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EFFECT OF KETANSERIN AND AMPHETAMINE ON NIGROSTRIATAL NEUROTRANSMISSION AND REACTIVE OXYGEN SPECIES IN PARKINSONIAN RATS. IN VIVO MICRODIALYSIS STUDY.

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5-$HT_{2A/2C}$ receptors are one of the most important in controlling basal ganglia outputs. In rodent models of Parkinson's disease (PD) blockade of these receptors increases locomotion and enhances the actions of dopamine (DA) replacement therapy. Moreover, previously we established that 5-$HT_{2A/2C}$ antagonist attenuate DA D_1 agonist mediated vacuous chewing movements (VCMs) which are considered as an animal representation of human dyskinesia. These findings implicate 5-HT neuronal phenotypes in basal ganglia pathology, and promote 5-HT_2 antagonists as a rational treatment approach for dyskinesia that is prominent in most instances of PD replacement therapy. In the current study we determined whether ketanserin (KET) and/or amphetamine (AMPH) affected dopaminergic neurotranssmision in intact and fully DA-denervated rats. Moreover, we looked into extraneuronal content of HO· of the neostriatum after AMPH and/or KET injection, assessed by HPLC analysis of dihydroxybenzoic acids (2,3- and 2, 5-DHBA) - spin trap products of salicylate. Findings from the present study demonstrated that there are no substantial differences in extraneuronal HO· generation in the neostriatum between control and parkinsonian rats. KET did not affect DA release in the fully DA-denervated rat's neostriatum and also did not enhance HO· production. As 5-$HT_{2A/2C}$ receptor-mediated transmission might prove usefulness not only in addressing motor complications of PD patients (dyskinesia) but also in addressing non-motor problems such depression and/or L-DOPA evoked psychosis, the findings from the current study showed that the use of 5-$HT_{2A/2C}$ receptor antagonists in Parkinson's disease does not impend the neostriatal neuropil to be damaged by these drugs. We concluded that 5-$HT_{2A/2C}$ receptor antagonists may provide an attractive non-dopaminergic target for improving therapies for some basal ganglia disorders.

Key words: *amphetamine, ketanserin, dopamine, reactive oxygen species, 6-OHDA lesioned rat, striatum, microdialysis.*

INTRODUCTION

Parkinson's disease (PD) is classically characterized by bradykinesia, rigidity and tremor et rest. All features seems to be the result of the degeneration of the nigrostriatal pathway, however involvement of serotoninergic and noradrenergic systems is also well documented (1). Replacement therapy with L-dihydroxyphenylalanine (L-DOPA) or direct dopamine (DA) agonists administration gives relief to many of those symptoms but the bulk of complications arise after 4-5 years of treatment. The frequently occurred are the wearing off phenomenon, on-off fluctuations, dyskinesia, psychosis, etc. Among these, dyskinesias are common complains of patients with PD, typically seen five to seven years after onset of L-DOPA administration. They can become so disabling as to negate any clinical benefit from treatment or even seriously complicate the management of patients with advanced PD (2).

The etiology of dyskinesia is very complex and still unknown, studies on nonhuman primates have shown that it seems to be brought about by enhancement of DA D_1- and/or DA D_2 receptor mediated signal transduction pathways, supersensitization of DA D_2 receptor or modifications in the functional links between DA receptors subtypes (D_1, D_2, D_3) (3-6). Our previous studies strongly support that 5-HT receptors may be involved in dyskinesia as well. More specifically, 6-OHDA lesioned rats display oral movements (vacuous chewing movements, VCMs) in response to the DA D_1 agonist (SKF 38393) challenge. Conversely, VCMs may be considered as an animal representation of human dyskinesia that is prominent in most instances of PD replacement therapy (7, 8). Previously we established that mCPP a 5-$HT_{1B/2C}$ agonist enhances VCMs to a greater extent than does SKF 38393 (9). The effect was not attenuated by DA D_1 receptor antagonist while diminished by mianserin (the largely 5-HT_2 receptor antagonist) or by partial 5-HT fiber damage in adult rats (5,7-DHT icv injection). Although the imbalance in DA D_1/D_2 receptor stimulation was invoked as a mechanism of dyskinesia, our earlier findings (10, 11) as well as Deuwaerdere and Chesselet (12) study provided strong evidences that the serotoninergic system seems to be implicated in these abnormalities as well. Finally, the above mentioned experimental data are in line with clinical observation; Fox and Brotchie (13) found that 5-HT_{2C} receptor binding was increased in the output regions of the basal ganglia in PD patients with L-DOPA - induced dyskinesia, suggesting a compensatory up-regulation of receptors levels in response to decreased stimulation by endogenous neurotransmitter.

