Ethanol-Oxidizing and NO-Synthesizing Enzymes in Monoaminergic Nuclei of Human Brain

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 136, No. 8, pp. 231-234, August, 2003
Original article submitted February 10, 2003

Serotonin-, dopamine-, and noradrenergic nuclei in human brainstem were examined histochemically for alcohol dehydrogenase, aldehyde dehydrogenase, and NADPH diaphorase. The findings indicate that monoaminergic centers are characterized by different repertoire of NO-ergic and ethanol-oxidizing enzymes, whose distribution correlates with the transmitter specialization of neurons.

Key Words: *nitric oxide; monoamines; ethanol*

Physiological disturbances in alcoholism are caused by the effect of ethanol on the cerebral NO-ergic system [1,3,14]. Ethanol potentiates the neurotoxic effects of NO and regulates monoamine production and release [14]. Both effects of ethanol are complementary and are determined by the efficiency of ethanol-oxidizing, NO synthesizing, and aminergic mechanisms. The factors and mechanisms of neuronal damage in different brain nuclei are not identical because of different location and different activity of NO synthase and ethanol-oxidizing enzymes [1,4]. We studied the location of alcohol dehydrogenase (ADH), aldehyde dehydrogenase (AlDH), and NADPH diaphorase (NADPH-d) activities in monoaminergic nuclei in human brainstem.

MATERIALS AND METHODS

Specimens of human brain was obtained at autopsy no later than 3 h postmortem from 6 cadavers without narcological or neurological pathologies. Histochemical analysis of ADH activity (EC 1.1.1.1) was carried out as described previously [7]. The brainstem was placed on glass, cut into fragments, frozen in a cryostat, and 25-30-μ sections were prepared. The sections were incubated for 2 h at 37°C in a medium containing 1 ml 0.2 M sodium phosphate buffer (pH 7.4), 0.15 ml 20% furfuryl alcohol, 0.3 ml 0.5% nicotinamide adenine dinucleotide (NAD, Calbiochem), 0.3 ml 0.5% nitroblue tetrazolium (NBT, Sigma), and 1.25 ml distilled water. Furfuryl alcohol is better than ethanol as the substrate, because human cerebral ADH more actively catalyzes oxidation of alcohols with long carbohydrate chains [4].

Brain sections for detection of AlDH (EC 1.2.1.3) were incubated for 20 min at 37°C in 1 ml warm incubation medium containing 0.1 M sodium pyrophosphate buffer (pH 7.4), 5 mM NAD, 2 mM amital, 0.1 mM pyrasol, 5 mM magnesium chloride, 0.025% NBT, 4% polyvinylpyrrolidone, and 30 mM acetaldehyde [2].

For the detection of NADPH-d activity (EC 1.6.99.1) the material was fixed for 2 h at 4°C in 4% paraformaldehyde solution in 0.1 M Na phosphate buffer (pH 7.4), washed in 15% sucrose solution for 24 h. Cryostat sections (25 μ) were incubated in a thermostat for 1 h at 37°C in a medium containing 50 mM Tris-buffer, 0.2% Triton X-100, 0.8 mg/ml β-NADPH (Sigma), 0.4 mg/ml NBT, pH 8.0 [9].

Enzyme activities in serotoninergic raphe nuclei (*n. raphe pontis, n. raphe dorsalis, n. raphe pallidus, n. raphe medianus, n. raphe magnus, and n. raphe obscurus*), noradrenergic nucleus of locus coeruleus, and dopaminergic structures of the substantia nigra

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0007-4888/03/1362-0202$25.00

and ventral tegmental field were determined by the density of histochemical precipitate on a Vickers cytodensitometer and expressed in optical density units (opt. dens. units).

RESULTS

Neurons with positive histochemical reaction to ADH, AlDH, and NADPH-d are characterized by the presence of formazan granules of different structure and density in the cytoplasm. Topochemical heterogeneity of the studied enzyme activities allows us to identify three groups of nerve centers in human brain (Table 1).

Group 1 includes dopaminergic nuclei with neurons exhibiting high activity of ADH, AlDH, and NADPH-d. The cell cytoplasm contains bright blue precipitate of very high density, evenly filling the perikaryon and passing into the processes. Enzyme activities in these neurons vary from 67 to 60 opt. dens. units and are located mainly in neurons of the substantia nigra reticular nucleus (Fig. 1, *a*).

