Use of Sodium Hydroxybutyrate and Nooglutyl to Correct Dopamine Release in the Striatum of Prenatally Alcoholized Rat Pups

G. I. Kovalev, E. A. Budygin, R. R. Gainetdinov, V. S. Kudrin, S. S. Trofimov, and R. U. Ostrovskaya

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Alcohol consumption by pregnant females is known to result in the development of mental abnormalities and mental retardation in their offspring [8]. It has been found experimentally that substances with nootropic activity such as sodium hydroxybutyrate and nooglutyl are capable of correcting these abnormalities resulting from prenatal alcoholization [3,4]. As shown by Trofimov *et al.* [4], the neurochemical mechanisms responsible for the pathology as well as those by which the pharmacotherapeutic effect of nooglutyl is achieved, involve alterations in the levels of serotonin and dopamine and of their main metabolites 5-HIAA and DOPAC in the cortex and hippocampus of young rats [4]. It has also been shown that slowed dopamine turnover in the striatum is a characteristic neurochemical sign of the ethanol withdrawal syndrome [7].

In the study described here the prenatal alcoholization model was used to explore how the functional activity of the presynaptic dopaminergic system of the striatum in rat pups is influenced by (a) maternal alcoholization and (b) sodium hydroxybutyrate and nooglutyl.

Institute of Pharmacology, Russian Academy of Medical Sciences, Moscow. (Presented by G. N. Kryzhanovskii, Membre of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

The experiments were conducted on the offspring of random-bred female rats that had been receiving, throughout the period of pregnancy, either ethanol (20% solution) in a daily dose of 5 g/kg body weight (to obtain alcoholized offspring) or water (to obtain normal offspring) administered intragastrically via a stomach tube. Sodium hydroxybutyrate (50 mg/kg per day), nooglutyl (25 mg/kg per day), or physiological saline was injected subcutaneously both to normal (groups 2, 3, and 1, respectively) and alcoholized (groups 5, 6, and 4, respectively) pups from the 8th to the 20th day after birth.

For a study of the release of dopamine (DA) or DOPAC in the striatum, 5 male pups from each group were decapitated on the 20th day of life and their striata were dissected out in the cold from both halves of the brain and cut transversely. Each striatum was then placed in a separate thermostatically controlled (37°C) chamber containing a carbogenized buffer of the following composition (nmol/liter): $CaCl_2$, 2.5; $MgSO_4$, 1.64; $NaNCO_3$, 25; KH_2PO_4, 1.2; EDTA, 0.054; and ascorbic acid, 0.28. After a 60-minute incubation the buffer was changed; following a further incubation for 10 min, the buffer was collected and DA and

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DOPAC were precipitated on preactivated aluminum oxide from a Katekholkhrom kit (manufactured by DIA-M, Moscow) in the presence of 1 mol/liter Tris buffer, pH 8.6. After shaking for 10 min the aluminum oxide was washed three times with deionized water and the precipitated DA eluted with 0.1 mol/liter $HClO_4$. The samples were analyzed by HPLC using a reverse-phase column (3×150 mm, C_{18}, 5 μ) (Elsiko, Moscow) with 0.1 mol/liter citrate-phosphate buffer containing 0.025 mmol/liter sodium octanesulfonate, 0.1 mmol/liter EDTA, and 8.5% acetonitrile, pH 3.6. Detection was carried out on an LC-4B glass-carbon electrode (BAS, USA). The results were treated statistically by Student's *t* test.

RESULTS

Daily administration of ethanol to pregnant rats in the oral dose of 5 g/kg led to significant reductions in both basal (Fig. 1) and K^+-stimulated (Fig. 2) release of endogenous DA in the striata of their 20-day-old pups. In contrast, the level of DOPAC was not altered as a result of maternal alcoholization (group 4).

In control groups 2 and 3, sodium hydroxybutyrate and nooglutyl administered daily in doses of 50 mg/kg and 25 mg/kg, respectively, exerted opposite effects on DA release from striatal tissue: whereas the former drug induced accelerated basal release of DA (and of DOPAC), the latter slowed

