Ketamine-Induced Rotational Asymmetry in Evaluation of Motor Disturbances in Rats with Middle Cerebral Artery Occlusion

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 135, No. 5, pp. 500-504, May, 2003
Original article submitted November 12, 2002

Intraperitoneal injection of ketamine in subanesthetic doses to Wistar rats with unilateral occlusion of the middle cerebral artery caused ipsilateral rotation (2-10 rpm), which was recorded in an automatic rotameter. The optimal dose of ketamine was 50 mg/kg. The animals were examined in an automatic rotameter for 40 min. Motor asymmetry persisted for no less than 2 months after surgery. According to the neurological test (Menzies scale) motor asymmetry in animals with focal brain ischemia persisted for no more than 30 days. The degree of ketamine-induced motor asymmetry in intact rats was 0.10±0.03 rpm.

Key Words: *ketamine; middle cerebral artery occlusion; motor asymmetry; automatic rotameter*

Chronic stage of ischemic stroke is accompanied by persistent neurological deficit. This disorder is hardly correctable and often causes disability. Neurotransplantation of donor neural cells substituting lost nervous tissue holds much promise for the therapy of stroke consequences. The procedure was successful during the therapy of several patients with stroke [1,2]. Experiments on animals demonstrated the possibility of using this method for correction of focal brain ischemia caused by middle cerebral artery occlusion (MCAO) [2-4,5]. It is difficult to evaluate objectively the efficiency of correction of neurological deficit in rats with focal brain ischemia, because of rapid compensation of neurological disorders in these animals (within 3 weeks after MCAO) [5,6]. Moreover, traditional methods for studying motor and neurological disorders in rats with MCAO based on subjective evaluation (Menzies—Bederson and Hirakawa scales) [7,8].

It is necessary to elaborate the objective (instrumental) method for evaluation of motor disorders in rats with MCAO, which can be used to study the dynamics of pathological processes and efficiency of experimental therapy over a long time after surgery (several months). In our experiments functional load on brain receptors was produced by intraperitoneal injection of ketamine. Ketamine, a nonselective antagonist of N-methyl-D-aspartate (NMDA) glutamate receptors, binds to phencyclidine sites of these receptors and blocks Na^+ channels. This compound also interacts with opioid receptors and affects acetylcholinesterase and monoamine transporters. However, ketamine is characterized by highest affinity for NMDA receptors [9]. Autoradiography showed that ketamine in subanesthetic doses activates limbic structures in rodents (entorhinal, perirhinal, and piriform cortex and molecular layer of the dentate gyrus) [10]. The effects of ketamine on brain receptor systems involved in the genesis and performance of movements suggests that this compound aggravates imbalance in receptor systems and produces motor asymmetry under conditions of unilateral massive loss of the nervous tissue after MCAO. Here we tested this hypothesis.

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0007-4888/03/1355-0424$25.00

MATERIALS AND METHODS

Experiments were performed on 59 adult male Wistar rats weighing 290-310 g. The animals were kept under natural light-dark cycle and had free access to water and food. Surgery, cerebral perfusion, and euthanasia were performed under ketamine anesthesia (100 mg/kg). The experiment was performed in 3 stages.

In stage I focal brain ischemia was produced by MCAO. Changes in body weight and neurological status of rats were determined over 2 months after surgery (MCAO group, n=20) and compared with parameters in animals subjected to sham-operation of artery occlusion (SMCAO, n=10). Brain tissue damage was morphologically verified at various terms after surgery (MCAO, 10 rats; SMCAO, 6 rats). In stage II motor activity of intact rats receiving ketamine in subanesthetic doses (30 and 50 mg/kg) 5 days before focal brain ischemia was studied in an automatic rotameter.

In stage III ketamine-induced rotational asymmetry in rats of both groups was studied every 10 days for 2 months after MCAO or SMCAO. The direction of rotational asymmetry and distribution of animals depending on the degree of rotational asymmetry after treatment with ketamine (30 and 50 mg/kg) and duration of the test (30-60 min) were evaluated.

