Neurotransmitter Mechanisms of Morphine Withdrawal Syndrome

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Activity of the major neurotransmitter systems in the brainstem and cerebellum was studied in rats with morphine withdrawal syndrome. The most significant changes were found in the brainstem of animals by the 36th hour and 7 days after morphine withdrawal. Dysfunction was revealed in the dopaminergic, noradrenergic, and serotoninergic neurotransmitter systems. Changes in cerebellar neurotransmission were most pronounced by the end of the first week of morphine withdrawal syndrome and manifested in the prevalence of inhibitory processes.

Key Words: *morphine; brain; dopamine; norepinephrine; γ-aminobutyric acid*

Narcotic withdrawal after its long-term consumption is followed by the development of withdrawal syndrome. The severity of mental, autonomic, somatic, and neurological disorders depends on the drug type and duration of abuse. Morphine-like narcotics are most abundant [8] and therefore morphine withdrawal symptom are most prevalent in medical practice. Much attention was paid to studying the mechanisms of opium withdrawal syndrome [2,3]. However, the relationship between behavioral, somatic, and biochemical disturbances remains unknown.

Long-term use of morphine contributes to the formation of specific opioidergic neurotransmission [2]. The major property of this structure is activation of the reinforcement system in the brain, which results in massive release of catecholamines from depots [7]. The termination of morphine intake is followed by uncoupling of activity of this functional unit and appearance of well-known withdrawal symptoms (emotional discomfort, irritability, *etc*.). A close relationship exists between the major neurotransmitter systems in the brain. Therefore, any change in one of these systems induces changes in others. Dysfunction of the dopaminergic system in some brain structures of rats was revealed after prolonged intake and withdrawal of morphine [11]. Published data show that γ-aminobutyric acid (GABA) plays a role in the development of some symptoms of morphine withdrawal syndrome in the brain cortex of experimental animals [1]. However, there is no general agreement on a variety of neurotransmitter changes in the brain during withdrawal syndrome.

Here we measured the content of some neurotransmitters, their metabolites, and neurotransmitter amino acids in the brainstem and cerebellum of rats during the progression of morphine withdrawal syndrome.

MATERIALS AND METHODS

Experiments were performed on 40 male outbred albino rats weighing 180-200 g. They were divided into 5 groups (8 animals per group). The animals were housed in a vivarium and had free access to water and standard diet. Withdrawal syndrome was induced by an intraperitoneal injection of 1% morphine hydrochloride in doses of 10 mg/kg (days 1-2), 20 mg/kg (days 3-4), and 40 mg/kg (days 5-7). The animals were decapitated after 1 h (group 2), 36 h (group 3), 3 days (group 4), and 7 days (group 5) after the last injection of the narcotic. An equivalent volume of physiological saline (NaCl) was injected intraperitoneally to control rats of group 1. After decapitation, the brainstem and cerebellum were removed on ice and frozen in liquid nitrogen.

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The content of biogenic amines, their derivatives, and neurotransmitter amino acids was measured in chlorine extracts. Tissue samples (20-80 mg) were weighted and homogenized in 10 volumes of 0.2 M $HClO_4$ with internal standards for biogenic amines and their derivatives (vanillic acid, 400 nM) and amino acids and their derivatives (δ-aminovaleric acid, 0.25 mM), 50 mg/liter EDTA, and 50 mg/liter $Na_2S_2O_5$ (antioxidant).

The content of biogenic amines and their precursors and metabolites was measured on a Waters high-performance liquid chromatography (HPLC) system. It consisted of a M501 pump system and pulsation damper, TCM column thermostat, Rheoolyne 7125 injector, and M460 amperometric detector (Waters Assoc.) [5,10].

