PHYSIOLOGY

Effect of Cerebrospinal Fluid Preparation from Opiate Addicts on Anxiety Level in Rats

N. E. Ordyan, V. Yu. Vasil'ev, N. N. Nesterov, V. V. Rakitskaya, I. A. Garina, S. G. Pivina, P. A. Pirumov, and V. G. Shalyapina

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 128, No. 7, pp. 13-16, July, 1999
Original article submitted June 26, 1998

Intranasal administration of a preparation isolated from the cerebrospinal fluid of opium addicts in the initial stage of withdrawal to rats reduced their exploratory activity and increased anxiety and nociceptive threshold in a tail-shock test.

Key Words: *opium addiction; rats; anxiety; nociception*

The role of cerebrospinal fluid (CSF) in the regulation of brain functions in the norm and pathology attracts now considerable attention. CSF contains a variety of bioactive compounds, in particular peptides and immunoglobulins identical to those of blood plasma [6], neurospecific proteins, neurohormones, unsaturated fatty acids, and other substances secreted by the ependyma and periventricular structures [2]. The neurotropic substances synthesized in the brain during drug or alcohol abuse can also be present in the CSF. Being products of drug addiction, these substances, on the other hand, can contribute to the maintenance of persistent pathology. This is evidenced by positive detoxifying effects of liquorosorption in drug addicts, alcoholics, and patients with different brain disorders. Preparations obtained during liquorosorption can be used for modelling of the corresponding pathology in animals and for identification of compounds responsible for pathological dominance. This study was designed to assess the effect of a CSF preparation isolated from opiate addicts on the level of anxiety in rats. Anxiety is one of the most important psychoemotional characteristics of the higher nervous activity in humans and animals [12]. We also assessed the effect of this preparation on the nociceptive threshold of electrical stimulation assuming that opioid peptides of endogenous or exogenous origin can constitute one of the active components of CSF in opiate addicts.

I. P. Pavlov Institute of Physiology, Russian Academy of Medical Sciences; City Hospital No. 32, St. Petersburg

MATERIALS AND METHODS

Male opiate addicts in the early period of withdrawal were treated by liquorosorption. All subjects were alert during the procedure. The preparation isolated after the first sorption procedure was used. Mass-exchangers (the columns with liquorosorbent) were washed with 150 ml distilled water at a rate of 5 ml/min and then centrifuged for 3-5 min at 2000 rpm to remove residual fluid. The absorbed compounds were eluted with 3 ml 0.05 M ammonia in ethanol. The eluate was transferred into test tubes and dried in a vacuum desiccator. The active fraction was extracted by double elution with 1 ml 96% ethanol. The total preparation was dried and stored at room temperature. Before testing the preparation was diluted with saline to physiologically optimal concentrations [3].

Experiments were carried out on male Wistar rats weighing 200-250 g. The animals were maintained under standard conditions with free access to food and water. The total preparation was administered intranasally in a volume of 5 μl. The control rats received

0007-4888/99/0007-0668$22.00

the same volume of saline. The level of anxiety was assessed in an elevated plus-maze (EPM). The maze was raised to a height of 75 cm and consisted of two open and two closed arms with the floor lined by 10×10 cm squares. The animals were placed in the EPM for 5 min and the following indices were recorded: horizontal (the number of crossed squares) and vertical (the number of rearings) motor activities, the time spent in the open arms, and the number of overhangs out of them, which is inversely proportional to anxiety level [11].

The nociceptive threshold was determined by the first tail flick after electroshock.

The data were analyzed statistically with Student's *t* test.

RESULTS

Bilateral intranasal administration of the preparation from CSF significantly reduced the exploratory activity, ambulation, and rearing in rats (Fig. 1, *a*). The time spent by animals in the closed arms considerably increased (Fig. 2, *b*), while the number of overhangs out of the open arms significantly decreased compared to the control rats (Fig. 2, *c*). The preparation significantly elevated the nociceptive threshold (Fig. 1, *b*).