Group 2 includes serotoninergic neurocytes of the raphe nuclei with high ADH activity, high or medium NADPH-d activity, and low AlDH activity (Fig. 1, *b*, *c*). If the enzyme activity is moderate (42-60 opt. dens. units), granular blue precipitate is seen in the neuron, more dense in the perinuclear zone forming a blue ring. The cytoplasm of cells with low activity of AlDH (37-40 opt. dens. units) contains disperse evenly blue precipitate.

Group 3 includes norepinephrine- and dopaminergic centers with high activity of ADH, but lacking AlDH and NADPH-d activities. These neurons are detected in the locus coeruleus, compact nucleus of substantia nigra (Fig. 1, *a*), and ventral tegmental field.

Heterogeneous localization of ADH, AlDH, and NADPH-d determines the formation of neuroprotective and cytotoxic mechanisms essential for the pathogenesis of alcohol dependence. It is known that shifts in monoamine activity developing in the brain in acute alcohol intoxication stimulate the development of ethanol tolerance [8]. High activities of NADPH-d and ADH in aminergic neurons in the absence of AlDH activity indicate that NO is a metabolic component mediating the cytotoxic effect of acetaldehyde. This mechanism plays a very important role under conditions of chronic alcohol intoxication, when NO production sharply increases [3]. In case of production of endogenous or massive entry of exogenous acetaldehyde into the brain these neurons loose enzyme defense and die [14].

Inability of norepinephrinergic cells in the locus coeruleus to express AlDH can be explained by the fact that aldehyde-oxidizing and norepinephrine-producing functions exclude each other. Local production of acetaldehyde surpasses here the rate of its elimination and becomes the key factor of ethanol intoxication. We cannot rule out the possibility of inactivation exogenous acetaldehyde by catalase because of low activity or absence of AlDH in these neurons [14]. We found no cells expressing AlDH in the substantia nigra compact nucleus and neurons of the ventral tegmental field. However, we cannot assert that all dopaminergic neurons cannot oxidize aldehydes, because we identified neurons expressing AlDH in the reticular nucleus of the substantia nigra (group 1, Table 1).

NO-ergic factor with pronounced cytotoxic and neuromodulatory effects is involved in the neurotropic effects of ethanol [1,6,13]. Previous studies [11,13,15] showed that acute ethanol poisoning decreased the level of NO production in brainstem nuclei, while NO synthase inhibitors prevent the increase in tolerance during the formation of chronic alcohol dependence. Involvement of NO as a diffuse messenger in the transmission of interneuronal bonds is explained by its capacity to facilitate or inhibit the neurotransmitter release

TABLE 1. Activities of AlDH, ADH, and NADPH-Diaphorase in Human and Rat Brainstem Monoaminergic Nuclei (*M*±*m*, in opt. dens. units)

Group, nucleus		AlDH	ADH	NADPH-d
1	*substantia nigra* (*pars reticularis*)	76.3±2.4	80.3±1.2	68.7±3.6
	n. raphe pontis	37.2±1.4	69.2±1.3	60.1±3.4
	n. raphe dorsalis	41.1±2.1	66.1±1.4	59.4±3.2
2	*n. raphe pallidus*	41.2±2.8	75.2±3.8	42.2±2.8
	n. raphe medianus	40.4±1.1	70.1±2.4	72.3±2.2
	n. raphe magnus	37.1±2.2	60.4±2.4	80.4±3.4
	n. raphe obscurus	40.6±2.5	68.4±3.3	56.2±2.9
3	*ventral tegmental area*	—	68.2±3.1	—
	substantia nigra (pars compacta)	—	79.3±2.8	—
	locus coeruleus	—	72.1±4.2	—

