Effects of D-Amphetamine on Extracellular Dopamine Content and Generation of Hydroxyl Radicals in the Striatum of Freely Moving Rats

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Four intraperitoneal injections of D-amphetamine induced dose-dependent effects on extracellular dopamine content and generation of hydroxyl radical in rat striatum. Neurotoxic effects of D-amphetamine were probably mediated by intense generation of hydroxyl radicals.

Key Words: *D-amphetamine; dopamine; hydroxyl radicals; intracerebral microdialysis*

Psychomotor effect of D-amphetamine is associated with accumulation of dopamine in the synaptic gap due to suppression of its reuptake, stimulation of dopamine release from vesicles, and inhibition of monoamine oxidase.

High doses of D-amphetamine or its repeated administration causes various effects, including neurotoxic neuronal damage. Neurotoxic effects of D-amphetamine, methamphetamine, and (methylenedioxy)methamphetamine are specific in relation to the dopamine- and serotoninergic systems of the brain [3,11]. Fine neurochemical mechanisms of neuronal damage induced by D-amphetamine and its derivatives are still unclear.

It is assumed that generation of reactive oxygen species, including hydroxyl radicals ($OH^{\bullet}$) plays a role in the mechanisms of D-amphetamine-induced neurotoxic effects [5,7,9].

The goal of the present study was to reveal the relationship between dopamine content and $OH^{\bullet}$ generation under the effect of various doses of D-amphetamine.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 220-280 g. Intracerebral microdialysis of rat striatum was used to study the release of dopamine and $OH^{\bullet}$ [12]. The rats were narcotized with chloral hydrate (400 mg/kg intraperitoneally), and concentric microdialysis probes were stereotactically implanted into the striatum (AP +0.5, L -3.0, and DV -7.5) and fixed to the skull. Sodium salicylate in a concentration of 5 mM was added to the perfusate for estimating $OH^{\bullet}$ generation. $OH^{\bullet}$ interacts with sodium salicylate with the formation of 2,3-dihydroxybenzoic acid (2,3-DHBA) detected by high-performance liquid chromatography (HPLC) with electrochemical detection (HPLC-ED) [8]. Twenty-four hours postoperation, artificial cerebrospinal fluid containing 150 mM Na^+, 3 mM K^+, 1.4 mM Ca^{2+}, 0.8 mM Mg^{2+}, 31 mM PO_4^{3-}, and 155 mM Cl^- (pH 7.4) was perfused at a rate of 2 μl/min by a syringe pump (Braun Perfusor IV). Dialysate samples were collected at 20-min intervals. After obtaining 3 basal samples, the animals received 4 intraperitoneal injections of 0.85% NaCl (control group) or D-amphetamine (Sigma) in doses of 2.5 mg/kg and 5 mg/kg at 2-h intervals (experimental groups). Dialysate samples were analyzed by HPLC-ED (BAS LC-4B) [1]. Dopamine and 2,3-DHBA were separated on a reverse-phase Ul'trasfera ODS column (5 μ, 4.6×150 mm) using 0.1 M citrate-phosphate mobile phase containing 1.1 mM octanesulfonic acid, 0.1 mM EDTA, and 9% acetonitrile (pH 3.7). A glass-carbon electrode (0.8 V) was used as the detector (against an Ag/AgCl reference electrode).

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Mean contents of dopamine and 2,3-DHBA in basal samples were considered as 100% (initial levels). The results were analyzed by Student's *t* test.

RESULTS

Dopamine content in the intercellular space gradually increased to 250% of the initial level over 240 min after the first injection of 2.5 mg/kg D-amphetamine and reached 400% after the third and fourth injections (Fig. 1, *a*).

When higher doses of D-amphetamine (5 mg/kg) were used, the content of dopamine sharply increased to 950% of the initial level 20 min after the first injection and then rapidly decreased the to control level. Repeated injections of D-amphetamine produced significant, but less pronounced effects: dopamine concentration increased to 300% after the second and third injections and to 250% after the fourth injection of the preparation (Fig. 1, *a*). After the last injection of D-amphetamine, the content of dopamine in the dialysate decreased and then returned to the initial level (Fig. 1, *a*).

The content of 2,3-DHBA increased after the first injection of 2.5 mg/kg D-amphetamine and remained at the level of 200% for 7 h (Fig. 1, *b*). The content of 2,3-DHBA reached 700% of the initial concentration 180 min after the second injection of 5 mg/kg D-amphetamine, remained unchanged for more than 2 h, and slightly decreased after the fourth injection of the preparation (Fig. 1, b).

D-Amphetamine and its analogues displayed considerable neurotoxic effects, reduced dopamine content and the number of binding sites of synaptic dopamine transporter, and caused degeneration of dopaminergic terminals [9,11]. However, fine mechanisms of these effects and subsequent neurodegenerative processes in the striatum remain unclear.

