# CHRONOPHARMACOLOGY OF DEVELOPMENT OF TOLERANCE TO THE NARCOTIC EFFECT OF ETHANOL IN A HOT CLIMATE

**T. Khodzhagel'diev, P. A. Khadzhiev, and O. D. Ovvadov**

UDC 615.212.7:547.262].015.4.07

**Key Words:** chronopharmacology; tolerance; narcotic effect; ethanol; behavioral activity.

The biorhythmologic approach to the study of the pharmacodynamics and pharmacokinetics of therapeutic substances is an important trend in contemporary clinical pharmacology. In Turkmenia acute ethanol poisoning is found more often in summer than in winter. In man, the psychophysiological state [7], sensitivity to pain [4], and effects of drugs [9] exhibit significant circadian and seasonal [12] fluctuations.

The aim of this investigation was to study the chronopharmacology of development of tolerance to the narcotic effect of ethanol relative to the behavioral activity of animals at different times of day and in different seasons or the year under the hot climatic conditions of Turkmenia.

## EXPERIMENTAL METHOD

Experiments were carried out on 400 noninbred male albino rats weighing 150-250 g. In the experiments of series I the intensity of the depressivelike state (DLS) arising in a situation of forced swimming for a period of 600 sec was studied at different times of the day in January (100 rats), and July (100 rats) [2, 3]. Rats whose total immobilization time (TIT) was under 100 sec were classified as high activity (HA), those with a TIT of over 240 sec as low activity (LA). The rats were tested at different times of day, using the same animals at the following tinle intervals: in the morning from 9:30 a.m. to noon (in the first week of January and July), in the afternoon from 2:00 to 4:30 p.m. (in the second week of January and July), and in the evening from 6:00 to 8:30 p.m. (in the third week of January and July). This schedule of study has been adopted in biorhythmology [6]. In the experiments of series II the same method was used to test HA and LA rats in January and July. The animals were tested from 9:30 a.m. to 12:30 p.m., starting on the day after subdivision of the rats into groups on the basis of their behavioral activity. For 10 days they received an intraperitoneal injection of 25% ethanol solution (4.5 g/kg) at 11:00 a.m. The intensity of alcohol narcosis was assessed by measuring the latent period and the duration of adoption of the side position, and changes in the body weight of the rats also were recorded. In the experiments of series III the plasma concentrations of noradrenalin, dopamine, adrenalin, and their metabolites were determined in rats tested in January, in LA and HA rats of the control group, and in the experimental group. The animals were killed and blood was taken from the experimental rats on the 10th day of the experiments after awakening from narcotic sleep. Concentrations of monoamines and their metabolites in the plasma were determined in the Laboratory of Biochemical Pharmacology, Research Institute of Pharmacology, Academy of Medical Sciences of the USSR, by the method in [1]. The results were subjected to statistical analysis by Student's $t$ test [10].

## EXPERIMENTAL RESULTS

Comparison of the behavioral activity of the animals in winter and summer revealed fluctuations (Fig. 1). DLS was observed from 2:00 to 4:30 p.m. in January (287.2 ± 10.7) in 40% of animals, whereas in the morning (262.5 ± 12.8) and evening (274.0 ± 9.69) the number of depressive (LA) animals fell to 25%. In summer (July) there was an even more marked increase in the number of LA animals, especially from 2:00 to 4:30 p.m. (to 50%; 298.9 ± 15.9), but in the morning (272.0 ±

Turkmenian Medical Institute, Ashkhabad. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 9, pp. 304-306, September, 1989. Original article submitted February 9, 1989.

  

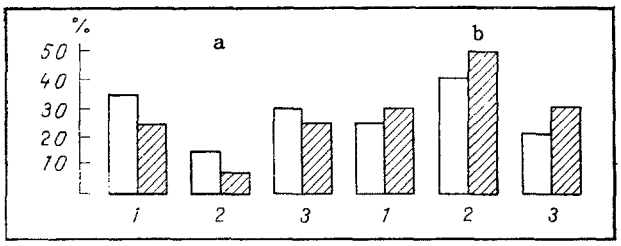


Fig. 1. Relations between animals tested by Porsolt's method at different times of day. a) HA rats, b) LA rats; 1) from 9:30 a.m. to noon; 2) from 2:00 to 4:30 p.m.; 3) from 6:00 to 8:30 p.m. Unshaded columns — January, shaded columns — July.

