Effect of the Calcium Channel Blocker Nifedipine on Anxiety and Seizure Manifestations of the Abstinent Syndrome in Rats after Discontinuation of Prolong Diazepam Administration

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Discontinuation of prolong administration of diazepam to Wistar rats led to enhancement of anxiety in the conflict situation test and to rise in convulsive readiness in the pentetrazolum kindling test. The blocker of calcium channels nifedipine removed the manifestations of the withdrawal syndrome: it moderated anxiogenesis and the convulsive reactivity in rats abstinent to diazepam. The data testify to participation of L-type Ca channels in the development of dependence to benzodiazepine anxiolytics.

Key Words: *benzodiazepine anxiolytics; withdrawal syndrome; Ca channel blockers; convulsive reactions; anxiogenesis*

The last decade yielded increasing amount of data indicating that Ca channel blockers (CCB), and specifically, the dihydropyridine derivatives nimodipine, nifedipine, nicardipine, and others, in addition to basic antiangiospastic and antihypertensive properties have the central effects. When penetrating into the brain, CCBs bind to the specific recognition sites (receptors) which are an integrative part of the voltage-dependent Ca channels. There is a number of experimental data of participation of Ca channels in the mechanisms of drug action and alcohol abuse. It is also corroborated by the evidence on the ability of CCBs to prevent and arrest the withdrawal syndrome to morphine, barbiturates, and alcohol [2,3,12].

Prolong usage of benzodiazepine anxiolytics provokes the development of tolerance, while drastic discontinuation of the treatment may result in the withdrawal syndrome, which is manifested in anxiety, aggressiveness, an enhanced convulsive readiness, weight loss, and decrease in food consumption [6,15].

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Our aim was to study the effect of the CCB nifedipine on anxiety and convulsive manifestations of the withdrawal syndrome in rats provoked by drastic discontinuation of prolong administration of diazepam.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats weighing 200-250 g at the beginning and 250-300 g at the end of the experimental protocol. Diazepam (Sigma) was suspended in Tween-80 and was administered intraperitoneally in a dose of 4 mg/kg at the same daytime during 28 days. The control rats obtained only 5% Tween-80. The manifestations of the withdrawal syndrome were evaluated 24, 48, 72, and 96 h after the last injection of diazepam. The level of anxiety was determined in the conflict situation [13]. After a 24-h deprivation, the rats were placed into a chamber where during 5 min they were conditioned to take water from a fixed drinking bowl. On the next day, electrical current (0.5 mA) was applied to the drinking bowl, and the number of punished drinkings was counted during 5 min.

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Convulsive readiness was evaluated in the pentetrazolum kindling test. A subthreshold dose of pentetrazolum (25 mg/kg), which provoked convulsive seizures in 5% of rats, was injected intraperitoneally during 4 days repeatedly with the interval between injections of no less than 24 h. The intensity of seizures was evaluated using a five-point scale: no manifestations (0), single seizures, tremor (1), repetitive twitching, "roll of a drum" (2), tonic seizures, fall on a side (3), clonic seizures, extension (4), and death (5).

Thirty minutes prior to testing, nifedipine (Sigma) suspended with Tween-80 was injected intraperitoneally in a dose of 10 mg/kg.

The results were statistically analyzed using Student's t test.

RESULTS

Discontinuation of prolong administration of diazepam modified rats' behavior. In the conflict situation, the number of water takings decreased to 11.7±1.9 compared with the control value of 22.7± 4.0 ($p<0.05$). This testifies to the development of anxiety. Nifedipine completely eliminated the manifestations of enhanced anxiety and significantly increased the number of punished drinkings to 40.1± 14.1, which was more than the absolute control value ($p<0.05$).

The spontaneous convulsive abstinent syndrome did not appear under the conditions of prolong administration of diazepam for 28 days in a daily dose of 4 mg/kg. When convulsive reactions were evoked by pentetrazolum in the kindling model, they developed after discontinuation of diazepam in a cascade manner and at much greater rate than in the control. Nifedipine injected to the abstinent rats 30 min prior to pentetrazolum prevented the withdrawal syndrome; the indices of seizure intensity were virtually the same as in the control (Fig. 1).

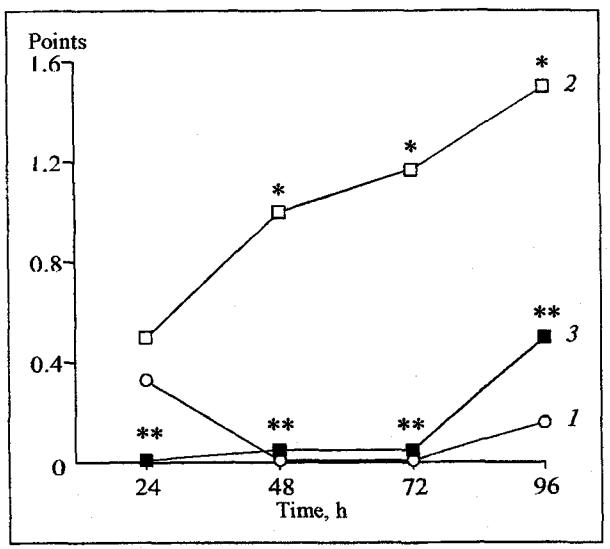

Fig. 1. Effect of nifedipine on intensity of seizures in the pentetrazolum kindling test in rats after discontinuation of diazepam. *1*) control group; 2) after discontinuation of prolong administration of diazepam, 3) after administration of nifedipine (10 mg/kg) against the background of discontinuation of diazepam. $p<0.05$: *in comparison with control; **in comparison with 2.

Thus, nifedipine (10 mg/kg) removed high-level anxiety and convulsive reactivity occurring in rats after discontinuation of diazepam.

The observed anxiogenic and convulsive manifestations of the withdrawal syndrome after discontinuation of a prolong diazepam administration were also found in the study of benzodiazepine anxiolytics in various behavioral models [1,4,15]. According to the hypothesis on neurochemical heterogeneity of the benzodiazepine abstinent syndrome, each of its manifestations is based on an individual neurochemical (i.e. neurotransmitter) [1]. The ability of the dihydropyridine CCB nifedipine to arrest anxiety and convulsive readiness in diazepam-abstinent rats, prevention of convulsive reactions in flurazepam-abstinent mice by nitrendipine [4], and the ability of verapamil to antagonize the anxiogenesis in rats after discontinuation of diazepam [7] imply the participation of L-type Ca channels in basic mechanisms of adaptation of CNS to prolong administration of benzodiazepines. It should be noted that nifedipine and nitrendipine have neither anticonvulsive no anxiolytic potency in intact animals [4,11], which testifies to selectivity of the CCB effects in relation to abstinent symptoms caused by discontinuation of benzodiazepines. Another evidence of participation of Ca channels in the development of benzodiazepine dependence is that nifedipine arrests both anxiogenic and convulsive manifestations of the withdrawal syndrome. These hypotheses are confirmed by the significance of functional state of Ca channels in realization of some effects of benzodiazepines, as well as the effect of prolong administration on the voltage-dependent calcium current [8].

Calcium channel blockers prevent tolerance and withdrawal syndrome to alcohol under conditions of competitive administration [14], while their combined administration with benzodiazepines has no essential affect on the development of tolerance [4]. One can conclude that realization of adaptive processes in the CNS in response to prolong administration of benzodiazepines involves the voltage-dependent Ca channels to a lesser degree than in case of alcohol.

Our results provide additional evidence for the modern hypotheses that modification of the Ca channel functions may be one of the general mechanisms of CNS tolerance to depressants, the benzodiazepine anxiolytics included [9,10].

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