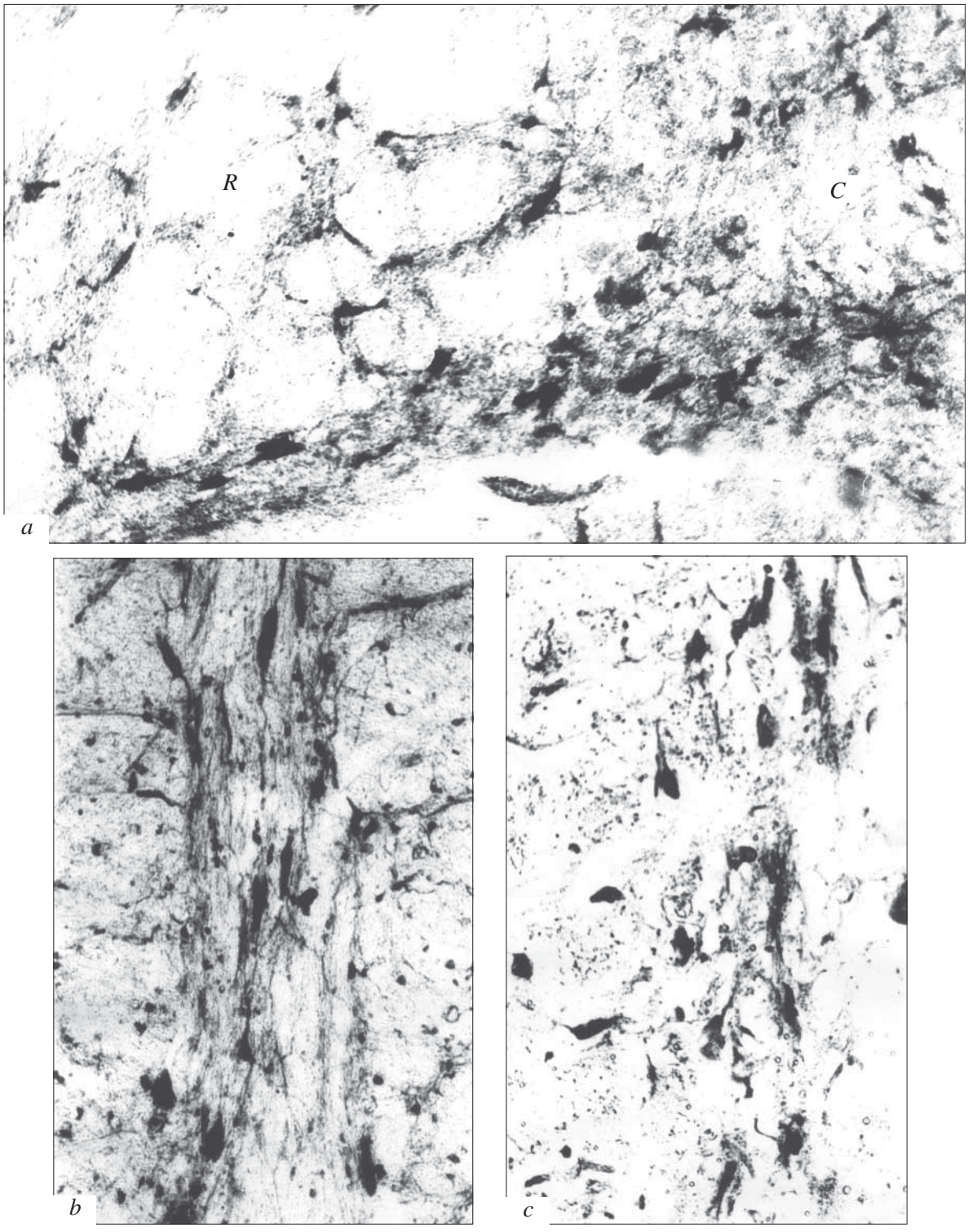

Fig. 1. Location of alcohol dehydrogenase, aldehyde dehydrogenase, and NADPH-diaphorase in human brainstem monoaminergic nuclei. *a*) neurons of the reticular (*R*) and compact (*C*) nuclei of substantia nigra with high activity of alcohol dehydrogenase, ×200. *b*) NADPH diaphorase in *n. raphe obscuris*, ×250.; *c*) *n. raphe obscuris* neurons with positive reaction to aldehyde dehydrogenase, ×250.

from the surrounding synapses [10]. Thus, intracellular production of NO stimulates activity of dopaminergic neurons [12] and inhibits transmitter release in serotoninergic neurocytes of the raphe nuclei [5].

Hence, monoaminergic nuclei possess different sets of neuromodulatory (NO-ergic) and ethanol-oxidizing mechanisms, which correlates with different transmitter specialization of neurons and can be sig-

nificant for understanding of the pathogenesis of alcoholism.

The study was supported by the Russian Foundation for Basic Research (grant No. 02-04-49644).

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