MCAO was modeled by the method of Tamura with modifications of Bederson [8]. A 5-6-mm segment of the middle cerebral artery (MCA) was electrocoagulated (from its branching from the left internal carotid artery to the point localized 3-4 mm distally to the line of MCA intersection with the olfactory tract). SMCAO included all surgical manipulations except electrocoagulation.

For evaluation of motor asymmetry the rats were intraperitoneally injected with 5% ketamine (30 and 50 mg/kg) and placed in a half-spherical automatic Ungerstend rotameter for 60 min. The computerized device separately recorded rotations in both directions. We calculated the difference between the number of leftward and rightward rotations per 1 min (K-test) during 30-, 40-, 50-, and 60-min testing.

Neurological status of rats was estimated by the method of Menzies [8] and expressed in points: no deficit (0), tonic flexion of the contralateral forelimb during tail suspension (1), low resistance of the contralateral forelimb to passive movement during tail stretch on a horizontal surface (2), contralateral movement during tail pinch (3), and spontaneous contralateral rotation of animals on a horizontal surface (4). The total score of neurological deficit was determined.

For histomorphological examination the rats were consecutively perfused with 200 ml phosphate buffered saline (PBS) and 400 ml 4% paraformaldehyde in PBS through the ascending aorta. The brain was removed and placed in 4% paraformaldehyde. Brain samples were kept in 20% sucrose and serial sections were prepared on a freezing microtome. Histomorphological examination included light microscopy of sections (20 μ) stained with cresyl violet by the method of Nissl.

The results were analyzed by Student's t test and Fischer's U test.

RESULTS

MCAO produced focal brain ischemia, which was morphologically manifested in extensive infarction in the caudal putamen and cortex followed by its organization and formation of scar or cavity. Body weights in rats subjected to MCAO and SMCAO significantly differed starting from day 5 after surgery (p>95%). This difference progressively increased throughout 60-day observations. The rats of the MCAO group were characterized by prolonged postoperation decrease in body weight over the first 15 days, while in animals with SMCAO this parameter returned to normal on days 2-3 after surgery.

Neurological status differed in rats of MCAO and SMCAO groups (Fig. 1). One day after surgery neurological deficit in rats with MCAO was 3-4 points in 18 animals and 2 points in 2 rats. Then neurological status spontaneously recovered: on day 15 after surgery neurological deficit in MCAO rats decreased by 2 times and was <1 point by the middle of the second month. Mild neurological abnormalities in animals of the SMCAO group observed over the first week after surgery (<1 point) were related to transitory local edema of the brain.

Preliminary experiments on 10 intact rats showed that intraperitoneal injection of ketamine in subanesthetic doses produced locomotor excitation. These changes developed 1-2 min after treatment and persisted for 15-20 (30 mg/kg) or 30-40 min (50 mg/kg). Testing in a rotameter for 40 min revealed no laterali-

TABLE 1. Rotametry of Intact Rats after Administration of Ketamine in Subtherapeutic Doses

Rotation, rpm	Ketamine, dose			
	30 mg/kg		50 mg/kg	
	n	%	n	%
0.0-0.5	15	52	11	35
0.6-1.0	6	21	8	27
1.1-1.5	5	17	5	18
>1.6	3	10	6	20

Note. Ratio of rats with various degrees of rotational asymmetry, %.

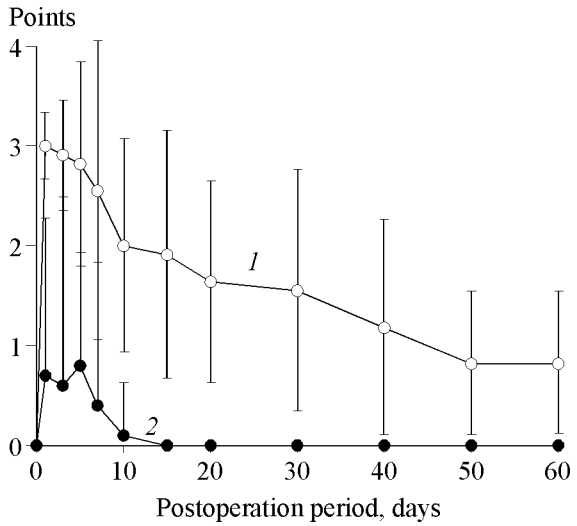

Fig. 1. Neurological status of rats after middle cerebral artery occlusion (MCAO) and sham-operation of occlusion (SMCAO, X_m, points).