The content of biogenic amines and their metabolites was measured by ion-pair HPLC (Separon SGX C_{18} column, 5 μM, 3×15 mM (Elsico); mobile phase, 0.1 M KH_2PO_4; 17 mM CH_3COOH (pH 3.55); 200 mg/liter sodium heptyl sulfonate; 200 mg/liter sodium octyl sulfonate; 0.1 mM EDTA; and 11.5 vol % methanol) under the following conditions: 0.5 ml/min flow rate, 27°C column temperature, electrochemical detection, 0.78 V potential of the main electrode, and 2 sec time constant [5]. Calibration was conducted using a mixture of standards, which contained 100 μM Tyr, 10 μM Trp, and 1 μM other substances.

GABA was identified by the method of reverse-phase chromatography after precolumn derivatization with O-phthalic aldehyde and β-mercaptoethanol. Isocratic elution and fluorescence detection were performed using the same chromatographic system and M 420 fluorescence detector (Waters Assoc.) [10].

Chromatograms were recorded and analyzed on a Multi-Khrom-1 hardware-software system. Processing of chromatograms was performed by the internal standard method.

The results were analyzed by Student's *t* test (Statistica 7.0 software). Intergroup differences were significant at $p<0.05$.

RESULTS

Morphine withdrawal syndrome was accompanied by dysfunction of the major neurotransmitter systems in the brainstem and cerebellum of rats. It was manifested in variations in the content of some neurotransmitters, their metabolites, and neurogenic amino acids during various periods after narcotic withdrawal (Tables 1 and 2).

Dopamine content in the brainstem decreased by 29% ($p<0.05$) 1 h after the last injection of morphine (chronic morphine intoxication). The observed changes were accompanied by an increase in the concentration of dopamine metabolites, 3,4-dihydroxyphenylacetic

TABLE 1. Concentration of Neurotransmitters and Neurotransmitter Amino Acids (nmol/g tissue) in the Brainstem of Rats with Morphine Withdrawal Syndrome (*M*±*m*)

Parameter	Control (group 1)	Period of morphine withdrawal			
		1 h (group 2)	36 h (group 3)	3 days (group 4)	7 days (group 5)
Dopamine	0.883±0.060	0.627±0.055*	0.769±0.085	0.767±0.089	0.693±0.072
3,4-Dihydroxyphenylacetic acid	0.102±0.014	0.243±0.023*	0.321±0.035*	0.193±0.028	0.366±0.031*
Homovanillic acid	0.244±0.027	0.455±0.050*	0.539±0.042*	0.339±0.047	0.396±0.035*
Norepinephrine	2.711±0.240	1.723±0.140*	3.886±0.290*	3.14±0.37	1.698±0.130*
5-Hydroxytryptophan	0.460±0.048	0.541±0.064	0.633±0.052*	0.656±0.055*	0.897±0.077*
Serotonin	0.703±0.081	0.327±0.029*	0.520±0.076	0.352±0.029*	0.349±0.031*
5-Hydroxyindoleacetic acid	0.817±0.080	0.921±0.123	0.995±0.134	0.794±0.068	1.236±0.107*
GABA	2138.3±188.3	3387.6±291.5*	2571.8±309.8	2636.4±325.1	2479.2±286.4
Glutamate	6311.1±573.4	6953.4±686.1	3753.6±402.6*	3419.1±318.7*	4250.4±403.8*
Aspartate	1190.9±126.1	1237.3±195.9	2734.6±256.7*	1628.5±214.7	1373.8±221.8
Glycine	2081.5±182.3	2117.6±247.2	2156.3±295.7	1912.5±214.7	3219.7±240.6*

Note. Here and in Table 2: *$p<0.05$ compared to the control.

TABLE 2. Concentration of Neurotransmitters and Neurotransmitter Amino Acids (nmol/g tissue) in the Cerebellum of Rats with Morphine Withdrawal Syndrome (*M*±*m*)