Rodents are known to avoid open space and height instinctively [10] and these innate behavioral characteristics allow to use EPM for assessing the level of anxiety in rats. The decreased time of stay in the open arms and the reduced number of overhangs indicate an increased level of anxiety. Thus, the preparation not only suppressed exploratory activity but also increased anxiety in rats. Similar inhibitory activity of the preparation after its intranasal and intracerebroventricular administration was observed in the open field test [3].

When considering the mechanisms of the effects of CSF preparations on adaptive behavior, it should be kept in mind that catecholamines, in particular, dopamine, significantly contribute to the formation of drug addiction [4,14]. Therefore, the observed effects can primarily be attributed to the elevated content of dopamine and its metabolites involved in the regulation of mental activity and anxiety [12]. An important part can also be played by opioid peptides, which participate in the regulation of drug tolerance and addiction [7,9]. Intracerebroventricular administration of β-endorphins and other opioids was reported to affect motor activity in a dose-dependent manner causing immobilization followed by catalepsy [15]. Long-term intravenous administration of opiates, e. g. heroine, to rats did not change significantly the content of β-endorphin in the brain, but elevated its plasma concentration [7]. Similar changes in the content of opioid peptides probably occur in the CSF. The involvement of the

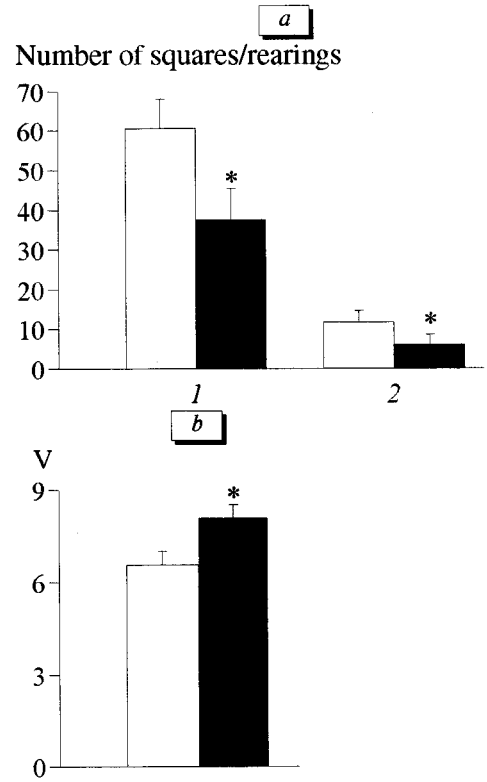

Fig. 1. Exploratory activity (*a*) and nociceptive threshold (*b*) in rats after intranasal administration of preparation from cerebrospinal fluid of opiate addicts. *a*: *1)* number of crossed squares; *2)* number of rearings. Here and in Fig. 2: Open bars: control (n=20); shaded bars: experiment (n=20). *p<0.05 in comparison with the control.

brain opioid system in the effect of CSF can also be suggested taking into consideration the intranasal route of drug administration. Sensory neurons of the nasal epithelium send their projections to the olfactory bulbs [13], a part of the brain opioid system which includes limbic and striopallidar structures, raphe nuclei, central grey matter, and nuclei of the trigeminal nerve [1]. These structures are functionally integrated in the system regulating nociception and analgesia and inhibiting motor activity and visceral functions. The increased nociceptive threshold in rats receiving the CSF preparation supports this suggestion. Thus, the observed effects of the preparation from CSF of opiate addicts can be explained by the presence of opiates which exert their inhibitory effects either directly or through the regulation of the release of GABA and excitatory amino acids. The same mechanisms can be implicated in its effect on anxiety regulated by the dopamine-, opioid- and GABA-ergic systems of the brain [12]. It cannot be excluded that some other components of the CSF of drug addicts contribute to the inhibitory action. They can include, for instance, unsaturated fatty acids, in particular arachidonic acid, a well-known inhibitory transmitter [5,8].