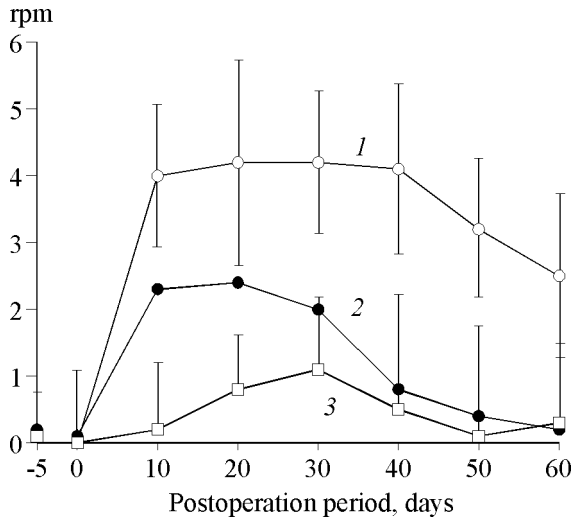

Fig. 2. Rotational asymmetry in rats with focal brain ischemia after intraperitoneal injection of ketamine in various doses (X_m, rpm): MCAO, 50 mg/kg ketamine (*1*); MCAO, 30 mg/kg ketamine (*2*); and SMCAO, 50 mg/kg ketamine (*3*). Here and in Fig. 3: 0 days, surgery.

zation of rotations (leftward or rightward movements). After injection of ketamine in doses of 30 and 50 mg/kg the mean values of K-test were 0.10±0.03 and 0.10± 0.02 rpm, respectively (p<95%). Locomotor activity of 59 intact animals was studied in a rotameter over 40 min after treatment with ketamine 5 days before modeling of focal brain ischemia. The degree of physiological motor (rotational) asymmetry produced by ketamine in a dose of 30 mg/kg (n=29) was lower than that observed after treatment with 50 mg/kg ketamine (n=30, Table 1). The number of rats with rotational asymmetry 0.6-1.0, 1.1-1.5, or >1.6 rpm was higher after injection of ketamine in a dose of 50 mg/kg. After treatment with ketamine in a dose of 50 mg/kg the percent of animals with motor asymmetry >0.5 rpm surpassed that observed after administration of 30 mg/kg ketamine (65 and 48%, respectively). It should be emphasized that in 3 intact rats (5%) the value of K-test was >2 rpm (50 mg/kg, n=2; 30 mg/kg, n=1). These animals were excluded from further observations.

On day 10 after MCAO injection of 50 mg/kg ketamine produced general locomotor excitation (5-10 min), which was followed by a short period of depression (5-7 min). Then locomotor activity increased. These changes were not observed after injection of 30 mg/kg ketamine. It should be noted that locomotor excitation produced by ketamine in both doses was accompanied by lateralization of movements toward the injury side (2-10 rpm). The animals rotated ipsilaterally in relation to the side of MCAO. The frequency of rotations was maximum 40 min after administration of 50 and 30 mg/kg ketamine (4.0±0.6 and 2.3±0.7 rpm, respectively). After injection of 50 mg/kg ketamine the percentage of rats with rotation frequency >2 rpm 2-fold surpassed that observed after treatment with 30 mg/kg ketamine (80 and 40%, respectively, P_Δ 92%).

On day 10 after SMCAO the value of K-test in rats receiving 50 mg/kg ketamine did not differ from the control (0.20±0.01 rpm). These animals demonstrated ipsilateral rotation. In 9 of 10 animals the frequency of rotation did not exceed 2 rpm; only in 1 rat this parameter was >2 rpm.

Parameters of K-test were recorded over 2-month recovery after MCAO (Figs. 2 and 3).

