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Central and Peripheral Nitric Oxide Metabolites and BDNF Content in Rats with Alcohol Dependence and Under Intranasal Administration of Sodium Nitroprusside

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Abstract. The aim of the study was to investigate the possibility of correcting the nitric oxide (NO) and BDNF deficiency in alcohol dependent rats by intranasal administration of the NO donor sodium nitroprusside (SNP). Intranasal administration of SNP to rats with alcohol dependence was carried out at a dose 8 μg/kg body weight twice a day on the background of alcohol withdrawal for 3 days. Nitric oxide metabolites (nitrites, nitrates; NOx) and BDNF concentration were measured in the brain structures and serum. Chronic alcohol consumption leads to a decrease of NOx and BDNF content in brain structures and BDNF concentration in serum in rats under alcohol withdrawal. SNP restores NOx level in the brain and BDNF concentration in serum on the background of NOx level decrease in serum. Intranasal administration of SNP causes recovery of NO cerebral functions and BDNF level in serum impaired as a result of chronic alcoholization.

Keywords: nitric oxide metabolites, BDNF, brain structures, alcohol dependence, sodium nitroprusside.

Центральні та периферійні метаболіти оксиду азоту і вміст BDNF у щурів з алкогольною залежністю та при інтраназальному введенні нітропрусиду натрію

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Резюме. Метою дослідження було вивчити можливість корекції дефіциту оксиду азоту (NO) та мозкоспецифічного нейротрофічного фактору (BDNF) у щурів з алкогольною залежністю шляхом неінвазивного методу інтраназального введення низьких доз донору NO нітропрусиду натрію. Алкогольну залежність моделювали шляхом добровільного прийому алкоголю в дозі 1,25 г/кг маси тіла протягом 40 днів. Інтраназальне введення нітропрусиду натрію в дозі 8 мкг/кг маси тіла здійснювали двічі на добу на тлі відміни прийому алкоголю протягом 3 днів. Концентрацію метаболітів NO (нітрити, нітрати; NOx) вимірювали спектрофотометричним методом у гомогенатах гіпоталамусу, гіпокампу, мигдалини, фронтального неокортексу та у сироватці крові щурів. Концентрацію BDNF в гомогенатах гіпокампу, фронтального неокортексу та сироватці крові тварин визначали за допомогою набору для імуноферментного аналізу «BDNF (Brain BDNF (Brain Derived Neurotrophic Factor) Kit». Хронічна алкоголізація призводить до зниження вмісту NOx та BDNF у всіх досліджених структурах мозку та концентрації BDNF у сироватці крові щурів, які перебувають у стані відміни прийому алкоголю. Інтраназальне введення нітропрусиду натрію відновлює рівень NOx у гіпоталамусі, гіпокампі, мигдалині, фронтальному неокортексі та концентрації BDNF у сироватці крові на тлі зниження також рівня NOx у сироватці крові. Таким чином, інтраназальне введення низької дози нітропрусиду натрію призводить до відновлення мозкових функцій NO та рівня BDNF у сироватці крові, порушених внаслідок хронічної алкоголізації.

Ключові слова: метаболіти оксиду азоту, BDNF, структури мозку, алкогольна залежність, нітропрусид натрію.

INTRODUCTION

Nitric oxide (NO) is well known as an important regulator of neurotransmission, synaptic plasticity, vascular homeostasis, smooth muscle relaxation. This makes it a necessary participant in regulatory processes in the formation and transformation of states of dependence on psychoactive substances. Alcohol has its effect on nitrergic processes depending on dose and duration of administration. Low concentrations of alcohol induce increased synthesis and release of NO from the endothelium and nervous cells due to activation and expression of endothelial nitric oxide synthase (eNOS) or neuronal nitric oxide synthase (nNOS) accordingly. In contrast, administration of high concentrations of alcohol or its chronic intake impairs endothelial functions and reduces NO bioavailability in parasympathetic perivascular nerves and brain neurons [1, 2].

NO activates soluble guanylyl cyclase in vascular smooth muscle cells and nervous cells to produce cyclic GMP that is mainly involved in the regulatory actions of NO. This causes muscle relaxation effects and mediates, in particular, the anxiolytic properties of alcohol. Cerebral neurovascular dysfunction and decreased bioavailability of brain NO contribute to cognitive decline and neurodegenerative processes caused with alcohol abuse [3]. However NO plays key role in mediating adult neurogenesis by inducing neural stem cells to generate newborn neurons for replacing damaged neurons [4].