It must be also considering that 5-$HT_{2A/2C}$ receptors have also attracted particular interest in view of their broad role in regulating mood, emotional behavior and cognition function, addressing in this way non-motor problems of PD like depression and psychosis. The prevalence of hallucinosis and psychosis in PD has increased substantially with the use of L-DOPA treatment for motor symptoms (2). Clozapine a 5-$HT_{2A/2C}$ receptor antagonist, represents a class of atypical

antipsychotic drugs with reduced incidence of extrapyramidal side-effects compared to the classical neuroleptics and is the most widely studied and recommended medication used for treatment of hallucinations and delusions in PD (14, 15). While psychosis is associated with dopaminomimetics administration, depression occurs "naturally" in the course of the PD and has been present more often in patients with PD than in age-match samples ranging approximately 40 % of PD patients. It must also be added that involvement of the 5-HT_2 receptor in depressive disorder has been confirmed by animal and human study (16-18). In conclusion, manipulation of 5-HT_{2C} receptor-mediated transmission might prove useful not only in addressing motor problems of PD (e.g. dyskinesia) but also in addressing non-motor problems such as depression and/or psychosis.

However, much less is known about the serotoninergic system and its role in reactive oxygen species (ROS) production. There are scarce data which have implicated 5-HT as the progenitor for ROS (19-21). Moreover, an ongoing debate over the past decade relates also to the concern of weather L-DOPA or other dopaminomimetics promote or reduce ROS formation in brain, thereby possibly accelerating or decelerating the progression of PD. Furthermore, in recent studies we found (22) that 5-HT innervation promotes HO· production and actually suppresses the in vivo microdialysate (extraneuronal) concentration of DA in the neostriatum of rat brain. These findings established the intricate role played by 5-HT nerves not only in regulating receptor sensitivity status but in modulating DA release and HO· production in partially DA-denervated striatum.

Therefore, in this paper we investigated the role of ketanserin (KET) and amphetamine (AMPH) on extracellular DA and the respective DA metabolite levels in the neostriatum of 6-OHDA lesioned rats. Furthermore, we looked into extraneuronal content of HO· of the neostriatum after AMPH and/or KET injection, assessed by HPLC analysis of dihydroxybenzoic acids (2,3- and 2, 5-DHBA) - spin trap products of salicylate.

MATERIALS AND METHODS

Subjects

Male Wistar rats were obtained from the University Animal Department (Katowice, Poland) and were housed in a well-ventilated room, at 22 ± 2°C under a 12h light:12h dark cycle (lights on 7:00 a.m.), with free access to food and water. Rats were weaned at 21days. All procedures, reviewed and approved by the Institutional Animal Care Committee, are in accord with principles and guidelines described in the NIH booklet *Care and Use of Laboratory Animals.*

Neonatal treatment

At 3 days after birth animals were pretreated with desimipramine HCl (20 mg/kg, IP, base; 1 h) (Sigma Chemical Co., St. Louis, MO, USA) and pargyline HCl (50 mg/kg, IP, salt form; 0.5 h) (Sigma) and were given bilateral ICV injection of 6-OHDA HBr (66.7 µg, base form, on each side)

or the vehicle saline (0.85%) - ascorbic acid (0.1%). This procedure has been described in detail (7). 6-OHDA and control rats were housed until 10 weeks of age for further experiments.

It must be mentioned that at present various Parkinson's disease animals models are introduced (23, 24). That one applied in this study is posed as a near-ideal model of severe Parkinson's disease, because of the non-lethality of the procedure, near-total destruction of nigrostriatal dopaminergic fibers, near-total DA-denervation of striatum and reproducibility of effect (6).

Tissue sample preparation

Rat neostriatum was rapidly dissected and placed on dry ice, weighed and stored at -70°C, pending assay. The samples were homogenized in ice-cold trichloracetic acid (0.1M) containing 0.05 mM ascorbic acid, for 15 - 20 sec. After centrifugation (5,000xg for five min), the supernatants were filtered through 0.2 µm cellulose membranes (Titan MSF Microspin Filters, scientific resources Inc., Eatontown GB) and supernatants injected onto the HPLC/ED column. Levels of dopamine (DA), 3.4-dihydroxyphenylacetic acid (DOPAC), homovanilic acid (HVA), 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) were measured (25, 26).

