microsomes, which was estimated from accumulation of malonic dialdehyde (MDA) in the microsomes after incubation for 60 min at 37°C on a water bath (with constant stirring), either without the addition of pro-oxidants (spontaneous LPO) or in the presence of Fe^{++} and ascorbate—ascorbate-dependent LPO (ADP), or of NADPH—dependent LPO (NDP), in the medium [1]. The MDA concentration in the samples was determined by the method in [1, 14] and expressed in nanomoles per milligram protein.

Institute of Nutrition, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 4, pp. 428-429, April, 1985. Original article submitted July 16, 1984.