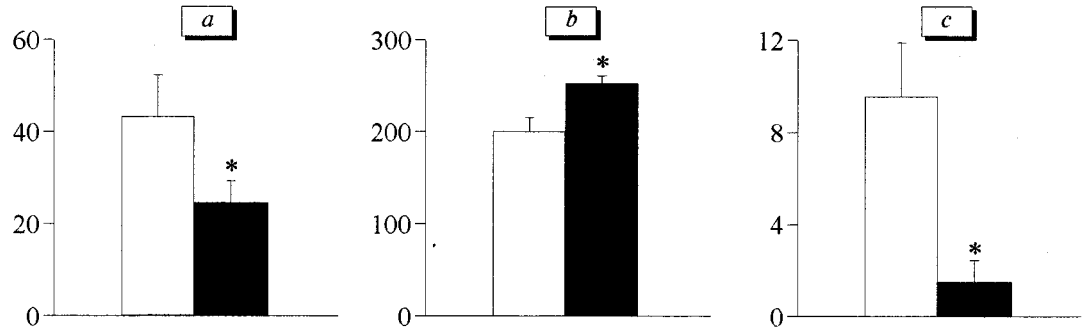

Fig. 2. Effect of intranasal administration of the CSF preparation on rat behavior in elevated plus-maze. *a:* time spent in open arms, sec; *b:* time spent in closed arms, sec; *c:* number of overhangs out of the open arms.

The data on the effects of the preparation isolated from CSF of opiate addicts on rat behavior indicate that this approach is promising for the analysis of factors involved in the formation of nosological symptoms typical of opium addicts.

REFERENCES

1. I. G. Akmaev and L. B. Kallimulina, *Amigdaloid Complex of the Brain: Functional Morphology and Neuroendocrinology* [in Russian], Moscow (1993).
2. G. A. Vartanyan and B. I. Klement'ev, *Chemical Symmetry and Asymmetry* [in Russian], Leningrad (1989).
3. V. Yu. Vasil'ev, I. B. Ivanova, N. N. Nesterov, *et al., Vestn St. Peterb. Univer., Ser Biol.*, No. 1, 25-31 (1998).
4. N. N. Ivanets, I. P. Anokhina, and N. V. Strelets, *Zh. Nevropatol. Psikhiatr.*, **97**, No. 9, 4-10 (1997).
5. A. N. Klimov and N. G. Nikul'cheva, *Lipids, Lipoproteins, and Atherosclerosis* [in Russian], St. Petersburg (1995).
6. E. Einstein, *Brain and Cerebrospinal Fluid Proteins in Norm and Pathology* [in Russian], Moscow (1998).
7. V. Berridge, *Annu. N. Y. Acad. Sci.*, **398**, 1-11 (1982).
8. H. R. Borne, *Nature*, **376**, 727-728 (1995).
9. D. De Wied and J. P. H. Burbach, *Acta Physiol. Scand., Suppl.*, **140**, 595 (1990).
10. J. A. Gray, *The Physiology of Fear and Stress*, Cambridge (1987).
11. S. Pellow, P. Chopin, S. File, and M. Briley, *J. Neurosci. Methods*, **14**, 149 (1985).
12. R. J. Rodgers, *J. Psychopharmacol.*, **5**, 316-319 (1991).
13. Th. F. A. Schoenfield, A. N. Clansy, W. B. Forbes, and F. Macrides, *Brain Res. Bull.*, **34**, 183-210 (1994).
14. T. Suzuki, M. Tsuda, M. Narita, *et al., Life. Sci.*, **58**, 349-357 (1996).
15. C. G. J. Sweep, V. M. Wiegant, J. De Vry, and J. M. Van Ree, *Ibid.*, **44**, 1133-1140 (1989).