The composition of the mobile phase was 75 mM NaH_2PO_4 $2H_2O$ (Avocado Research Chemicals Ltd), 1.7 mM 1-octanesulphonic acid (Avocado), 5 µM EDTA (Avocado), 100 µl triethylamine (Sigma), 9.5% acetonitril (Lab-Scan), pH 3 adjusted with phosphoric acid (Fluka). The flow rate was 0.7 ml/min at 22°C, and the oxidizing potential was +750 mV, 5 nA/V sensitivity. Peaks were automatically integrated by the universal chromatographic interface UCI-100 (Dionex, Germany).

The instrumentation used included: an electrochemical detector Gilson (France) model 141 with flow cell, piston pump Gilson (France) model 302 with head 5SC, manometric module Gilson (France) model 802, thermostat for STH 595 column (Dionex, Germany), precolumn Hypersil BDS C18, 10x4 mm, 3 µm (ThermoQuest GB) and chromatographic column Hypersil BDS C18, 250x4.6 mm, 3 µm (ThermoQuest GB).

In vivo Microdialysis study

Rats were first placed in a stereotaxic frame. Under anesthesia, Relanium (Polfa) (10 mg/kg IP) and ketamine (Parke-Davis) (80 mg/kg IP) the skin and tissue were retracted to expose the skull overlying the STR. A small hole was drilled to allow implantation of the dialysis probe, with 4 mm active membrane (ID 75 µm, OD 150 µm, Polymicron Technologies, USA) into the right STR (A +0.7, L +3.0, V -7.0) according to Paxinos and Watson (1986). Two stainless steel screws were mounted to the cranium near the probe, and this assembly was fixed in place with dental cement (Duracryl Plus, Spofa, Praha).

For the catecholamine assay, on the following day the free ends of the probe were connected with teflon tubes and continuously perfused with artificial cerebrospinal fluid (Na^+ 145 mM, K^+ 2.7 mM, Ca^{2+} 1.2 mM, Cl^- 151.7) at a flow rate of 2.0 µl/min (Micodialysis pump, Harvard Apparatus Model 22, GB). Samples were collected every 22 min and injected directly onto a microdialysis column, MD 150/RP-18, 150x3mm, 3 µm (ESA, USA), using a mobile phase consisting of 1.7 mM 1-octanesulfonic acid, 25 µM EDTA, 100 µl trietylamine/1000 ml, and 10% acetonitrile in 75 mM phosphate buffer at pH 3 and flow rate of 0.6 ml/min. A guard cell (+250mV), and flow-through electrochemical cell (E1 +250; E2 -175) were used for analysis, with a Coulochem (ESA, USA) data analysis system to integrate peak areas of DA, DOPAC (27-29).

When dialysate DA levels became constant (at about 1.5 h from the beginning of perfusion) rats of two groups (control and 6-OHDA) were injected with saline 1.0 ml/kg IP and after 66 min (3 collections) with AMPH (1.0 mg/kg IP). Separate groups of control and 6-OHDA lesioned animals were challenged with KET (5.0 mg/kg IP) and after 66 min with AMPH (1.0 mg/kg IP). The study was continued until 198 min.

For trapping OH· radicals the striatum of separate groups of rats was perfused with aCSF containing 5 mM salicylic acid (sodium salt). Flow rate was set identical as in catecholamine assay. Samples were collected every 22 min and injected directly onto a DHBA-250, 250x4mm, 5 µm column (ESA, USA), using a mobile phase consisting of 50mM sodium acetate, 50mM citric acid, 25% methanol, 5% isopropanol, pH 2.5 with phosphoric acid. Flow rate was set 0.5 ml/min. A guard cell (+775mV), electrochemical cell (E1 +250; E2 +750) were used for analysis, with a Coulochem (ESA, USA) data analysis system to integrate peak areas of 2.3- and 2.5-dihydroxybenzoic acids isomers (2.3- and 2.5-DHBA) (30, 31). Drugs were administered according the same procedure as in catecholamine assay.

The indirect, salicylate trap method for assessing HO· is reliable and sensitive. It is based upon the measurement of 2.3- and 2.5-DHBA formed when HO· reacts with salicylic acid. Of the products from the salicylate trap, the 2.3-DHBA is a more reliable indicator of non-enzymatically formed product (32, 33). In the absence of a change in 2.3-DHBA, 2.5-DHBA has been used as an index of cytochrome P450 metabolism (34).