TABLE 1. Duration of Narcotic Sleep in Different Seasons of the Year for 10 Days after Injection of Ethanol (4.5 g/kg intraperitoneally)

| Day of experiment | Duration of narcotic sleep, min | | | |
|---|---|---|---|---|
| | January | | July | |
| | HA rats | LA rats | HA rats | LA rats |
| 1 | 87,3±5,0 | 133,5±19,0 | 132,4±9,5 | 162,1±10,3 |
| 2 | 78,9±12,0 | 142,9±8,9 | 122,5±11,2 | 153,9±8,3 |
| 3 | 80,0±7,8 | 110,0±9,8* | 180,6±18,6 | 142.8 (three rats)+ |
| 4 | 84,9±8,6 | 107,2±5,9** | 187,2±19,1 | 183,0 (one rat)+ |
| 5 | 63,5±5,0*** | 87,7±6,7* | 220,4 (four rats)+ | All animals died |
| 6 | 66,8±7,9* | 75,9±8,1* | 230,4 (two rats)+ | |
| 7 | 56,7±7,5*** | 68,0±9,7** | All animals died | |
| 8 | 61,9±5,8** | 81,1±5,0** | | |
| 9 | 41,7±5,2** | 84,9±4,8* | | |
| 10 | 47,6±3,0** | 68,9±6,5** | | |

11.1) and evening (259.8 ± 8.67) their number fell to 25 and 20%, respectively. In winter, from 2:00 to 4:30 p.m. the number of HA rats fell by comparison with the other times of day, and the fall was greatest in summer also.

The behavioral activity of the animals was found to depend on climatic conditions and on the circadian rhythm. It thus follows that when animal behavior is studied under Turkmenian conditions special attention must be paid to the time when the experiments are carried out.

The study of the development of tolerance to the narcotic effect of ethanol showed that in winter the food intake was significantly reduced and all animals showed a smaller increase in their body weight from the 4th-5th days of the experiment, and the effect was more marked in the HA rats. Significant lengthening of the latent period of onset of narcotic sleep was observed on the 9th-10th day of ethanol administration (in HA rats from 2.6 ± 0.2 to 6.5 ± 0.4 sec and in LA rats from 2.9 ± 0.2 to 6.4 ± 0.3 sec, respectively). No difference was observed in the rate of onset of ethanol narcosis in the rats tested in winter. However, a significant difference was found in the rate of development of tolerance to the narcotic effect of ethanol, for the duration of the side position of the HA in rats was reduced with effect from the 5th day of the experiment, whereas in LA rats the duration of narcotic sleep was appreciably reduced with effect from the 3rd day of ethanol administration (Table 1). The chronic toxicity of ethanol in narcotic doses was increased in winter for LA rats, for 28.6% of the animals died in the course of the experiment, especially on the last few days. In summer, ethanol in narcotic doses exhibited high toxicity, especially for LA rats (Table 1). Of 14 LA rats 11 had already died on the 3rd day of the experiment. HA rats were better adapted than LA rats to the toxic action of ethanol.

It can thus be concluded that in winter tolerance to the narcotic effect of ethanol develops under the conditions of Turkmenistan, irrespective of the level of the animals' behavioral activity. However, in animals with a high level of behavioral activity this process developed more slowly than in animals with a low level of behavioral activity. The results confirm the views expressed in [11] that behavioral biorhythms of animals can be used to predict the activity of psychotropic drugs, especially in relation to the development of tolerance to them. In summer the effective dose of ethanol (4.5 g/kg), accepted in