Parameter	Control (group 1)	Period of morphine withdrawal			
		1 h (group 2)	36 h (group 3)	3 days (group 4)	7 days (group 5)
Dopamine	0.392±0.042	0.519±0.038*	0.569±0.049*	0.441±0.045	0.426±0.053
3,4-Dihydroxyphenylacetic acid	0.161±0.018	0.196±0.023	0.324±0.035*	0.203±0.023	0.212±0.028
Homovanillic acid	0.211±0.022	0.363±0.029*	0.439±0.036*	0.274±0.033	0.291±0.037
Norepinephrine	0.907±0.121	1.066±0.161	1.199±0.173	0.973±0.128	0.961±0.134
5-Hydroxytryptophan	0.290±0.037	0.318±0.041	0.309±0.045	0.244±0.033	0.263±0.040
Serotonin	0.225±0.019	0.303±0.035	0.246±0.037	0.170±0.015*	0.152±0.017*
5-Hydroxyindoleacetic acid	0.152±0.018	0.294±0.026*	0.231±0.036	0.247±0.032	0.317±0.029*
GABA	1060.3±131.6	2271.3±189.5*	1206.3±137.5	1113.6±147.2	1105.2±139.4
Glutamate	9422.3±909.4	5988.7±620.7*	8361.0±1032.1	8772.5±926.2	6495.5±610.5*
Aspartate	1048.4±127.3	839.6±96.8	937.8±121.9	956.7±134.6	631.2±66.3*
Glycine	1152.2±133.1	1235.3±207.6	1317.2±104.9	1009.6±164.1	1235.4±119.4

acid (by 138%, $p<0.001$) and homovanillic acid (by 86%, $p<0.02$). These changes are probably related to the increased degradation of catecholamines during forced administration of the narcotic [8] and realized via catecholamine depletion in the brain. In group 2 rats, the concentrations of norepinephrine and serotonin decreased significantly (by 37 and 54%, respectively), while GABA content in the brainstem increased (Table 1).

Neurotransmitter changes in the cerebellum of group 2 animals were similar to those in the brainstem (Table 2). The concentrations of GABA and homovanillic acid increased by 114 and 72%, respectively. As differentiated from the brainstem, dopamine concentration was elevated in the cerebellum of these rats. It was probably associated with different density of the corresponding neurons in some regions of the brain.

Neurotransmitter changes in the brainstem were more pronounced in group 3 animals, which correlated with behavioral symptoms of withdrawal. Although the concentration of dopamine returned to normal, the content of dopamine metabolites remained high (Table 1). The concentration of one of the key neurotransmitters, norepinephrine, was elevated in rats of this group (as differentiated from group 2 animals). Despite the increase in 5-hydroxytryptophan concentration (by 37%), serotonin content in group 3 rats did not differ from that in control animals.

Change in norepinephrine concentration in group 3 rats (compared to group 2 animals) is of particular interest. In addition to dopamine, this neurotransmitter plays a key role in the adrenergic system [6]. Dopamine is an immediate precursor of norepinephrine. The increase in norepinephrine concentration after 36-h withdrawal probably results from the decrease in dopamine content in an earlier stage of morphine withdrawal (Table 1). The bodies of noradrenergic neurons are mainly located in the brainstem [9]. Therefore, much attention should be paid to the observed changes. The concentration of glutamate decreased, while the content of aspartate increased in the brainstem of group 3 animals (Table 1).

As differentiated from the brainstem, neurotransmitter content in the cerebellum remained practically unchanged after 36-h withdrawal (Table 2). Dysfunction of the dopaminergic system manifested in a statistically significant increase in the concentration of dopamine (by 45%) and its metabolites, 3,4-dihydroxyphenylacetic and homovanillic acids (by 101 and 108%, respectively).

Relative compensation of neurotransmitter changes in brain structures was observed by the end of the 3rd day of morphine withdrawal. The concentration of 5-hydroxytryptophan increased (by 42%), while the content of serotonin and glutamate in the brainstem decreased during this period (by 50 and 46%, respectively). Serotonin concentration was reduced in the cerebellum of group 4 animals (Table 2).

The reduction of neurotransmitter changes on day 3 of morphine withdrawal is consistent with published

data that withdrawal syndrome proceeds in stages [3]. The period of severe symptoms of withdrawal syndrome (group 3) is followed by unstable equilibrium. During this stage, any exogenous factor can cause exacerbation of withdrawal symptoms.