Data Analysis

Group differences in monoamines and metabolites were analyzed by student's *t*-test. Group differences in DHBA products were assessed by an analysis of variance (ANOVA) and the post-ANOVA test of Newman-Keuls. A P value <0.05 was taken as the level of significant difference.

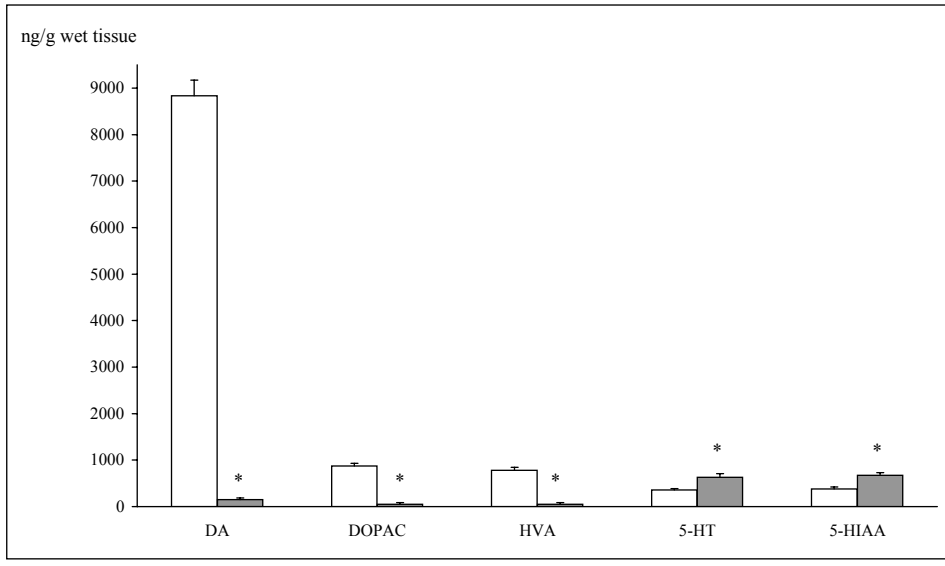

Fig. 1. Levels of dopamine (DA), 3.4-dihydroxyphenylacetic acid (DOPAC), homovanilic acid (HVA), 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in control and 6-OHDA lesioned animals (n=6)

Explanations:

□ Control

▒ 6-OHDA lesioned rats

* p<0.05

Tab. 1. Effect of AMPH 1.0 mg/kg and/or KET 5.0 mg/kg on DA and DOPAC microdialysate concentration in the striatum of control rats.
* p<0.05

DA microdialysate concentration in the striatum (pg/20µl)	DRUG APPLY											
	WITHOUT TREATMENT (base line)			SALINE 1.0 ml/kg IP			AMPHETAMINE 1.0 mg/kg IP					
CONTROL	4.3 ±1.3	4.6 ±1.3	5.1 ±1.2	5.1 ±0.9	5.1 ±1.2	5.3 ±1.2	36.4 ±8.1	56.9 ±6.2	40.0 ±7.3	25.3 ±4.5	15.8 ±2.4	9.6 ±2.0
	WITHOUT TREATMENT (base line)			KETANSERIN 5.0 mg/kg IP			AMPHETAMINE 1.0 mg/kg IP					
CONTROL	4.4 ±2.0	4.8 ±1.9	5.0 ±1.7	6.6* ±1.1	7.7* ±0.9	7.8* ±0.8	22.2* ±3.1	41.7* ±6.3	23.0* ±3.3	12.7 ±2.3	7.7* ±1.5	6.2 ±1.6
DOPAC microdialysate concentration in the striatum (pg/20µl)	DRUG APPLY											
	WITHOUT TREATMENT (base line)			SALINE 1.0 ml/kg IP			AMPHETAMINE 1.0 mg/kg IP					
CONTROL	2255.2 ±278.1	2220.1 ±288.3	2209.5 ±345.6	2254.5 ±330.4	2239.9 ±341.7	2268.2 ±357.3	1951.3 ±355.3	1224.5 ±153.8	908.0 ±116.4	849.5 ±126.1	1001.3 ±119.1	1114.9 ±176.4
	WITHOUT TREATMENT (base line)			KETANSERIN 5.0 mg/kg IP			AMPHETAMINE 1.0 mg/kg IP					
CONTROL	2399.9 ±342.2	2333.4 ±333.7	2316 ±325.5	2220.5 ±304.0	2242.9 ±303.2	2193.6 ±321.0	2104.6 ±310.4	1540.2 ±207.2	1318 * ±152.2	1238 * ±163.2	1191.3 ±108.4	1355.9 ±146.7

RESULTS

Tissue assay

In neonatally 6-OHDA lesioned animals, the DA content of the neostriatum was reduced by 98.1% in comparison with intact rats (8832.4 *vs* 92.2ng/g). The main DA metabolites DOPAC and HVA were diminished to a similar extent, respectively by 98.9% (873.8 vs 8.87ng/g) and 98.5% (782.3 *vs* 11.9ng/g).

