Modulating Role of NO in Haloperidol-Induced Catalepsy

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Injection of haloperidol during catalepsy modeling decreased the content of NO in rat cerebral cortex. NO precursor L-arginine arrested catalepsy and prevented the decrease in NO content.

Key Words: *catalepsy; haloperidol; L-arginine; nitrogen oxide; electron paramagnetic resonance*

According to modern concepts, the main property of neuroleptics determining their efficiency in the treatment of psychoses is their capacity to inhibit dopaminergic neurotransmission [5]. On the other hand, a serious drawback of the majority of clinically used neuroleptics is the development of extrapyramidal disorders during long-term therapy [10]. Therefore, along with the development of new antipsychotic agents of atypical profile [5], a perspective trend in psychopharmacology is the search for agents correcting side effects of neuroleptic therapy.

Research aimed at elucidation of the role of NO, a pathogenetic factor in neurodegenerative diseases of the CNS, ischemia, brain stroke, and convulsive disorders, acquire special importance [2,7]. Many studies demonstrated that NO content significantly increased in the cerebral cortex of adult rats with induced convulsive conditions of different origin [1,3].

First reports indicating that inhibitors of NO synthase (NOS), the main enzyme of NO synthesis, can induce extrapyramidal symptoms similar to the effects of neuroleptics, appeared in the middle of the 1990s. It was shown that N-nitro-L-arginine, a nonselective NOS inhibitor, causes dose-dependent catalepsy in mice after acute injection and potentiates the effects of antipsychotic agents [14]. Similar results were obtained after subchronic 4-day [8,9] and longer (7 day) treatment with L-NNA [11].

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In parallel with investigations of NOS inhibitors, great attention was paid to studies of psychotropic effects of NO donors. It was found that L-arginine (an amino acid serving as the substrate for the formation of NO) arrested haloperidol (HP)-induced catalepsy in rats [12]. On the other hand, NO content under conditions of this experimental model was not measured in any of the above-mentioned studies. Though NO is intensively studied for more than 20 years, the direct quantitative method of electron paramagnetic resonance (EPR) [3] used in our study and allowing evaluation of the rate of NO generation in biological tissues was only recently introduced into practice.

We studied possible participation of NO in the mechanisms of anticataleptogenic effect of NO precursor L-arginine in HP-induced catalepsy.

MATERIALS AND METHODS

Experiments were carried out on 32 Wistar rats (180-220 g). In order to rule out the effects of circadian rhythms on behavioral parameters, all experiments were carried out at 9.00-14.00 at 22±1°C.

HP (2 mg/kg, Janssen Pharmaceutica) and L-arginine (100 and 300 mg/kg, Sigma) were injected intraperitoneally. L-arginine was administered 3 h 50 min after HP. L-arginine was dissolved in saline, HP in a drop of glacial acetic acid and then in 0.9% NaCl.

Catalepsy was determined by the time during which the fore paws of an animal stayed on the horizontal bar at the height of 10 cm from the podium ("lecturer posture").

Catalepsy was scored using Morelli scale [6], where 1 point corresponded to 15-29 sec of posture retaining,

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TABLE 1. Effect of L-Arginine on HP-Induced Catalepsy Score and NO Content (*M*±*m*)

Substance, dose	Catalepsy, score	NO content, nmol/g wet tissue/30 min
Control, 0.9% NaCl	0	1.65±0.60
HP, 2 mg/kg+0.9% NaCl	2.67±0.21	0.75±0.28
L-arginine, 300 mg/kg+0.9% NaCl	0	1.8±0.5
HP+L-arginine, 300 mg/kg	0.5±0.2*	1.87±0.15+
HP+L-arginine, 100 mg/kg	0.40±0.24*	1.98±0.26+

Note. *$p<0.001$ compared to the control; +$p<0.001$ compared to HP+NaCl group.

2 points to 30-59 sec, and 3 points to 60 sec and longer. Catalepsy testing was carried out for 1 min.

The content of NO in the brain was directly measured by EPR with the use of diethyldithiocarbamate as a radical trap [1,3]. This substance reacting with endogenous NO and Fe^{2+} forms paramagnetic mononitrosyl iron complexes. The animals of all experimental groups were injected with Na-diethyldithiocarbamate (500 mg/kg intraperitoneally) and a mixture of $FeSO_4$ (37.5 mg/kg) with sodium citrate (165 mg/kg) subcutaneously 30 min before behavioral experiments. Then the rats were decapitated, the cortex was isolated, and specimens were frozen in liquid nitrogen. NO content was measured on a Radiopan radiospectrometer.

RESULTS

Injection of HP induced stable catalepsy 4 h postinjection (Table 1). L-arginine in both doses reliably eliminated extrapyramidal disorders caused by HP.

The generation of NO in the cerebral cortex at the peak of catalepsy (4 h after HP injection) decreased 2-fold compared to the control. The level of NO in the cerebral cortex after injection of L-arginine alone did not differ from the control. Injection of NO precursor in doses of 100 and 300 mg/kg after HP abolished the decrease in NO generation. In both groups NO content in the brain tended to increase compared to the control. No differences in the effects of different doses of L-arginine were detected.

Recently NO modulators, particularly their psychotropic effects, attracted special interest. Some substances regarded as NO donors (L-arginine, isosorbide dinitrate, linsidomine, and S-nitro-N-acetylpenicillamin) can prevent the development of neuroleptic catalepsy; this effect is sex-dependent [15]. The behavioral effects of L-arginine (100 mg/kg) observed in our study are quite in line with previous data [12]. On the other hand, the capacity of NO donor to prevent neuroleptic catalepsy was not dose-dependent, which could be, primarily, due to relative similarity of the selected doses. On the other hand, the differences in the effects of L-arginine on the intensity of HP-induced catalepsy can manifest only after long-term treatment.

Our data on the content of NO in rat cortex under conditions of neuroleptic catalepsy are principally new and are priority data. Injection of HP seems to inhibit NOS activity, which can indicate a relationship between the nitroergic and nigrostriate dopaminergic systems. Injection of HP for 21 days appreciably increased NOS activity and expression of D2-dopamine receptor in the striatum and calmodulin kinase II activity [13]. The differences between the effects observed in our study and previously reported results [13] seem to be due to different protocols of HP treatment. The neurotoxic effect developing in chronic treatment with HP is also probable. Hence, it seems that normally the content of NO in the brain is maintained at a constant level, and the described increase in NOS activity is a compensatory response to decreased level of NO induced by HP.

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