CHANGES IN THE BIOGENIC AMINE LEVEL IN THE RAT BRAIN DURING ETHANOL INTOXICATION

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The dynamics of the concentration of biogenic amines in the brain stem and forebrain of rats was investigated after a single dose (1.5 and 6 g/kg, intraperitoneally) and chronic administration (for two months, ethanol solution in concentrations rising from 10 to 40%) of ethanol and after deprivation of the animals of its action (1, 4, and 7 days). In acute ethanol intoxication the noradrenalin and dopamine concentrations fell in the forebrain (by 15-25%). In the brain stem the dopamine level rose (by 20-48%). The serotonin concentration rose sharply both in the forebrain (by 59-136%) and in the brain stem (by 36-49%). In chronic ethanol poisoning the noradrenalin and dopamine levels rose in both structures, whereas the serotonin concentration was indistinguishable from the control. Deprivation of the animals of ethanol (one day) led to accumulation of noradrenalin and dopamine in their brain, whereas the serotonin concentration was lowered (by 12%). The role of the coupled functioning of monoamine-containing neurons in changes in the excitability of the CNS under the influence of ethanol is discussed.

KEY WORDS: biogenic amines; monoamine-containing neurons; ethanol.

Concentrations of biogenic amines during studies of the action of ethanol are usually determined in the whole brain or in individual brain structures, and this may perhaps partly explain the extremely contradictory results obtained [1, 7, 10]. Changes in activity of the CNS as a result of ethanol intoxication, however, are determined not only by total changes in the concentration of biogenic amines in the whole brain, but also by their redistribution between the bodies of neurons and nerve endings. Cell bodies of monoamine-containing neurons in the brain are located in the brain stem and medulla, whereas synapses of ascending axons are located in the fore- and end-brain [3].

With these considerations in mind, it was considered important to undertake a comparative study of the dynamics of the serotonin, dopamine, and noradrenalin concentrations in the region of the bodies and nerve endings of monoamine-containing neurons in acute and chronic ethanol intoxication and after the end of exposure to ethanol.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 180-220 g. Ethanol (40%) was injected intraperitoneally into the animals 30 min before sacrifice in doses of 1.5 and 6 g/kg body weight. Control rats received injections of the same doses of physiological saline. In the course of chronic intoxication the animals received a solution of ethanol, the concentration of which was increased gradually from 10 to 40%, as the sole source of drinking fluid.

The concentrations of the biogenic amines were determined in the forebrain and brain stem fluorometrically [6]. The forebrain included caudate nucleus, septum, preoptic region, part of the amygdala, anterior commissure, and corpus callosum. The brain stem included the substantia nigra, interpeduncular nucleus, mesencephalic raphe nucleus, red nucleus, mesencephalic reticular formation, and cerebral peduncles.

Amines were extracted from samples of brain tissue by the use of butanol and heptane. The intensity of fluorescence of oxidation products of noradrenalin was measured at 365/485 nm, of dopamine at 313/390 nm, and of serotonin at 365/470 nm, on the ÉF-3MA fluorometer with an M-95 microammeter.

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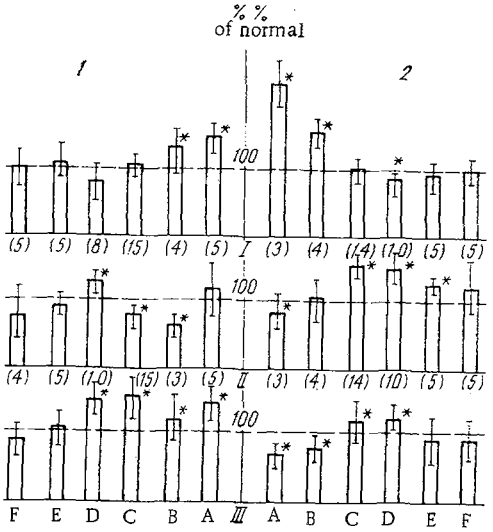

Fig. 1. Dynamics of serotonin (I), noradrenalin (II), and dopamine (III) concentrations in brain stem (1) and forebrain (2) of rats during ethanol intoxication and after deprivation of animals of ethanol (in percent of control). A, B) single injection of ethanol in doses of 1.5 and 6 g/kg respectively (acute intoxication); C) injection of ethanol for two months in increasing concentrations from 10 to 40% (chronic intoxication); D, E, F) 1, 4, and 7 days respectively after deprivation of rats with chronic intoxication of ethanol. Number of experiments shown beneath each column (the number of experiments to determine noradrenalin and dopamine was the same). Asterisk) difference from corresponding control is significant ($P < 0.05$). Control values taken as 100%.