As is well known, ontogenetic destruction of DA innervation is accompanied by hyperinnervation of the neostriatum by 5-HT fibers originating from the median raphe nucleus (8). In the current study, 5-HT content of neostriatum in 6-OHDA lesioned animals was increased by 76.3% when compared to control (357.5 vs 630.8ng/g), and the principle 5-HT metabolite 5-HIAA was elevated by 78.6% (378.3 *vs* 674.2ng/g) (*Fig. 1.*)

In vivo Microdialysis study

Catecholamine assay

The striatal extraneuronal (i.e., microdialysate) level of DA in two control groups (intact rats) before drugs injection (baseline) varied between 4.3 - 5.1pg/20 µl (saline

Tab. 2. Effect of AMPH 1.0 mg/kg and/or KET 5.0 mg/kg on DA and DOPAC microdialysate concentration in the striatum of 6-OHDA lesioned rats.

DA microdialysate concentration in the striatum (pg/20µl)	DRUG APPLY											
	WITHOUT TREATMENT (base line)			SALINE 1.0 ml/kg IP			AMPHETAMINE 1.0 mg/kg IP					
6-OHDA	0.9 ±0.4	1.0 ±0.4	1.1 ±0.4	1.1 ±0.4	1.1 ±0.4	1.1 ±0.3	1.4 ±0.5	1.3 ±0.5	1.3 ±0.5	1.4 ±0.5	1.5 ±0.6	1.2 ±0.4
	WITHOUT TREATMENT (base line)			KETANSERIN 5.0 mg/kg IP			AMPHETAMINE 1.0 mg/kg IP					
6-OHDA	0.9 ±0.1	0.8 ±0.2	0.7 ±0.2	0.8 ±0.2	0.8 ±0.2	0.7 ±0.2	0.7 ±0.1	0.6 ±0.1	0.5 ±0.1	0.7 ±0.2	0.6 ±0.2	0.6 ±0.2
DOPAC microdialysate concentration in the striatum (pg/20µl)	DRUG APPLY											
	WITHOUT TREATMENT (base line)			SALINE 1.0 ml/kg IP			AMPHETAMINE 1.0 mg/kg IP					
6-OHDA	29.2 ±2.5	25.2 ±2.4	22.7 ±3.1	22.2 ±2.7	22.7 ±3.2	21.8 ±2.0	22.7 ±2.8	19.8 ±2.9	19.2 ±2.3	19.1 ±4.0	19.2 ±1.4	20.91 ±2.2
	WITHOUT TREATMENT (base line)			KETANSERIN 5.0 mg/kg IP			AMPHETAMINE 1.0 mg/kg IP					
6-OHDA	32.3 ±11.3	24.1 ±7.4	23.8 ±7.0	24.8 ±7.0	25.8 ±7.1	26.0 ±7.3	27.0 ±7.7	24.5 ±9.4	19.8 ±6.1	19.2 ±5.3	19.3 ±5.1	18.4 ±4.5

+ AMPH group) and 4.4 - 5.0pg/20 µl (KET + AMPH group). AMPH (1.0 mg/kg IP) acutely produced in control animals robust increase in DA microdialysate concentration up to 56.9pg/20 µl in 110 min; then gradual decrease of DA was observed. KET (5.0 mg/kg IP) injected to intact animals evoked approximately ~ 45% increase in DA in comparison with saline treated rats. Moreover, KET injected before AMPH challenge significantly diminished AMPH evoked DA release in all examined intervals except the last one, compared to control (AMPH alone) (*Tab. 1*).

In this study the microdialysate level of DA metabolites was also analyzed. AMPH decreased DOPAC microdialysate concentration in control intact rats while KET partially antagonized this effect. Similar data were obtained with final DA metabolite HVA (not presented).

The basal extraneuronal level of DA in 6-OHDA lesioned rats was diminished to approximately 18 - 20% of control (intact) animals. In DA denervated rats, neither AMPH, KET nor both affected DA, DOPAC and HVA microdialysate concentrations (*Tab. 2*).

