Effect of Bromantane, a New Immunostimulating Agent with Psychostimulating Activity, on the Release and Metabolism of Dopamine in the Striatum of Freely Moving Rats. A Microdialysis Study

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It is demonstrated that bromantane induces a pronounced and prolonged (8 h) increase in the release of dopanime. Tetrodotoxin (10^{-6} M) perfused via a microdialysis probe partially inhibits the bromantane-induced release of dopamine. The extracellular content of the dopamine metabolites 3,4-dioxyphenylacetic and homovanillic acids is slightly decreased. The possible mechanisms of action of bromantane on the dopamin- and serotoninergic systems of the brain are discussed.

Key Words: *psychostimulators; bromantane; dopamine, striatum, microdialysis*

Bromantane (2-[n-bromphenyl]-aminoadamantane) is a new immunostimulating drug [3] with psychoactivating and adaptogenic properties [5-7]. Its mechanism of action has not been studied in sufficient detail. The dopaminergic and serotoninergic systems play an important role in the realization of the effects of adamantane derivatives [1,2,4,8,11, 12,14]. In a concentration range of 50-500 μM bromantane notably reduces serotonin reuptake by rat brain synaptosomes, inhibiting to a lesser extent the reuptake of dopamine. The fact that bromantane eliminates catalepsy induced by haloperidol and tryphtazin indicates that the dopamin- and serotoninergic brain structures play an instrumental role in the the pharmacological effect of bromantane. From this viewpoint, a detailed examination of the effect of bromantane on the function of these two neurotransmitter brain systems is of undoubted interest.

Our objective was to examine the effect of bromantane on the release of dopamine and to measure by intracerebral dialysis the extracellular content of the bromantane metabolites 3,4-dihydroxyphenylacetic and homovanillic acids, DOPAC and HVA, in the dorsal striatum of the brain of freely moving rats [10]. The method of intracerebral dialysis makes it possible to evaluate the state of a particular neurotransmitter system while preserving the brain neuroregulatory mechanisms.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 200-250 g. The animals were anesthetized with chloralhydrate (400 mg/kg intraperitoneally) and were placed in a stereotaxic apparatus (David Kopf Instruments). A dialysis membrane (the length of membrane exposure relative to the bregma was 10 mm, outer diameter was 0.32 mm,

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permeability 15,000 D, AN69 - HF, Hospal-Dasco, Italy) with an inner tungsten core was implanted in the dorsal striatum. After the operation, the rats were maintained in cages and had free access to food and water. A 24-h perfusion with Ringer solution was performed 24 h postoperation at a rate of 2.7 μl/min) using a syringe pump. The solution contained (in mM): NaCl 147, $CaCl_2$ 1.5, KCl 4, pH 6.0). Dialyzate was collected 1 h after the start of perfusion. After 3-4 baseline samples had been obtained, the test compound was administered *per os*. The content of dopamine, DOPAC, HVA, and 5-oxyindoleacetic acid (5-OIAA) in the dialyzate was determined by high performance liquid chromatography with electrochemical detection. Citrate-phosphate buffer (0.1 M) containing 1.1 mM sodium octanesulfonate, 0.1 mM EDTA, and 9% acetonitryl (pH 3.7) was used as a mobile phase. Detection was carried out on the working electrode at +0.8 V against Ag/AgCl on the comparisong electrode. Under these conditions the detection limit for dopamine was 4 pg. Bromantane (Institute of Pharmacology, Russian Academy of Medical Sciences, Moscow) was dissolved in polyethylene glycol-400 to a final concentration of 2.5% and administered to rats *per os* in a dose of 100 mg/kg. Tetrodotoxin (Sigma) was added to the perfusing solution to a final concentration of 10^{-6} M.

The content of dopamine and its metabolites in the baseline specimens was taken as 100%. The effects of the test compounds were assessed by the ratio to the baseline level, which under the conditions of this experiment was 21.1±1.7 pg/20 μl dialyzate for dopamine, 2.365±0.234 ng/20 μl dialyzate for DOPAC, and 1.825±0.247 ng/20 μl dialyzate for HVA. The results were analyzed using Student's *t* test. They were presented as $M \pm m$.

