EFFECT OF INDOMETHACIN AND NONACHLAZINE ON TYROSINE HYDROXYLASE ACTIVITY IN THE RAT MYOCARDIUM AND BRAIN

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Nonachlazine, a new Soviet antianginal preparation, has a marked action on central and peripheral adrenergic processes [3, 5, 6]. The central inhibitory effect of nonachlazine on sympathetic vascular tone and somatosympathetic reflexes is connected with activation of inhibitory adrenergic brain-stem structures [2]. At the same time, nonachlazine has a psychotropic action, reducing the feeling of anxiety during an attack of ischemic heart disease [4], possibly on account of its influence on brain structures which play a regulatory role in the formation of emotional responses, in which adrenergic processes play a significant role. There are indications that prostaglandins (PG), which exert a modulating action on the activity of adrenergic structures [15], may participate in the mechanism of the effects of nonachlazine [6].

When continuing research into the mechanism of action of nónachlazine an important step was to study its effect on the velocity of the tyrosine hydroxylase reaction, the limiting and regulatory stage of catecholamine biosynthesis [11], in brain and heart structures under ordinary conditions and when PG biosynthesis is inhibited by indomethacin, an inhibitory of prostaglandin synthetase [14].

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats weighing 200-250 g. Nonachlazine and indomethacin were injected intraperitoneally in doses of 6 and 10 mg/kg respectively. A 0.9% solution of NaCl in the same volume was injected into control animals. The animals were decapitated 15 min after the injection and their heart and brain were removed and placed on ice. The hypothalamus and brain stem were isolated from the brain and the septum and adjacent tissues of the left ventricle, which are rich in nerve structures, from the heart. Weighed samples of tissues were homogenized in 10 volumes of 0.05 M K-phosphate buffer, pH 6.0, with 0.2% Triton X-100. The samples were centrifuged for 15 min at 15,000g. The supernatant was used to determine enzyme activity. The composition of the sample was: buffer 0.05 M Tris-maleate, pH 6.0, tissue extract corresponding to 20 mg of the original tissue, $9 \cdot 10^{-5}$ M tyrosine, $2.7 \cdot 10^{-4}$ M $DMPH_4$.* Soluble and membrane-bound enzyme were obtained by the method in [7, 11]. Tyrosine hydroxylase (TH) activity was determined by a direct spectrophotometric method [7], arachidonic acid cyclooxygenase activity was determined by a polarographic method [10], and total prostaglandin synthetase activity by biological testing on a strip of rat stomach [14].

When both indomethacin and nonachlazine were adminstered, indomethacin was injected first, followed by nonachlazine, in the same doses as when they were given separately.

*$DMPH_4$ — reduced form of 2,3-dimercaptopropanol.

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TABLE 1. Effect of Nonachlazine and Indomethacin, Injected Systemically, on TH Activity in Rat Brain and Heart Tissues (M ± m; n = 6)

Experimental conditions	TH activity, nmoles $DMPH_2$/min/mg protein		
	hypothalamus	brain stem	cardiac septum
Control	18,2±2,6	20,7±2,5	8,3±1,4
Nonachlazine (6 mg/kg)	10,0±1,2*	11,4±1,5*	9,9±1,4*
Indomethacin (10 mg/kg)	5,7±1,3*	13,4±2,5*	13,0±2,1*
Nonachlazine (6 mg/kg)+ Indomethacin (10 mg/kg)	9,0±1,1*	9,7±1,6*	14,5±1,8*

*Here and in Table 2, $P < 0.05$ compared with control.

Legend. 0.1 M Tris-maleate buffer, pH 6.0; tyrosine $0.12 \cdot 10^{-3}$ M; $DMPH_4$ $0.18 \cdot 10^{-3}$ M; tissue extract corresponding to 20 mg tissue.

TABLE 2. Effect of Nonachlazine and Indomethacin *in vitro* on TH Activity (M ± m; n = 6)

Experimental conditions	TH activity, nmoles $DMPH_2$/min/mg protein	
	membrane-bound enzyme	soluble enzyme
Control	37,5±2,6	21,6±1,8
Nonachlazine (10^{-4} M)	64,7±5,4*	50,1±4,8*
Indomethacin (10^{-4} M)	35,4±3,6	24,0±2,1

Legend. 0.1 M Tris-maleate buffer, pH 6.0; tyrosine $0.10 \cdot 10^{-3}$ M; $DMPH_4$ $0.17 \cdot 10^{-3}$ M; protein 30 µg/ml sample.

EXPERIMENTAL RESULTS

The results of determination of TH activity in the hypothalamus, brain stem, and septum of the rat heart after injection of nonachlazine in a dose of 6 mg/kg, indomethacin 10 mg/kg, and a combination of both, are given in Table 1. The specific activity of the enzyme in both brain structures was reduced almost by half 15 min after injection of nonachlazine. Specific activity of TH in the heart was significantly increased by 20% after injection of nonachlazine, reflecting the effect of the drug on catecholamine biosynthesis in the nerve cells of the heart.

Meanwhile nonachlazine, unlike indomethacin, can have a direct action on the velocity of the tyrosine-hydroxylase reaction, as was shown by experiments *in vitro* on a preparation of membrane-bound and soluble enzyme from hypothalamic synaptosomes (Table 2). This fact is complementary to previous data on adrenergic mechanisms of the action of nonachlazine [5, 6]. Evidently nonachlazine can interfere not only with the uptake of catecholamines and interaction with the receptor [1], but also with the velocity of catecholamine biosynthesis.