2,3- and 2, 5-DHBA estimation

Basal concentration of 2.3- and 2.5-DHBA, spin trap products from interaction of HO· with salicylate were not substantially altered in the neostriatal

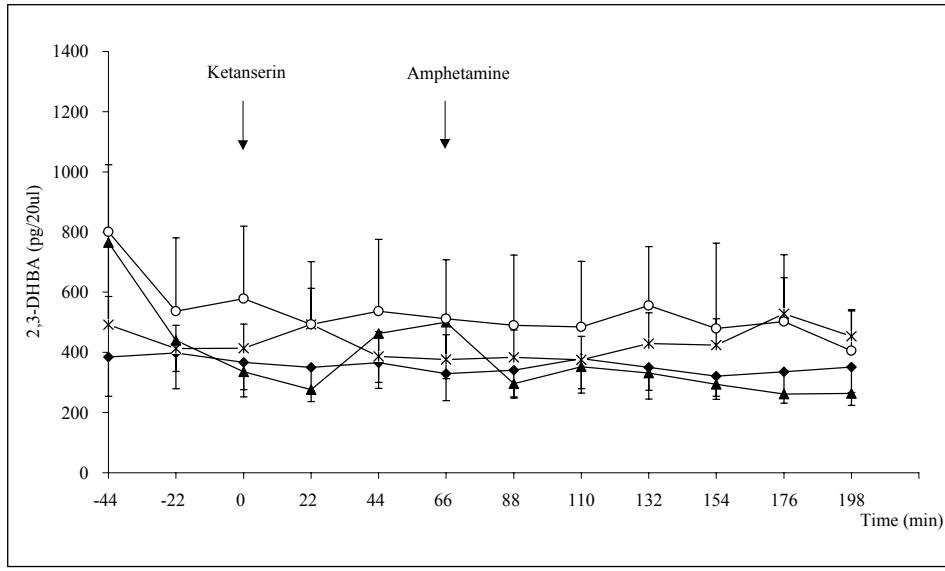

Fig. 2. Concentration of 2.3-DHBA in microdialysates in the striatum of control and 6-OHDA lesioned rats after AMPH and/or KET injection (n=7).
- ♦ - control (saline + AMPH)
- **X** - control (KET + AMPH)
- ▲ - 6-OHDA (saline + AMPH)
- ○ - 6-OHDA (KET + AMPH)

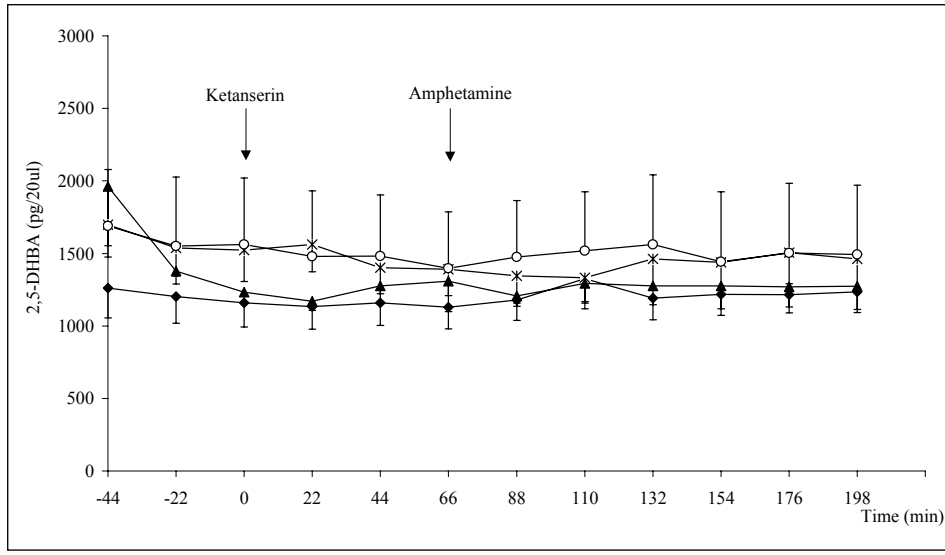

Fig. 3. Concentration of 2.5-DHBA in microdialysates in the striatum of control and 6-OHDA lesioned rats after AMPH and/or KET injection (n=7).
Explanations as in figure 2

microdialystes in 6-OHDA animals in comparison to control (intact rats). Neither AMPH nor KET affected HO· generation in the extraneuronal space (i.e. microdialysates) of neostriatum of all tested groups (*Fig. 2, 3*).