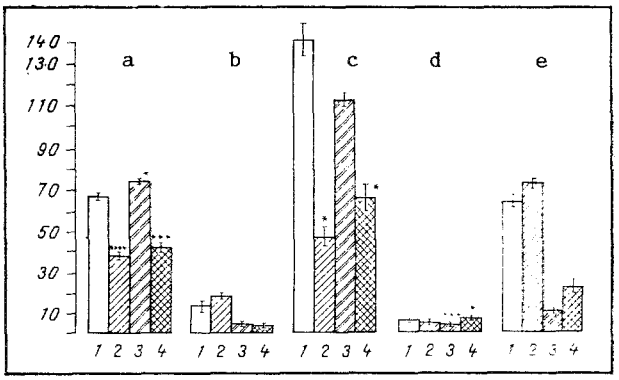


Fig. 2. Plasma concentration of noradrenalin (NA), adrenalin (A), dopamine (DA), and their metabolites (DOPA, dihydroxyphenylacetic acid — DHPAA) in rats after chronic administration of ethanol for 10 days in winter. a) NA, b) DOPA, c) A, d) DA, e) DHPAA; 1) LA rats; 2) LA rats receiving alcohol; 3) HA rats; 4) HA rats receiving alcohol. Ordinate, blood levels of mediators and their metabolites (in ng/ml).

Moscow at the Research Institute of Pharmacology, Academy of Medical Sciences of the USSR, is a toxic dose for animals under Turkmenian conditions. Ethanol has this kind of action also on animals with a low level of behavioral activity in winter.

The behavioral activity of animals is known to depend on their monoamine metabolism [5]. Mediators (noradrenalin, dopamine, adrenalin, serotonin, etc.) are known to play an important role in the regulation of animal behavior [5, 8]. We therefore studied concentrations of noradrenalin, dopamine, adrenalin, and their metabolites (DOPA, DHPAA) in the plasma of LA and HA rats, and also in the same rats after receiving daily injections of ethanol in narcotic doses for 10 days. The results showed that the blood noradrenalin level was raised in the HA rats but not in the LA animals (Fig. 2), while on the other hand, the dopamine concentration was significantly lowered. In animals with a low level of behavioral activity, increased destruction of mediators was observed in the blood. Under the influence of ethanol, significant falls in the plasma adrenalin and noradrenaain levels were observed in LA and HA rats. In HA rats after alcohol administration, however, the dopamine concentration was higher than in the control.

These investigations confirm the view that the behavior of animals depends on the ratio between their body monoamine levels. Ethanol changes relations between blood monoamine levels and behavioral activity of animals.

## LITERATURE CITED

1. N. A. Bondarenko, I. I. Miroshnichenko, V. S. Kudrin, and N. A. Bondarenko, Byull. Éksp. Biol. Med., No. 8, 168 (1988).
2. B. V. Burov, Vestn. Akad. Med. Nauk SSSR, No. 5, 72 (1982).
3. Yu. V. Burov, A. B. Kampov-Polevoi, and L. I. Kaminka, Zh. Vyssh. Nerv. Deyat., No. 5, 941 (1983).
4. E. O. Bragin and R. A. Durinyan, Patol. Fiziol., No. 5, 22 (1983).
5. A. V. Val'dman, Experimental Neurophysiology of Emotions [in Russian], Leningrad (1972).
6. G. D. Gubin and A. M. Durov, Chronobiology and Chronopathology [in Russian], Moscow (1981), p. 85.
7. V. P. Latenkov and G. D. Gubin, Biorhythms and Alcohol [in Russian], Novosibirsk (1987).
8. Yu. M. Ostrovskii, V. I. Satanovskaya, and M. N. Sadovnik, The Biological Component of Alcoholism [in Russian], Minsk (1986).
9. N. U. Plaksin and K. A. Meshcherskii, Abstracts of Proceedings of the 6th All-Union Congress of Pharmacologists [in Russian], Tashkent (1988), p. 253.
10. N. A. Plikhinskii, Biometrics [in Russian], Moscow (1970).
11. A. V. Popov and A. P. Popova, Abstracts of Proceedings of the 6th All-Union Congress of Pharmacologists [in Russian], Tashkent (1988), pp. 294-295.

