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PHYSIOLOGY

Endogenous Opioid Dependence Induced in Rats by Periodic Intake of 5% Ethanol Solution

S. K. Sudakov, N. G. Bogdanova, E. V. Alekseeva, and G. A. Nazarova

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We studied the possibility of formation of endogenous opioid dependence in rats during periodic intake of 5% ethanol solution. In the control group, both drinking bottles contained water. In the experimental group, the second bottle was filled with 5% ethanol solution for 12 h per day; in the following 12 h, these rats were deprived of food and ethanol. This regimen was maintained over 8 days. The rats were subdivided into alcohol- and water-preferring subgroups. Ethanol deprivation followed by naloxone injection evoked the signs of opiate withdrawal syndrome in both subgroups. These findings suggest that periodic voluntary intake of a weak ethanol solution over 8 days led to the formation of endogenous opioid dependence in rats irrespective of amount of the consumed alcohol.

Key Words: *ethanol; taste preference; endogenous opioid dependence; withdrawal syndrome; Wistar rats*

It is a common knowledge that intake of food with pleasant flavor induces positive taste perception and emotional feeling. These events activate the positive reinforcement mechanisms manifested by the release of β-endorphin in the midbrain ventral tegmental area and elimination of GABA-mediated inhibitory influences on dopamine neurons resulting in the release of dopamine from nerve terminals projected to various limbic and cortical structures [6,7,11]. Stimulation of positive reinforcement system can form psychic and physical dependence related, specifically, to essential alterations in the endogenous opioid system [8,10,14]. However, continuous intake of dissolved sucrose, sodium chloride, and other substances with pleasant flavor provokes no symptoms of physical dependence [3]. In 2002, a paper described the development of dependence on periodic intake of 25% glucose solution for 1 week [4]. After glucose deprivation coupled with a rather large dose of naloxone, the rats demonstrated the behavioral features typical of opiate withdrawal syndrome in animals with opiate dependence. Later, the possibility of formation of physical dependence on periodic intake of 10% sucrose solution over 1 month was demonstrated [2]. The researchers hypothesized that the use of delicious food forms the dependence based on the mechanisms similar to those underlying the narcotic drug dependence; logically, it was termed as natural endogenous opioid dependence [9].

Consumption of ethanol provokes positive reinforcement with the release of opioids from the nerve terminals in the midbrain [13]. Importantly, numerous alcoholic beverages have a pleasant flavor; they contain sugar and frequently have the balanced and harmonic combination of sweet, sour, and bitter taste modalities. The pleasant flavor of low-alcohol drinks can provoke the onset of alcoholic dependence, which initially can be manifested by formation of endogenous opioid dependence. It is an established fact that solutions with a low ethanol concentration

P. K. Anokhin Research Institute of Normal Physiology, Moscow, Russia. ***Address for correspondence:*** s-sudakov@nphys.ru. S. K. Sudakov

have a sweet taste, so the rats can prefer such solutions to water [5].

This work was designed to examine the possibility to form the endogenous opioid dependence in rats during periodic drinking of 5% ethanol solution.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats (n=40) weighing 200-220 g at the beginning of the experiments. In 3 days prior to experiments, the rats were placed into individual ventilated cages (Tecniplast) with 2 drinking bottles and a feeder. The animals received fodder (Profgryzun, 3 kcal/g) and water *ad libitum* with a 12/12 h day/night cycle under controlled illumination regimen (16.00 to 04.00). All procedures with animals were carried out in strict adherence to Directive No. 267 of Ministry of Health of the Russian Federation ("On Establishing the Rules of Laboratory Practice"; June 19, 2003) and to the "Guidance to Works Involving Animals in Experiments" approved by the Ethics Committee of P. K. Anokhin Research Institute of Normal Physiology (Protocol No. 1, September 3, 2005).