DISSCUSION

The present study examined the relationship between AMPH (DA releaser), KET (5-$HT_{2A/2C}$ antagonist) and their reciprocal effects on nigrostriatal dopaminergic neurotransmission as well as extraneuronal HO· formation in the neostriatum of intact and fully DA-denervated rats.

AMPH effects in intact and fully DA-denervated rats

It is well known that AMPH and its derivatives promote DA efflux from non-vesicular stores mainly by reverse transport through monoamine uptake transporter (DAT) (35, 36). As demonstrated be the microdialysis and voltametry studies an increase in DA efflux after AMPH attained its maximum response 20-40 min after injection with concomitant reduction in DOPAC and HVA efflux (29, 37). In the present study we confirmed the above findings, showing that the concentration of DA, DOPAC and HVA in striatal microdialysates are respectively altered. Conversely, no changes in DA release were observed in animals with DA -denervated neostriatum. The above-mentioned is in line with Herrera-Marschitz et al. (38), who determined that in rats injected into the right ventricle with 6-OHDA (at the age of 3-day), AMPH used in relatively high dose (2.0 mg/kg) increased (2-fold) acetylcholine levels but did not affect extraneuronal DA in denervated striatum. The abolition of AMPH evoked DA release seems to be a consequence of vast dopaminergic terminals devastation (98 - 99% reduction in endogenous DA content of neostriatum) and subsequent decline in AMPH targets throughout the caudate-putamen (lesion-induced loss of DAT binding sites).

Effectiveness of DA-denervation

In this study ontogenetic 6-OHDA treatment was associated with 99% reduction in endogenous DA content of neostriatum at 10 weeks (tissue examination) but the basal extraneuronal level of DA was diminished only to approximately 18 - 20% of control (intact) animals. Several reports have shown that extensive loss of dopaminergic neurons accompanied by dramatic loss of DAT, elicits compensatory changes in the residual dopaminergic neurons such as increased synthesis, enhanced turnover and a reduction of DA inactivation (39, 40). Interestingly loss of DAT binding sites may paradoxically supports maintenance of basal concentration DA in synaptic cleft on nearly physiological level (4). However, in rats in which the striatum was DA-depleted by 80% or more, the basal extraneuronal DA level was reduced reaching only 10 - 30% of

intact rats. The compensatory mechanisms (i.e. safety factor) are not more sufficient to enable surviving DA terminals to maintain DA in synaptic cleft in physiological concentration which is in line with our data. Moreover, as shown by the present study there is no more capability for additional DA release in response to AMPH challenge.

Reciprocal interconnection between dopaminergic and serotoninergic system in intact and fully DA-denervated rats

There is a body of evidence that in the basal ganglia DA release is substantially modified by 5-HT acting through putative heteroreceptors (e.g 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2C}) (41-43). It was determined that 5-HT_{2C} antagonist and agonist enhance and inhibit, respectively, DA release at terminals of intact rats (44). In line with the above report we observed in control animals approximately 45% increase in DA extraneuronal concentration in the neostriatum after KET injection. Conversely, similar response to 5-$HT_{2A/2C}$ antagonist were not more observed in 6-OHDA lesioned animals. It is interesting to know that Maeda et al. (45) demonstrated that quinpirole (D_2/D_3 receptor agonist) dose dependently inhibited striatal DA release even in animals with DA denervated striatum and this way they proved that autoregulation by presynaptic DA D_2 receptors (in spite of tremendous DA terminals devastation) is preserved and still operative on endogenous DA in extraneuronal space. If we take into account that the large number of 5-HT_2 receptors are heteroreceptors located on dopaminergic terminals, and lesioning studies (with 6-OHDA) showed decrease in 5-HT_2 receptors binding (41), thus it may explain an abolishment of 5-$HT_{2A/2C}$ antagonist mediated effects in 6-OHDA lesioned rats. There is a body of evidence indicating that 5-HT_{2C} receptor inhibits nigrostriatal transmission (44). Conversely, 5-$HT_{2A/2C}$ antagonism enhances the anti-parkinsonian action of DA receptor agonists, an effect observed in behavioral studies (46). Fox and Brotchie (13) implied that anti-parkinsonian action of 5-$HT_{2A/2C}$ antagonists may involve reducing the overactivity of the substantia nigra pars reticulata at the receptor level only, but not by the influence on DA release, which was proved by the present study (KET did not affect DA release in the neostriatum in 6-OHDA-lesioned animals).