This hypothesis is conformed by the results of our experiments. Dysfunction of the neurotransmitter systems in the brainstem and cerebellum was observed not only 36 h after narcotic withdrawal, but also on day 7 of the post-withdrawal period (Tables 1 and 2). These changes were typical of all neurotransmitter systems, particularly in the brainstem. The concentrations of 3,4-dihydroxyphenylacetic and homovanillic acids in group 5 animals were 358 and 162%, respectively, compared to the control level (Table 2). These data serve as indirect evidence that the dopaminergic system plays a primary role in the development of morphine withdrawal symptoms [2]. Our findings illustrate the latency and regional distribution of neurotransmitter changes in the brain. The concentration of norepinephrine decreased (by 38%), while the content of 5-hydroxytryptophan increased in group 5 animals. These changes were accompanied by a 51% decrease in serotonin concentration. The decrease in glutamate concentration and increase in glycine content in group 5 animals reflect the prevalence of inhibitory processes in the brainstem on day 7 of morphine withdrawal syndrome (Table 1).

Neurotransmitter changes in the cerebellum were less pronounced than in the brainstem on day 7 of withdrawal (Table 2). Functional activity of the dopaminergic and noradrenergic system remained practically unchanged under these conditions. The decrease in serotonin concentration (by 33%) was followed by the increase in 5-hydroxyindoleacetic acid content (Table 2). Symptoms of inhibition were revealed in the cerebellum of group 5 animals (similarly to the brainstem). It was manifested in a decrease in the concentration of major excitatory amino acids, glutamate and aspartate (Table 2).

We conclude that morphine withdrawal syndrome is accompanied by neurotransmitter changes in the brainstem and cerebellum of rats. The most significant changes in the brainstem are revealed by the end of the 36th hour and 7 days after narcotic withdrawal. These changes are found in the dopaminergic, noradrenergic, and serotoninergic neurotransmitter systems. Changes in cerebellar neurotransmission are most pronounced by the end of the first week of morphine withdrawal syndrome and manifested in the prevalence of inhibitory processes. These data provide new knowledge on the role of brain neurotransmitters in the appearance of morphine withdrawal symptoms. Our results hold promise for the planning and conduction of therapeutic procedures.

REFERENCES

1. Kh. Abazid and V. V. Lelevich, *Vestsi NAN Belarusi. Ser. Med. Navuk*, No. 2, 44-48 (2005).
2. I. P. Anokhina, A. G. Veretinskaya, G. N. Vasil'eva, and I. V. Ovchinnikov, *Fiziol. Chel*., **26**, No. 6, 74-81 (2000).
3. A. B. Gekht, A. G. Polunina, E. A. Bryun, and E. I. Gusev, *Zh. Nevrol. Psikhiatr*., **103**, No. 2, 9-15 (2003).
4. A. I. Golovko, D. A. Konoplin, Yu. A. Romanenko, and M. A. Strel'skii, *Neirokhimiya*, **17**, No. 1, 3-12 (2000).
5. E. M. Doroshenko, L. I. Nefedov, and I. I. Klimovich, *Zdravookhr. Belarusi*, No. 12, 20-23 (1994).
6. V. V. Lelevich, *Neurochemistry* [in Russian], Grodno (2008).
7. S. V. Lelevich, *Metabolic Aspects of Morphine Addiction* [in Russian], Grodno (2007).
8. A. F. Meshcheryakov and S. K. Sudakov, *Vopr. Narkol*., No. 2, 33-39 (1991).
9. *Neurochemistry* [in Russian], Eds. I. P. Ashmarin and P. V. Stukalov, Moscow (1996).
10. L. I. Nefedov, E. M. Doroshchenko, and V. Yu. Smirnov, *Ukr. Biokhim. Zh.*, **68**, No. 1, 21-26 (1996).
11. A. Herz, T. S. Shippenberg, R. Bals-Kubik, and R. Spanagel, *Arzneim-Forsch*, **42**, No. 2A, 256-259 (1992).