The experiments were conducted according to the method described elsewhere [4]. In all groups, one of two drinking bottles was filled with water *ad libitum*. In control group (n=10) both drinking bottles contained water. For experimental rats, the second drinking bottle was filled with 5% ethanol solution for 12 h starting from 4 h after the onset of dark time. During other 12 h, the experimental rats were deprived of food and ethanol. This drinking regimen was administered for 8 days. The body weight and the amount of consumed food and fluid were measured daily. The ethanol preference ratio was calculated as the ratio of consumed ethanol to total amount of consumed fluid during 12 h. This parameter was used to subdivide the experimental rats into 3 subgroups: ethanol-preferring rats (n=8), water-preferring rats (n=8), and the intermediate group rats (n=14). In the following, only two first subgroups were used as the experimental rats. On experimental day 9 and after standard 12-h food deprivation (as in the control group), the rats were placed individually in a round open field for 20 min, where motor activity and individual symptoms of withdrawal syndrome (teeth-chattering, head shaking, forelimb tremor, and wet-dog shakes) were scored [12]. The total withdrawal syndrome index was calculated as the sum of individual symptom scores. In 30 min, the rats received intraperitoneal injection of 20 mg/kg naloxone and in another 30 min, they were again placed in the open field for 20 min to record the above indices.

The data were analyzed statistically using Statistica 10.0 software. Significance of differences was assessed using non-parametric Mann–Whitney U test and Pearson correlation test. The results are presented as m±SE.

RESULTS

Under conditions of periodic access to ethanol solution, Wistar rats demonstrated different preferences for water or ethanol. In ethanol-preferring group, the rats drank persistently no less than 30 ml ethanol solution for 12 h. On experimental day 8, the ethanol preference ratio in this group was 0.86±0.10. In water-preferring group, the rats persistently preferred water. On experimental day 8, the ethanol preference ratio in this group was 0.20±0.03. The rats of intermediate group sporadically drank water or ethanol solution, so the ethanol preference ratio in these animals varied near 0.5. These rats were excluded from the further study. The volumes of water and ethanol consumed during 8 days are shown in Figures 1 and 2.

When placed in the open field, the control rats (who drank only water) demonstrated only teeth-chattering and head shaking. After injection of naloxone, the rats demonstrated only rare episodes of teeth-chattering and wet-dog shaking (Table 1). At the same time, naloxone had no effect on total score.

The ethanol-preferring rats demonstrated the symptoms of withdrawal syndrome typical of opiates both prior to and after naloxone injection, and the score of these symptoms was greater than in the control rats (Table 1). In ethanol-preferring rats, naloxone elevated the total index of withdrawal syndrome from 5.1±0.4 to 9.6±1.2 (Fig. 3). At this, the latent period to the onset of motor activity in open field was significantly greater, whereas the total motor activity and the number of rearings were significantly smaller in ethanol-preferring rats in comparison with the control group (Table 2).

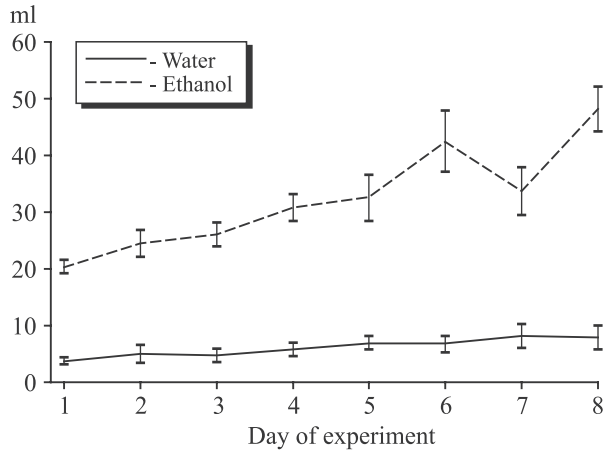

Fig. 1. Consumption of water and ethanol solution by ethanol-preferring rats.

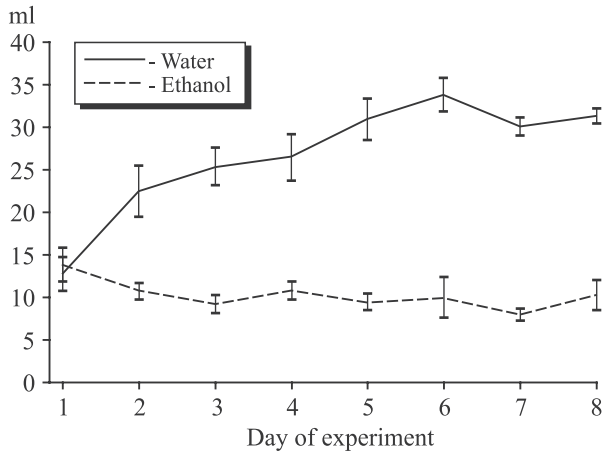

Fig. 2. Consumption of water and ethanol solution by water-preferring rats.