The animals with focal brain ischemia were characterized by ipsilateral ketamine-induced rotational asymmetry. Ketamine in a dose of 50 mg/kg produced more pronounced changes compared to 30 mg/kg ketamine. The differences were significant on days 40, 50,

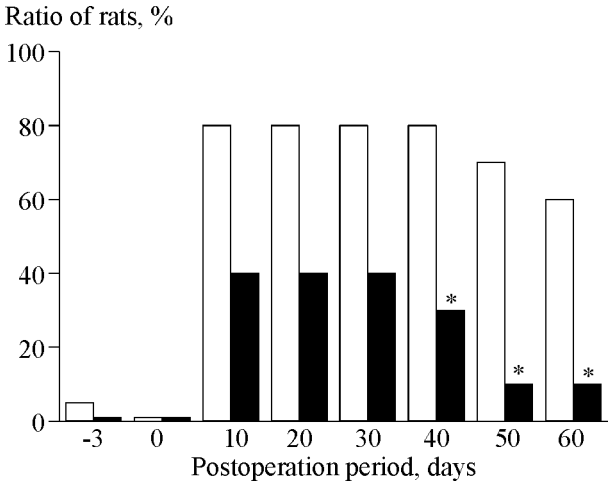

Fig. 3. Ratio of rats (%) with rotational asymmetry >2 rpm after intraperitoneal injection of ketamine in doses of 50 (light bars) and 30 mg/kg (dark bars). *p>95%.

and 60 (p>95%). Rotational asymmetry persisted over 60 days after injection of 50 mg/kg ketamine. However, motor asymmetry was observed only for 30-40 days after treatment with ketamine in a dose of 30 mg/kg (Fig. 2). On days 40-60 the number of animals with K-test >2 rpm was higher after treatment with 50 mg/kg ketamine (Fig. 3). After injection of ketamine in a dose of 50 mg/kg the percentage of rats with pronounced motor asymmetry (K-test >4 rpm) was higher than after treatment with 30 mg/kg ketamine (80 and 40%, respectively, p=92%). We revealed no considerable differences in postoperation changes in K-test in rats with focal brain ischemia tested in an automatic rotameter for 30-60 min. However, values of K-test after testing for 30 and 40 min were slightly higher compared to those observed after testing for 50 and 60 min.

In the group of sham-operated rats we observed only a tendency to ipsilateral asymmetry on days 20-40 after surgery. However, the degree of ipsilateral asymmetry did not exceed 1 rpm (Fig. 2).

These data show that physiological ketamine-induced rotational asymmetry was practically absent in adult intact Wistar rats. In individual specimens the value of K-test was relatively high. It necessitates preexamination of animals by K-test before the formation of experimental groups. Ketamine in subanesthetic doses induced ipsilateral rotational asymmetry in rats with focal brain ischemia. The optimal dose of ketamine was 50 mg/kg. The animals were examined in an automatic rotameter for 40 min.

The observed changes are probably related to receptor asymmetry that results from unilateral focal ischemia of the brain. MCAO is accompanied by injury to brain regions involved in the genesis and performance of movements, *e.g.*, somatosensory cortex, striatum, and limbic structures (entorhinal cortex) [8]. These changes are followed by a decrease in the number of receptors responsible for the realization of motor functions (NMDA and other receptors). Therefore, ketamine is suitable for evaluating the degree of receptor and motor imbalance during focal brain ischemia.

Our results show that studies of ketamine-induced rotational asymmetry can be useful in the diagnostics of motor asymmetry during cerebral dysfunction. K-test is more sensitive than traditional neurological methods. Motor asymmetry in rats with focal brain ischemia can be detected by standard methods over 20-30 days after MCAO. K-test allows us to evaluate these disturbances for at least 2 months.

Motor asymmetry caused by ketamine in subanesthetic doses can be used not only for modeling macrofocal organic damage to the brain, but also as a marker of the efficiency of experimental therapy and functional recovery of CNS in individuals with diffuse pathological processes in brain (*e.g.*, hypoxia/ischemia in the perinatal period).

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