Brain-derived neurotrophic factor (BDNF) is involved not only in neuronal development and plasticity, but also in regulation responses to drugs of abuse, including alcohol. Decreased neurotrophic activity may be involved in ethanol-induced neurodegeneration in the brain through decreased expression of BDNF or through inability of the receptor to transduce signals in the presence of ethanol [5, 6]. Moreover several studies have demonstrated a complex interplay between cerebral NO and BDNF signaling. These findings identify NO as a paracrine messenger stimulated by neurotrophin signaling in newly generated neurons to control the proliferation and differentiation of brain neural progenitor cells. Administration of NO donors can increase neuronal differentiation of neural progenitor cells and prevent impairments in recognition memory [7, 8, 9]. However, the effect of these drugs may be dose dependent. Different concentrations of sodium nitroprusside (SNP; NO donor) induced a dual effect on the excitability of neuronal membrane: 1 mM of SNP evoked membrane hyperpolarization and an outward current, whereas 10 μM induced depolarization of the membrane and an inward current [10].

The aim of the study was to investigate the possibility of correcting the NO and BDNF deficiency in rats with alcohol dependence by non-invasive method of intranasal administration of low doses of the NO donor sodium nitroprusside.

MATERIALS AND METHODS

The procedures with experimental animals were approved by the Commission on Ethics and Deontology of the SI «Institute of Neurology, Psychiatry and Narcology of National Academy of Medical Sciences of Ukraine» (protocol No. 12 of 04.12.2020) and performed in accordance with the «General Ethical Principles of Animal Experiments» (Kyiv, 2011), «The procedure for conducting experiments on animals by scientific institutions» (No. 249 of 01.03.2012), and the Law of Ukraine «On protection of animals from cruel treatment» (No. 3447 IV of 21.02.2006).

The studies were carried out on 38 nonlinear adult laboratory male rats weighting 250–300 g in a chronic experiment in four groups: intact rats (9), rats with alcohol dependence in the state of alcohol withdrawal (8), rats with alcohol withdrawal and intranasal administration of sodium nitroprusside (13), rats with intranasal administration of sodium nitroprusside (8). Rats were kept in groups of 6 per cage. The model of alcohol dependence was created by voluntary intake of food containing alcohol at a dose of 1,25 g/kg body weight of rat for 40 days. For this purpose, the rats were placed in individual cages for 10 min. Animals were also tested for alcohol preference in these cages for 10 min The rats were offered a choice of two pieces of bread soaked with alcohol or water. The latent period of the approach and selection of the appropriate piece of bread was recorded. Withdrawal of alcohol was carried out for 3 days (72 hours after the last alcohol consumption). When testing rats for alcohol preference in the state of alcohol withdrawal, they were offered only a choice between pieces of bread with alcohol and water. There was no alcohol intake in this case.

Sodium nitroprusside (SNP) was diluted with bidistilled water at a concentration of 0,1 % in a dark vial immediately prior to administration. Then this solution was diluted to a concentration of 0,017% and injected intranasally with a single-channel pipette, 10 µl into each nasal cavity. SNP was administered at a low dose of 8 µg/kg body weight of the animal (approximately 9,3 nmol per rat) twice a day (at 10. 00 a.m. and at 4.00. p.m.) on the background of alcohol withdrawal for 3 days. The intranasal route of administration was chosen due to the fact, that SNP is a compound with a small molecular weight that easily penetrates the olfactory epithelium and the blood-brain barrier (BBB). A low dose of the drug does not have the toxic effect of high doses of NO donor when directly delivered to the brain [10].

30–40 min after the last administration of SNP, the whole brain was removed and brain structures were extracted on ice according to the rat brain atlas [11], then were weighted and frozen in Eppendorf tubes at -80ºC. The concentrations of NO metabolites (nitrites and nitrates; NOx) in homogenates of the hypothalamus (Hpt), hippocampus (Hip), amygdala (Am), frontal neocortex (FC) and serum were studied by spectrophotometric method [12]. Brain tissue homogenates were prepared in 1,2m of bidistilled water on ice and after a freeze-thaw

cycle were used to determine NOx. Concentration of BDNF in homogenates of the Hip, FC and serum was determined using «Rat BDNF (Brain Derived Neurotrophic Factor) ELISA Kit» (Elabscience, China) in accordance with the instructions for the kit.