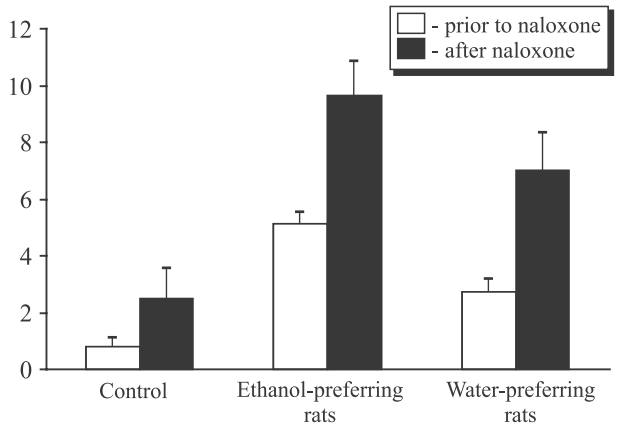

Fig. 3. Effect of naloxone on the total score of withdrawal syndrome in ethanol- and water-preferring rats.

Similarly, the water-preferring rats demonstrated significantly greater scores of withdrawal symptoms than the control animals (Table 1). In this group, naloxone elevated the total score of withdrawal syndrome from 2.8±0.4 to 7.0±1.4 (Fig. 3). In water-preferring rats, the latent period to onset of motor activity was significantly greater, whereas the total motor activity and the number of rearings were significantly smaller than the corresponding parameters in the control group (Table 2).

There was no correlation between ethanol preference ratio and the severity of withdrawal syndrome in ethanol- and water-preferring rats (r=-0.46 and r=-0.38, respectively), which suggests that the development of dependence is not related to taste preferences or amount of consumption of the preferred fluid.

Based on assumption that the key factor of development of endogenous opioid dependence is the release of β-endorphin and its binding to opioid receptors in the midbrain region, we further hypothesized that this dependence could develop during consumption of "tasty" solution despite the small amount of consumed fluid. However, it cannot be excluded that a large amount of endogenous opioids can release during waiting of reinforcement. Since the preferred solutions were presented in 4 h after the onset of dark period during 8 days, the rats could pleasantly anticipate the appearance of the tasty fluid for 4 h on day 9, which would be accompanied by binding of opioids with their receptors. Such mechanisms of "antedating reinforcement" were described previously [1]. At this, naloxone replaced the endog-

TABLE 1. Effect of Naloxone on Withdrawal Syndrome in Water- and Ethanol-Preferring Rats (m±SE)

Symptom	Control		Ethanol-preferring rats		Water-preferring rats	
	prior to naloxone	after naloxone	prior to naloxone	after naloxone	prior to naloxone	after naloxone
Head shaking	0.60±0.35	1.30±0.68	1.5±1.0	5.375±1.150	1.75±1.10	3.0±0.8
Forelimb tremor	0	0	0	0.20+0.13	0	0
Wet-dog shaking	0	0.070±0.35	1.75±0.64	4.125±0.490	0.625±0.350	2.875±1.000
Teeth-chattering	0.20±0.15	0.50±0.32	0.5±0.3	0.25±0.15	0.50±0.25	1.25±0.20

TABLE 2. Effect of Naloxone on Latent Period to Onset of Motor Activity, Total Motor Activity, and Rearing Score in Water- and Ethanol-Preferring Rats (m±SE)

Parameter	Control		Ethanol-preferring rats		Water-preferring rats	
	prior to naloxone	after naloxone	prior to naloxone	after naloxone	prior to naloxone	after naloxone
Latent period, sec	1.40±0.21	3.00±0.81	19.25±6.05	25.125±7.300	16.25±4.30	35.25±14.90
Locomotor activity, arb. units	72.9±7.70	8.8±1.8	14.50±3.05	5.375±1.400	7.5±1.4	6.25±1.60
Rearings	24.2±4.20	2.6±0.9	4.25±1.30	2.0±0.4	1.25±0.30	2.625±1.100

enous opioids and provoked the symptoms of withdrawal syndrome.

Thus, it seems logical to hypothesize that a mild dependence can develop in rats after persistent periodic voluntary intake of weak ethanol solution during 8 days. At this, the endogenous opioid dependence was formed not only in ethanol-preferring rats consuming a relatively large amount of ethanol solution but also in water-preferring rats consuming ethanol in small amounts.

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