RESULTS

Figure 1 shows that bromantane stimulates the release of dopamine, which was observed during an 8-h period. The release was maximal (553% compared with the control) 2 h after administration of bromantane. The release of dopamine was increased (240-355% over the control level) throughout the observation period. By the 8th hour of observation, the extracellular content of DOPAC and HVA was decreased to 68 and 60%, respectively. Perfusion with Ringer solution containing 10^{-6} M tetrodotoxin almost completely inhibited the spontaneous release and partially suppressed the bromantane-induced release of dopamine (Fig. 2). It is known that psychostimulating agents such as

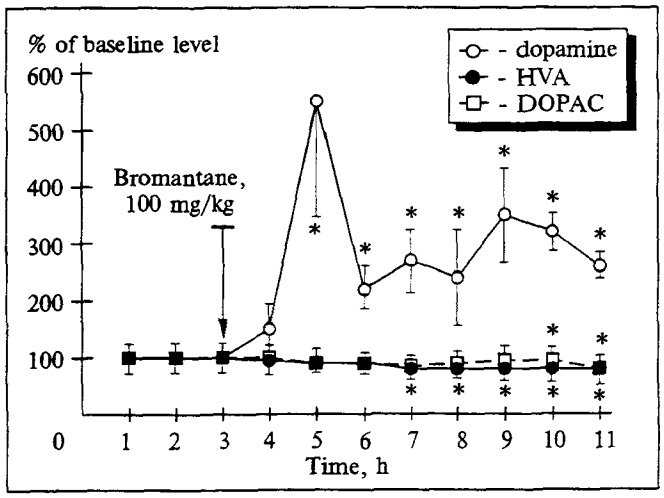

Fig. 1. Extracellular content of dopamine, DOPAC, and HVA in the dorsal striatum of freely moving rats ($n=5$) under the action of bromantane (100 mg/kg *per os*). Dialyzates were collected every hour. Asterisk indicates $p<0.05$ compared with the control. Here and in Fig. 2 the data are expressed as $M \pm m$.

amphetamine, methylphenidate, and nomifensine induce a pronounced increase in the release of dopamine in the striatum [9]. It has been assumed that amphetamine releases dopamine from nerve endings predominantly via a mechanism mediated by dopamine transmitters [15]. The increase in the extracellular dopamine concentration induced by methylphenidate and nomifensine is probably a result of a complex mechanism including an amphetamine-like effect and an inhibitory effect on the reuptake of the neurotransmitter [9,15].

The following possible mechanisms of action on dopaminergic neurons have been described: 1) an amphetamine-like release of newly synthesized cytoplasmic dopamine via the mechanism mediated by dopamine transmitters; 2) blockade of the neurotransmitter reuptake; 3) presynaptic antagonism [11,14]. The prolonged intensified release of dopamine observed in this study may result from a combination of these three mechanisms. The maximum increase in the release of dopamine observed by the 2nd hour may reflect the amphetamine-like effect of bromantane. The subsequent moderate increase in the release of dopamine is probably due to blockade of reuptake and putative antagonism to the dopamine receptors [11]. It is known that practically all the studied antagonists of dopamine receptors cause a maximum increase of dopamine release up to 200%. It has also been demonstrated that amphetamine blockade of the dopamine receptors results in the summation of the effects on dopamine release [13]. It is noteworthy that the derivatives of adamantane stimulate not only the release but also the synthesis of dopamine [12]. The prolonged release of dopamine

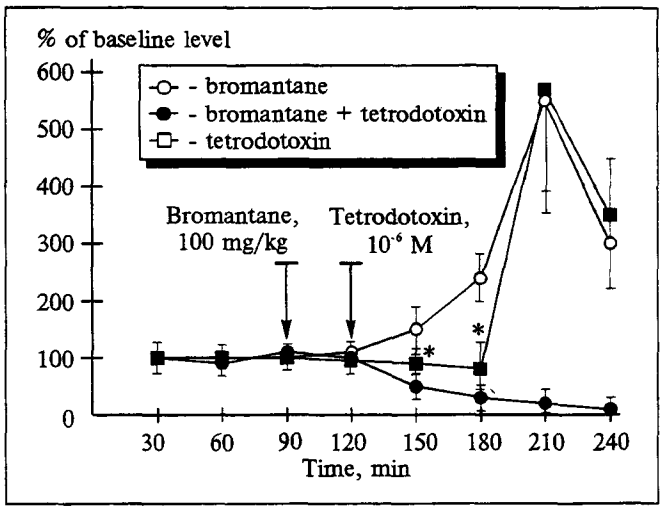

Fig. 2. Effect of bromantane (100 mg/kg *per os*) on the extracellular content of dopamine in the dorsal striatum of freely moving rats ($n=5$) against the background of local perfusion of the striatum tetrodotoxin (10^{-6} M). * $p<0.05$: reliable differences between the effect of bromantane + tetrodotoxin in comparison with the effect of bromantane.

against the background of bromantane results from the increased synthesis of dopamine thanks to the presumed presynaptic antagonism of the compound.