Statistical analysis was performed using the program «Statistics 6.0» (Statsoft Inc., USA, 2001) with the mean and standard deviation (x ± SD) for each group. One-way analysis of variance ANOVA was used to detect statistically significant differences between groups. Differences were considered significant at $p < 0,05$ according to the Turkey test.

RESULTS

Testing animals for the development of alcohol dependence showed that by 40 days of alcohol intake, 87,5% of rats preferred food with alcohol. After alcohol withdrawal 100% of animals chose food with alcohol, but 27,3% of them with a latent period up to 2 min. The level of NOx in this group was reduced in the Hpt – by 35%, in Hip – by 23,6%, in Am – by 26,3%, in FC – by 30% compared to the level of intact rats (Tab. 1).

Table 1

Concentrations of NO metabolites in brain structures and serum in rats under alcohol withdrawal and intranasal administration of sodium nitroprusside

Brain structures, serum	Intact (nM/g tissue; μM/l)	SNP (nM/g tissue; μM/l)	Alcohol Withdrawal (nM/g tissue; μM/l)	Alcohol Withdrawal + SNP (nM/g tissue; μM/l)
FC	131,3±12,3	156,3±14,5	92,0±10,7*	145,1±10,2**
Hip	274,2±17,9	274,9±21,2	209,6±13,5*	292,6±32,6**
Am	239,6±11,1	347,7±19,6	176,7±17,6**	324,5±23,1**
Hpt	319,5±23,8	311,4±30,7	207,7±21,9*	394,1±59,2**
Serum	16,6±1,2	19,4±2,2	13,4±1,2	8,5±0,5*, **

* $p < 0,05$ in comparison with control group
** $p < 0,05$ in comparison with group «alcohol withdrawal»
SNP – sodium nitroprusside

The level of BDNF was decreased in the Hip – by 44,1%, FC – by 41,3%, serum – by 32% in rats after alcohol withdrawal against its value in intact animals as well (Tab. 2).

Intranasal administration of a low dose of SNP on the background of alcohol withdrawal for 3 days led to increase of a latent period of choice of food with alcohol in the whole group of alcohol-fed rats up to 3 min (data not shown). These changes in alcohol intake were accompanied with increasing NOx level in the Hip – by 39,6%, in Hpt – by 89,7%, in Am – by 83,6%, in FC – by 57,7% compared to the level of rats with alcohol withdrawal. However, concentration of NOx in serum after SNP treatment was surprisingly decreased by 49% to 26,6% in comparison with its level in intact rats and rats with alcohol withdrawal, whereas the BDNF content in serum was restored (Tab. 1, Tab. 2).

Table 2

Concentrations of BDNF in brain structures and serum in rats under alcohol withdrawal and intranasal administration of sodium nitroprusside

Brain structures, serum	Intact (pg/g tissue; pg/ml)	Alcohol Withdrawal (pg/g tissue; pg/ml)	Alcohol Withdrawal + SNP (pg/g tissue; pg/ml)
FC	550,0±96,0	323,0±53,0*	411,0±54,0
Hip	1598,0±282,0	893,0±114,0*	852,0±82,0*
Serum	2055,0±209,0	1396,0±158,0*	1976,0±119,0**

* $p < 0,05$ in comparison with control group
** $p < 0,05$ in comparison with group «alcohol withdrawal»
SNP – sodium nitroprusside

SNP intranasal administration caused the increase of NOx level in non-alcohol-fed rats in the Am only (Tab. 1).