In the current experiment we also found that in fully DA-innervated (control) rats, KET diminished AMPH evoked DA efflux. Similar results were demonstrated by Auclair et al. (47) who showed decrease in AMPH evoked DA extracellular level (in the nucleus accumbens) after a specific 5-HT_{2A} antagonist challenge accompanied by simultaneous reduction in locomotor activity. Also Schmidt et al. (48) found that 5-HT_2 antagonist decrease DA release in the striatum induced by AMPH analogue (3,4-methylenedioxymethamphetamine). These observations are not only relevant for the examination of a various range of psychostymulants but might be also of particular interest in relation to Parkinson's disease (e.g. L-DOPA induced psychosis which is thought to be

related to overactivity of certain dopaminergic pathways). However, it must be stressed that we did not observed changes in DA efflux after combined AMPH and KET injection in DA - denervated rats. For better elucidating the role and usefulness of 5-HT_2 receptor ligands in PD replacement therapy further experiments with L-DOPA and KET are needed.

Biologic significance of extraneuronal HO· determination in the neostriatum of intact and 6-OHDA lesioned animals

The next important finding of the present study is determining that there are no substantial differences in extraneuronal HO· generation in the neostriatum between control and parkinsonian rats. The present data might seems to be in contrary with our earlier work (49). More specifically, previously we found that 2,3- and 2,5-DHBA, spin trap products from interaction of HO· with salicylate, were substantially altered in the neostriatum after DA-denervation. 2.3-DHBA content was increased more then 4-fold and 2,5-DHBA content was increased 2.5 fold (in comparison to fully DA-innervated rats). Moreover, L-DOPA treatment actually suppresses HO· formation in the neostriatum of those animals. However, there must be cautious to differentiate between extraneuronal and intraneuronal indices of free radical production. In the above-mentioned study we estimated mostly intraneuronal compartment (whole tissue DHBA content) whilst in the present paper only extraneuronal compartment (microdialysis effluent) was investigated. It must be also mentioned that DA itself can undergo both enzymatic and nonenzymatic reactions resulting in the formation of toxic radicals. The principal metabolic pathway of DA is intraneuronal oxidizing by monoamine oxidase (MAO). For every molecule of DA that is metabolized by MAO, one molecule of H_2O_2 is formed. Because striatum and substantia nigra are rich in iron (Fe2+), hydroxyl radical HO· will subsequently be generated *via* the Fenton reaction increasing this way the intraneuronal radicals level. This may in part elucidate apparent discrepancy between previously performed experiments (tissue study) and presented microdialysis data in this paper. It must be also considering that extraneuronal compartment is effectively protected by many antioxidants (e.g. ascorbic acid, uric acid, etc.) before ROS formation. Extracellular ascorbic acid concentration in the neostriatum ranges between 350 and 500μM (50) and increases by about 50-80% following systemic injection of AMPH. Furthermore, Miele et al. (48) found that subcutaneous AMPH (2.0 mg/kg) injection increased also uric acid in the neostriatum by more then 50%. It is worth observing that uric acid is also an active component of the neuronal antioxidant pool, it protects ascorbic acid and DA from oxidation (52, 53). The above-mentioned data may explain the fact that AMPH did not affected 2.3- and 2.5-DHBA concentration in the microdialysates of the neostriatum, the effect observed in the current study. However, it is generally accepted that AMPH or metamphetamine applied in very high doses (10.0 mg/kg or more) increased ROS

production in the intra- and extraneuronal compartment of the striatum (54, 55). As we indicated by the present work, KET (5.0 mg/kg ip) by itself did not rise the concentration of extraneuronal HO·. It is relevant because of some reports pointing out that DA acting drugs may either suppress or promote hydroxyl radical formation in the brain (56-58).

CONCLUSIONS

Findings from the present study demonstrate that there are no substantial differences in extraneuronal HO· generation in the neostriatum between control and parkinsonian rats. KET did not affect DA release in the fully DA-denervated rats neostriatum and also did not enhance HO· production. From the above we concluded that the use of 5-$HT_{2A/2C}$ receptor antagonists in Parkinson's disease does not impend the neostriatal neuropil to be damaged by these drugs. The above is important because on the basis of many studies pointing out that 5-$HT_{2A/2C}$ receptor-mediated transmission might prove usefulness not only in addressing motor complications of PD patients (dyskinesia) but also in addressing non-motor problems such as depression and/or L-DOPA evoked psychosis. This way 5-$HT_{2A/2C}$ receptor antagonists may provide an attractive non-dopaminergic target for improving therapies for some basal ganglia disorders as well as psychiatric disturbances.

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