Enhanced OH• generation, exhaustion of intracellular dopamine stores, and neuronal degeneration are characteristic of neurotoxic effects of D-amphetamine and its analogues. The increase in dopamine content in the synaptic gap is assumed to play a key role in activation of OH• generation. Under the effect of toxic doses of methamphetamine, changes in the level of OH• were preceded by accumulation of dopamine in the intercellular space [7]. Dopamine and the product of its autooxidation 6-hydroxydopamine are the main substances inducing nonenzymatic production of OH• in the Fenton reaction [2,5].

D-Amphetamine in a single dose of 5 mg/kg rapidly and markedly increased the extracellular content of dopamine. Stimulation of dopamine release induced by repeated administration of D-amphetamine was less pronounced than after the first injection of this preparation. However, the concentration of dopamine remained at a high level to the end of observations. Under the effect of 2.5 mg/kg D-amphetamine, dopamine content in the intercellular space gradually increased and attained maximum after the third injection of the preparation. Intensification of OH• generation depended on the dose of D-amphetamine. After injection of 5 mg/kg D-amphetamine, the content of 2,3-DHBA reached 700% of the initial level and remained high for more than 2 h. Changes in the content of 2,3-DHBA were probably mediated by intense dopamine release observed after the first injection of D-amphetamine. These results agree with previously reported data [6]. Our findings suggest that considerable inten-

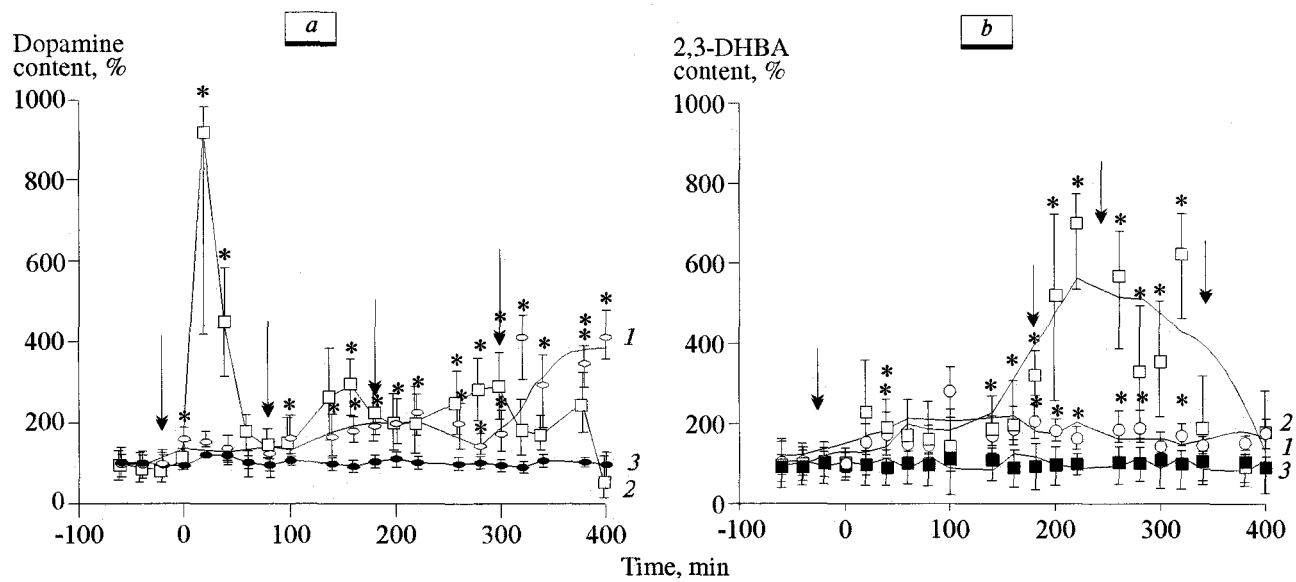

Fig. 1. Effects of D-amphetamine in doses of 2.5 (*1*) and 5.0 mg/kg (*2*) and 0.85% NaCl (*3*) on extracellular contents of dopamine (*a*) and 2,3-dihydroxybenzoic acid (*b*) in rat striatum. Arrows indicate time of injections. *$p<0.05$ compared with the control.

sification of OH• generation is determined by repeated injection of 5 mg/kg D-amphetamine.

2,3-DHBA content increased after the first injection of 2.5 mg/kg D-amphetamine and remained at a high level (200% of the initial concentration) to the end of observations. Therefore, accumulation of OH• in the intercellular medium damages dopamine transporter [8] and, together with blocking effects of D-amphetamine, inhibits cell reuptake of dopamine. This mechanism is probably involved in exhaustion of vesicular neurotransmitter stores.

Thus, our findings demonstrate the interrelation between changes in dopamine content and OH• generation induced by various doses of D-amphetamine.

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