12. T. Khodzhagel'diev, N. M. Mollaev, and M. A. Ataev, Abstracts of Proceedings of the First All-Union Congress of Toxicologists [in Russian], Rostov-on-Don (1986), pp. 508-509.

# ADAPTIVE CHANGES ON SIGMA- AND PHENCYCLIDINE RECEPTORS DURING LONG-TERM HALOPSRIDOL AND RACLOPRIDE TREATMENT IN RATS

**Ö. Ö. Vasar, A. É. Lang, J. E. Harro, and L. H. Allikmets**

UDC 615.214.2.015.4:612.82.014.467].076.9

**Key words:** haloperidol; raclopride; sigma receptors; phencyclidine receptors.

Derivatives of arylcyclohexylamine and benzomorphan possess a psychomimetic action [7, 9, 10]. Phencyclidine and other arylcyclohexylamines interact with phencyclidine receptors, whereas N-allylnormetazocine (SKF 10,047) and benzomorphans have highest activity for sigma receptors [8, 11]. Among the antipsychotic drugs, powerful antagonists of sigma receptors have been discovered; haloperidol has the highest affinity for these receptors [8, 12]. The view is held that selective antagonists of sigma receptors may prove to be potential antipsychotic drugs [13]. Neuroleptics do not interact with phencyclidine receptors in experiments in vitro [8, 12], but it has recently been shown that long-term haloperidol administration leads to a marked increase in number and reduction in affinity of phencyclidine receptors [2]. These facts are evidence of a possible role of sigma and phencyclidine receptors in the mechanism of action of neuroleptics.

The aim of this investigation was to study adaptive changes on sigma and phencyclidine receptors during long-term treatment with neuroleptics. Two different neuroleptics were chosen for this purpose: haloperidol, a high-affinity antagonist of sigma and dopamine$_2$-receptors, and raclopride, a selective dopamine$_2$-receptor antagonist.

## EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 200-250 g. Haloperidol (Gedeon Richter, Hungary) in a dose of 0.5 mg/kg daily, and raclopride (Astra Läkemedel AB, Sweden) in a dose of 1 mg/kg daily were injected intraperitoneally for 15 days. Experiments to study binding of $^3$H-thienylphenylcyclidine ($^3$H:TPC, "Dupont-NEN," USA; specific activity 60 mCi/mmole) and $^3$H-SKF 10,047 ("Dupont-NEN," USA; specific activity 40 mCi/mmole) were carried out 2 and 48 h after the last injection of the neuroleptics by the method in [8]. The forebrain of the rats (the brain stem and cerebellum were removed) was homogenized in 10 volumes of 50 mM Tris-HCl buffer (pH 7.7) at 20°C and centrifuged 3 times at 40,000*g* for 15 min. The membranes were incubated at 23°C for 30 min in the presence of 2 nM $^3$H-TPC, to study phencyclidine receptors, and in the presence of 10 nM $^3$H-SKF 10,047 to determine sigma receptors. Nonspecific binding of $^3$H-TFC was determined with the aid of ketamine (0.1-100 $\mu$M) and $^3$H-SKF 10,047, with the aid of haloperidol (2.5-10,000 mM). Binding was stopped by rapid filtration through Whatman 6F/B filters (treated with 0.1% polyethylamine solution), and the filters were then washed with 5 ml of cold incubation buffer (5 mM Tris-HCl, pH 8.1) at 20°C. The radioactivity of the samples was measured in Bray's scintillator by means of an LC-6800 beta counter ("Beckman," counting efficiency 50-53%).

In the behavioral experiments the action of ketamine (5 mg/kg), an agonist of phencyclidine receptors, and of apomorphine (0.15 mg/kg), an agonist of dopamine receptors, was investigated after long-term treatment with haloperidol and raclopride. The behavioral experiments were conducted 48 h after withdrawal of the neuroleptics. In the 15th minute after

Laboratory of Psychopharmacology, Tartu University. (Presented by Academician of the Academy of Medical Sciences of the USSR D. A. Eharkevich.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 9, pp. 306-309, September, 1989. Original article submitted October 10, 1988.

 0007-4888/89/0009-1270$12.50 