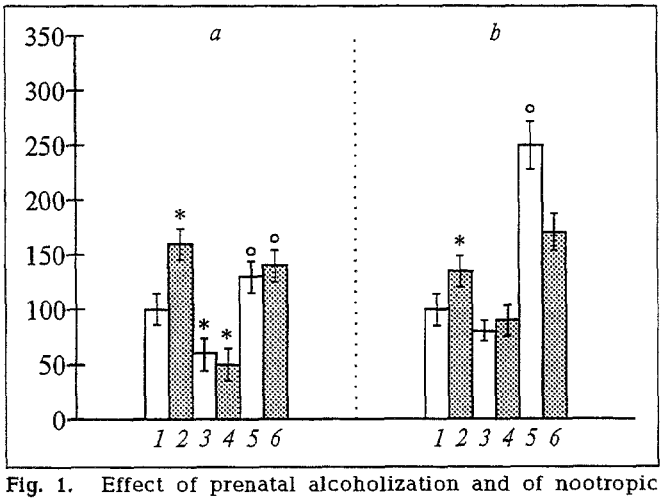

Fig. 1. Effect of prenatal alcoholization and of nootropic drugs (sodium hydroxybutyrate and nooglutyl) on basal dopamine (*a*) and DOPAC (*b*) release from perfused rat striata. Abscissa: *1*) females – water, offspring – physiological saline; *2*) females – water, offspring – sodium hydroxybutyrate; *3*) females – water, offspring – nooglutyl; *4*) females – ethanol, offspring – physiological saline; *5*) females – ethanol, offspring – sodium hydroxybutyrate; *6*) females – ethanol, offspring – nooglutyl. Ordinate: levels of DA and DOPAC release (in % relative to group 1). * and ° denote a statistically significant difference from groups 1 and 4) respectively.

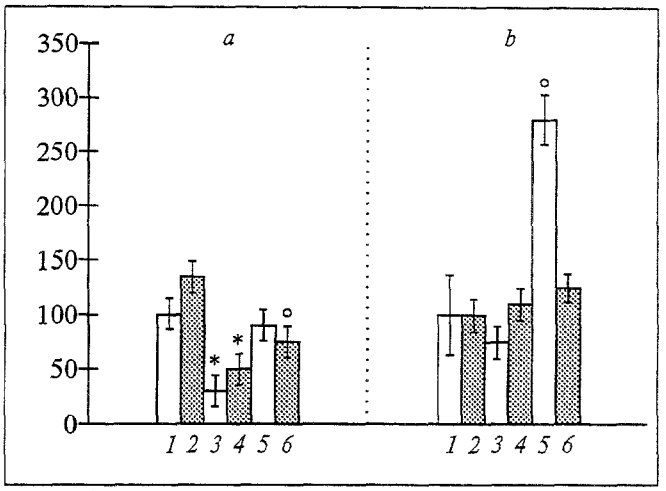

Fig. 2. Effect of prenatal alcoholization and of nootropic drugs (sodium hydroxybutyrate and nooglutyl) on K^+-stimulated dopamine (*a*) and DOPAC (*b*) release from perfused rat striata. For designations see Fig. 1.

both the stimulated and basal DA release without altering DOPAC levels. It may therefore be assumed that these two drugs each act by a distinct mechanism.

In contrast, sodium hydroxybutyrate and nooglutyl administered to prenatally alcoholized pups in the same doses as above (groups 5 and 6) normalized both the basal and stimulated DA release. Moreover, the basal DA release occurred at a higher rate than in the controls (group 1).

These findings suggest that the reduced motor and/or conditioned-reflex activity previously observed in rats subjected to prenatal alcoholization may be associated with a lowered efficiency of DA-ergic neurotransmission in various structures, including the striatum. As shown earlier [4], alcoholization also results in abnormal metabolism of this neurotransmitter in the hippocampus, which is believed to be one of the most sensitive targets for ethanol [6]. An active role in the ethanol-induced biochemical shifts appears to be played by a raised level of membrane polyphosphoinositides, which has been shown to occur with the prenatal alcoholization model [2].

On the other hand, it is apparent that sodium hydroxybutyrate and nooglutyl exert their beneficial effects in this animal model by acting on distinct neurochemical targets. It has now been established that gamma-hydroxybutyrate (GHB), an endogenous substance of the brain, is implicated in both the metabolic and neurotransmitter functions of neurons [12]. In particular, specific binding sites for ^{3}H-GHB have been identified in the frontal cortex, hippocampus, and striatum of rats [5] as well as of humans [10]. In rats injected with GHB, altered cGMP levels in the hippocampus

[11] and accelerated DA synthesis in the striatum [9] are reported.

These and other findings have led to the hypothesis that GHB can perform the functions of a neuromodulator or neurotransmitter in the rostral parts of the brain and metabolic functions in its caudal parts [5, 9, 10, 12]. If this is indeed so, then the mechanism of the stimulating effect exerted by sodium hydroxybutyrate may be visualized as consisting of the following chain of events: activation of putative GHB heteroreceptors located on a DA-synthesizing neurons - activation of cGNP - activation of DA synthesis - enhancement of DA release. Indeed, the efficiencies of all these processes which make possible the functioning of the striatal DA system were found to be reduced when ethanol was withdrawn after its prolonged consumption [7].

As regards nooglutyl, a nootropic agent recently produced in the CIS [1], the neurochemical mechanism mediating its activating effect on the striatal DA-ergic system has not been characterized. It may be assumed, however, that the primary pharmacological target for this agent will include the above-mentioned events.

In summary, prenatal alcoholization of rat progeny, which leads to motor, emotional, and mnestic abnormalities in later life, weakens DA-ergic neurotransmission in the striatum. Sodium hydroxybutyrate and nooglutyl administered to alcoholized rat pups from the 8th to the 20th day after birth both increased DA release to normal levels. In intact rat pups, they exerted opposite effects on DA, release which suggests that their neurochemical targets are not the same.

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