DISCUSSION

Many studies show that NO deficiency in the body can be compensated by administration of NO donors, in particular sodium nitroprusside. However, SNP produces a dose-dependent effect. Low doses of drug cause vasodilation, hypotension, muscle relaxation. High doses have a number of side effects such as excitotoxicity, cyanide toxicity, methemoglobinemia etc. [13]. SNP in therapeutic doses is administered by intravenous infusion. The idea of our study was to introduce very low doses of an NO donor directly into the brain. This is especially important for the correction of psycho-emotional and motivational states, in particular, states of anxiety or alcohol dependence. A convenient non-invasive way to administer low doses of drugs that penetrate the BBB is intranasal administration. We chose a water solution of SNP at a dose of 8 μg/kg body weight, which was injected into both nasal cavities of rats. This dose corresponds to approximately 9,3 nmol

SNP per rat. The NOx content in an intact rat Am in terms of its weight (35–50 mg) according to our data may be approximately 9,6 nmol per Am. These data suggest that the dose of the NO donor applied was physiological. The result of such a 5-fold exposure to SNP was an increase in the level of NOx by 1,5 times exactly in Am, which has a direct connection with the primary olfactory area of the brain.

Behavioral studies have shown that animals with high level of alcohol dependence took food with alcohol immediately. After 5 administrations of SNP for 3 days, the latency of choosing food with alcohol increased up to 3 minutes. This effect demonstrates an attenuation of alcohol motivation, although it was short-lived and disappeared a few days after SNP withdrawal (as shown by our unpublished data). These results may indicate that the intranasal use of SNP during the early days of alcohol withdrawal can alleviate the animal's alcohol-related craving.

Alcohol dependence in rats, as shown by our study, is formed against the background of a decrease in the level of NOx in all studied brain structures by 23–35%. Weakening of alcohol motivation was observed on the background of NOx level recovery in the brain after SNP administration. An excess of the normal level of NOx was noted in the Am and Hpt by 83,6% and 89,7%, respectively. This may be due to the fact that chronic alcohol intake leads to an increase in the permeability of the BBB, especially in the areas of the olfactory epithelium and Hpt. It was strongly supported the existence of a direct nose-to-brain transport rout for drugs in animals and humans. Similar to the BBB, the nasal mucosal barrier is permeable to different substances in different ways. SNP is a compound with a small molecular weight that easily penetrates the olfactory epithelium and the BBB due to absorption across the olfactory sustentacular epithelial cells by transcellular or paracellular mechanisms. The extracellular pathway that transports polar drugs through tight junctions appears to be very fast and allows to reach the brain structures within minutes after nasal application [14]. The axons of mitral cells of olfactory bulb project to primary olfactory areas which include the piriform cortex and Am, and then to the Hpt. Therefore, the observed greatest increase in the concentration of NOx in these brain areas may be related to the greater bioavailability of the SNP solution.

The Am plays a major role in the physiopathology of affective disorder and is activated in response to negative stimuli. This brain structure seems to be a strategic locus where olfactory and neuroendocrine stimuli are integrated, modulating in particular emotional behavior. An intraperitoneal administration of SNP was accompanied with the development of inhibitory processes in the Am [15, 16], due to an increase in the level of NO as we suppose. Thus, we suggest that an increase in the NOx level in the Am may lead to a decrease in its hyperexcitability and a weakening of rat alcohol motivation.

The intranasal rout of administering a low dose of SNP restores the level of NO in the subcortical emotiogenic zones of the brain and frontal neocortex, but not in the blood serum. This allows provide a targeted effect on the NO level in the brain.

There is evidence of direct effect of NO on BDNF and brain BDNF signaling dependence on cerebral endothelium-derived NO production [8, 17], but we did not find significant changes of BDNF level in the brain of alcohol-fed rats after SNP intranasal administration. NO is not the only factor that affects BDNF. For example, an increase in corticosteroid concentration due to alcohol withdrawal causes a decrease in the expression of BDNF in the brain [18]. That is why, administering small doses of SNP may simply not be enough to shift the BDNF balance reduced by chronic alcoholization. Large doses of NO donors can have a negative effect [13]. Therefore, we either have to choose other, more optimal concentrations or agree that this way of influencing the level of brain BDNF is ineffective.

However, the level of BDNF was restored in serum after SNP intranasal administration. This was accompanied with a decrease in the concentration of NOx in serum for which we did not find any explanations. This issue requires further research.

CONCLUSION

Intranasal administration of NO donors is an effective way to restore a reduced level of NO in brain structures. However, the selected dose was not effective enough to restore the reduced level of BDNF in the brain. Prospective are further developments of routs of targeted delivery of NO donors to correct the insufficiency of central nitrergic regulation and regulatory processes dependent on it.

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