The decrease in enzyme activity observed in the hypothalamus and medulla may reflect increased physiological activity of these structures [8]. The inhibitory effect of nonachlazine, in a dose of 6 mg/kg, when injected intravenously on tonic activity and reflex responses in the sympathetic nerves of the heart and kidney [3], due evidently to the activating effect of nonachlazine on inhibitory structures of monoaminergic neurons of the brain stem, was

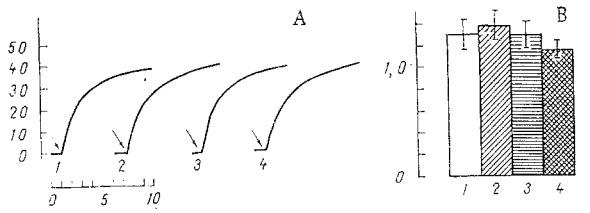

Fig. 1. Effect of nonachlazine on arachidonic acid cyclooxygenase activity. A) Absorption of oxygen by microsomes of sheep seminal vesicles; incubation medium contains 1.5 mg microsomal protein/ml in 0.1 M Tris-HCl buffer, pH 8.2, and sodium arachidonate (addition marked by arrow) in final concentration of 100 μM. Abscissa, time (in min), ordinate, oxygen absorption (in nmoles/mg protein); B) rate of oxygen absorption by microsomes (in nmoles/mg protein/min). 1) control, 2-4) addition of 10, 100, and 1000 μM nonachlazine respectively.

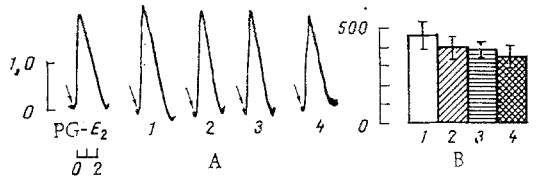

Fig. 2. Effect of nonachlazine on prostaglandin synthetase (biological testing) A) Reactions of isotonic contraction (in mm) of strip of rat stomach to injection (arrow) of 10 ng/0.2 ml PGE_2 (calibration) and 0.02 ml of incubated microsomal sample into surrounding solution. Surrounding solution: 40 ml Tyrode A solution + 20 ml Tyrode B solution + 1.5 g glucose + 2 mg propanolol + 0.5 mg atropine + 0.1 mg phenoxybenzamine + 0.0001 mg phenosamine (each in a volume of 1 ml) + H_2O to 1000 ml + 0.5 ml indomethacin (2 mg/ml). Incubation medium contains 1.6 ml 0.066 M phosphate-soda buffer, pH 8.0, + 0.1 ml glutathione (1 mg/ml) + 0.1 ml hydroquinone (0.1 mg/ml) + 0.1 ml microsomes from sheep's seminal vesicles + 0.1 ml arachidonic acid (200 μg/ml) + (if necessary) 0.1 ml nonachlazine solution; B) concentration of PGE_2 (in ng/mg protein). 1) Control; 2-4) nonachlazine added to incubation medium in concentrations of 1, 10, and 100 μg/2 ml, respectively, in a volume of 0.1 ml.

demonstrated previously. The increase in TH activity in the heart under the influence of nonachlazine may be the result of the regulatory effect of β-adrenoreceptors which, having lost their previous sensitivity to catecholamines, maintain a high level of activity in response to an increase in the catecholamine supply. These processes take place against the background of reduced noradrenalin uptake by the heart from the blood [5]. The increase in catecholamine biosynthesis proper in nerve cells of the heart, with a simultaneous decrease in their uptake from the blood, suggests that nonachlazine potentiates the regulatory influence of the sympathetic nerves of the heart and weakens humoral regulation of cardiac activity through the adrenals.

Meanwhile, the results of these experiments show that the PG system is involved in the mechanism of action of nonachlazine. For instance, after combined injection of nonachlazine and indomethacin, an inhibitor of PG biosynthesis, interference between the effects of these substances on TH was observed in the brain (Table 1), evidence of a common link in the mechanism of their action.

Combined injection of nonachlazine and indomethacin led to an increase in enzyme activity in the heart by almost 75% compared with the control, which was greater than the effect of each drug separately, and it suggests summation of the actions of nonachlazine and indomethacin.

No effect of nonachlazine on PG biosynthesis was discovered in experiments *in vitro*. In a concentration of 10^{-6}-10^{-4} M, for instance, nonachlazine did not change the velocity of oxidation of arachidonic acid by microsomal cyclooxygenase from sheep's seminal vesicles (Fig. 1). The absence of any direct effect of nonachlazine on prostaglandin synthetase activity also was demonstrated by biological testing (Fig. 2).

Much experimental evidence of close interaction between the prostaglandin and adrenergic system has now been obtained, and particularly of the modulating effect of PG on local regulation of adrenergic neurotransmission [12, 13, 15]. PG may have an inhibitory effect on catecholamine liberation, as has been found in experiments on the rabbit heart and rat vas deferens [9]. Potentiation of the effect of nonachlazine on the velocity of the tyrosine-hydroxylase reaction in the heart when injected together with indomethacin may perhaps be connected with this property of PG: Inhibition of PG biosynthesis by indomethacin may lead to an increase in the rate of secretion of the mediator which, in turn, could cause an increase in the velocity of the tyrosine hydroxylase reaction.

PG may thus participate in the action of nonachlazine on adrenergic processes regulating the activity of the cardiovascular system: inhibition of PG biosynthesis by indomethacin causes changes in catecholamine biosynthesis in the brain and heart.

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