The content of the dopamine metabolites DOPAC and HVA proved to be lowered, though to a lesser degree than under the action of amphetamine [15]. The extracellular levels of dopamine metabolites, particularly of DOPAC, are known to reflect the biosynthesis of dopamine rather than its release. The decrease in the level of these metabolites under the action of amphetamine is due to depletion of the newly synthesized cytoplasmic pool of dopamine, which leads to a decrease in the intraneuronal formation of DOPAC. An increase in the synthesis of dopamine on account of the presumed presynaptic antagonism of bromantane could compensate for the depletion of the newly synthesized pool of dopamine, probably inducing only a slight drop in the level of the metabolite.

These assumptions are confirmed by the effects of bromantane against the background of tetrodotoxin (Fig. 2), a compound that blocks sodium channels, thus interrupting the electrical activity of neurons, which manifests itself in a virtual cessation of exocytosis of dopamine. The amphetamine-induced release mediated by the transmitter is not tetrodotoxin-sensitive [15]. The partial tetrodotoxin sensitivity of the effect of bromantane on the release of dopamine allows us to divide the observed increase in the release of dopamine into two components: tetrodotoxin-sensitive and tetrodotoxin-insensitive. It has been shown that the increased release of dopamine *in vivo* induced by blockade of dopamine receptors and inhibition of re-uptake is tetrodotoxin-sensitive [15]. Presumably, the tetrodotoxin-sensitive component in the effect of bromantane on the release of dopamine in the striatum reflects both these mechanisms. Interestingly enough, bromantane had practically no effect on the extracellular concentration of 5-OIAA in the striatum of freely moving rats (data not presented). This, however, does not exclude the possibility of an effect on serotonin (not determined in this study). The involvement of the serotoninergic component in the mechanism of action of bromantane requires further investigation.

Thus, from our results it can be concluded that the pharmacological effect of bromantane is probably based on a multicomponent action on the release of dopamine in the striatum, a feature which distinguishes bromantane from the classical psychostimulators.

REFERENCES

1. D. A. Berzinya and A. A. Kimenis, in: *Experimental and Clinical Pharmacotherapy* [in Russian], Vol. 15, Riga (1986), pp. 50-53.
2. L. V. Devoino and R. Yu. Il'yuchenok, *Monoaminergic Systems in the Regulation of Immune Reactions (Serotonin, Dopamine)* [in Russian], Novosibirsk (1983).
3. I. S. Morozov, N. G. Artsimovich, T. A. Fadeeva, *et al.*, *Byull. Izobret.*, № 43-44, 184 (1993).
4. V. D. Petkov, in: *Pharmacology of Nootropic Agents*, Es. A. V. Val'dman and T. A. Voronina [in Russian], Moscow (1989), pp. 20-25.
5. G. Campanella, M. Roy, and A. Barbeau, *Ann. Rev. Pharmacol. Toxicol.*, **27**, 113-136 (1987).
6. Y. L. Hurd and U. Ungerstedt, *Europ. J. Pharmacol.*, **166**, 251-260 (1989).
7. F. Imperato and G. Di Chiara, *J. Neurosci.*, **4**, 966-977 (1984).
8. M. K. Menon, C. A. Vivonia, and V. G. Haddox, *Psychopharmacology* (Berlin), **82**, 89-92 (1984).
9. B. Scatton, A. Cheramy, M. J. Besson, and J. Glowinski, *Europ. J. Pharmacol.*, **13**, 131-133 (1970).
10. T. Sharp, T. Zetterstrom, T. Ljunberg, and U. Ungerstedt, *Ibid.*, **129**, 411-415 (1986).
11. J. W. Tilley and M. J. Kramer, *Prog. Med. Chem.*, **18**, 2-37 (1981).
12. B. H. C. Westerink, J. Tuntler, G. Damsma, *et al.*, *Naunyn Schmiedebergs Arch. Pharmacol.*, **336**, 502-507 (1987).