The blood alcohol level of the rats was determined spectrophotometrically at 340 nm on the SF-4A spectrophotometer, using an alcohol dehydrogenase method.

The significance of differences between the results for the experimental and control groups of animals was determined by Student's t-test.

EXPERIMENTAL RESULTS

After injection of ethanol in doses of 1.5 and 6 g/kg it accumulated in the rats' blood in amounts of up to 0.05 and 0.2% of the injected dose. The animals developed depression. The experimental results are illustrated in Fig. 1.

The decrease in the concentration of noradrenalin (by 15-40%) and dopamine (by 25-15%) discovered in the region of the nerve endings of monoamine-containing neurons in acute ethanol intoxication (1.5 and 6 g/kg) agrees with observations of other workers [1, 2] and is attributable to activation of their liberation and decomposition [2]. This action of ethanol is the result of its interaction with nerve cell membranes and its effect on Na, K-ATPase activity [11]. The dopamine level in the forebrain and brain stem changed in opposite direction during acute ethanol intoxication. Previously, the turnover of this amine in the substantia nigra was found to be depressed, but in the olfactory tubercles it was increased after injection of a 20% solution of ethanol [9]. A local increase in the dopamine level in the bodies of dopamine-containing neurons (Fig. 1) probably contributes to their autoinhibition [14]. This counteracts any further exhaustion of the mediator in the region of the nerve endings.

The increase in the dopamine concentration in the brain stem, with a simultaneous fall in the noradrenalin level after a single injection of ethanol in a dose of 6 g/kg (Fig. 1) was probably connected also with depression of dopamine-β-hydroxylase activity in noradrenalin-containing neurons [2], and also with activation of tyrosine hydroxylase [13]. The reciprocal change in the serotonin and noradrenalin concentrations in response to injection of ethanol in doses of 1.5 and 6 g/kg (Fig. 1), not previously reported, agrees with the view that functions of serotonin- and noradrenalin-containing neurons are coupled [5].

Chronic ethanol intoxication (2 months) led to the development of compensatory processes in the CNS to maintain homeostasis, which were characterized by an increase of 23-53% in the noradrenalin concentration and an increase of 58-22% in the dopamine concentration in the brain stem and forebrain respectively.

It has been shown [7, 10] that prolonged administration of ethanol causes a sharp increase in the concentrations both of noradrenalin and dopamine and of serotonin. We found no significant changes in the serotonin level in either part of the brain studied (Fig. 1), but this agrees with data showing relative stabilization of the serotonin system in chronic ethanol intoxication [8, 12].

Deprivation of the rats of ethanol after chronic administration led to a fall in their serotonin concentration and to accumulation of noradrenalin and dopamine in the brain after 24 h (Fig. 1). This imbalance of the biogenic amines persisted for several days, demonstrating loss of lability of the adaptive mechanisms of the CNS. The noradrenalin level remained high in the forebrain, even seven days after ethanol deprivation. This indicates that noradrenalin-containing neurons are the most sensitive to ethanol and that considerable after-changes occur in them.

Accumulation of noradrenalin and dopamine in the brain of animals after deprivation of ethanol was probably associated with activation of tyrosine-hydroxylase by end-products of the reaction by a feedback mechanism [4], as a result of the chronic administration of ethanol. Noradrenalin and dopamine, in turn, are inhibitors of tryptophan-5-hydroxylase – an enzyme which limits serotonin formation [4], and this may perhaps explain the reciprocity of changes in the levels of these amines. The discovery that changes in the serotonin and noradrenalin concentrations are opposite in direction to changes in the dopamine concentration in acute ethanol poisoning and after deprivation of the rats of ethanol suggests that it is this coupling of the function of monoamine-containing neurons which plays a role in the changes in CNS excitability under the influence of ethanol.

The decrease in the noradrenalin and dopamine concentrations in the region of the nerve endings of monoamine-containing neurons, with simultaneous accumulation of serotonin, is the cause of depression of the CNS arising as a result of acute ethanol poisoning. Conversely, the fall in the serotonin level and accumulation of noradrenalin and dopamine in the brain lead to the development of hyperexcitability of nervous structures in rats deprived of ethanol after chronic intoxication with it.

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