Neurotoxicity Research, 2008, VOL. 13(3,4). pp. 231-240

F.P. Graham Publishing Co.

Histamine H_3 Receptor Ligands Modulate L-dopa-evoked Behavioral Responses and L-dopa-derived Extracellular Dopamine in Dopamine-denervated Rat Striatum

PRZEMYSLAW NOWAK[a,*], ALEKSANDRA BORTEL[a], JOANNA DABROWSKA[a], IZABELA BIEDKA[a], GRZEGORZ SLOMIAN[a], WOJCIECH ROCZNIAK[a], RICHARD M. KOSTRZEWA[b] and RYSZARD BRUS[a]

[a]*Department of Pharmacology, Medical University of Silesia, 41-808 Zabrze, Poland;* [b]*Department of Pharmacology, Quillen College of Medicine, East Tennessee State University, Johnson City, TN, USA.*

(Submitted 30 March 2007; Revised 11 January 2008; In final form 15 January 2008)

To explore a recently established association between histaminergic and dopaminergic neuronal phenotypic systems in brain, we determined the effect of the respective histaminergic H_3 receptor agonist and antagonist/inverse agonist, imetit and thioperamide, on L-DOPA - derived tissue and extracellular dopamine (DA) and metabolite levels in the striatum of 6-hydroxydopamine (6-OHDA) - lesioned rats (*i.e.*, parkinsonian rats). We also examined the influence of histamine H_3 ligands on L-DOPA evoked behavioral responses (locomotor activity, number of rearings, stereotyped behavior and motor coordination). Using HPLC/ED and *in vivo* microdialysis technique, imetit (5 mg/kg, i.p.) but not thioperamide (5 mg/kg, i.p.) was shown to attenuate an L-DOPA-evoked (15 mg/kg, i.p.; carbidopa, 30 min pretreatment) increase in extracellular DA in the neostriatum of 6-OHDA-lesioned rats. However, both imetit and thioperamide increased microdialysate levels of DOPAC and HVA, probably by enhancing intraneuronal DA utilization. As indicated by neurochemical analysis of the striatum imetit produced a decrease in tissue DA content. These findings support the hypothesis that central H_3 histaminergic receptors have a modulatory role in the storage, metabolism and release of DA derived from exogenous L-DOPA challenge. Furthermore, evidence from behavioral studies indicate that histamine H_3 receptor block markedly improved motor coordination. Conversely, histamine H_3 receptor stimulation, being without effect on motor coordination, enhanced vertical activity in rats. From the above we conclude that histamine H_3 agonism may augment motor dyskinesia in Parkinson's disease (PD) patients and presumably worsen L-DOPA therapy. Consequently, the histaminergic system represents a viable target for modulating the effectiveness of L-DOPA therapy in Parkinson's disease.

Keywords: Histamine H_3 receptor; L-DOPA; Dopamine; 6-OHDA-lesioned rat; Microdialysis; Brain biogenic amines

INTRODUCTION

A revolution in the treatment of Parkinson's disease (PD) occurred with recognition that L-dihydroxyphenylalanine (L-DOPA), the metabolic precursor to dopamine (DA), replenishes neuronal DA stores and in this way improves motor features of PD. Unfortunately, long-term L-DOPA administration results in

*Corresponding author: Tel./FAX: +48 32 272 2683; E-mail: pnowak@slam.katowice.pl

ISSN 1029 8428 print/ ISSN 1476-3524 online. www.NeurotoxicityResearch.com

the emergence of adverse effects and a gradual loss of its effectiveness (Widnell, 2005). Therefore, many studies have been performed to examine factors that modify L-DOPA metabolism and biotransformation, as well as agents that affect L-DOPA derived extracellular DA in the neostriatum. Recognition of these factors serves a useful purpose for better elucidation of mechanisms underlying the gradual reduction of antiparkinsonian effects of L-DOPA, and understanding mechanisms of adverse effects linked to L-DOPA or other dopaminomimetic treatments (Brus *et al.*, 2003; Kostrzewa *et al.*, 2004; 2005).

In the past 10-15 years, the neuronal histaminergic system in brain has become reasonably well characterized (Cumming *et al.*, 1991; Pollard *et al.*, 1993; Brown *et al.*, 2001). Cell bodies of histaminergic neurons are located exclusively in the tuberomammillary nuclei of the hypothalamus and give rise to widespread projections throughout the central nervous system, including subcortical nuclei and cerebral cortex. Four subtypes (H_1, H_2, H_3, H_4) of histamine receptors are currently recognized. The histamine H_3 subtype is the least defined, although histamine H_3 receptors are now known to be predominately located presynaptically, functioning as an autoreceptor that regulates the synthesis and release of histamine (Arrang *et al.*, 1988; Sanchez-Lemus and Arias-Montano, 2004). Recently, histaminergic agonists and antagonists were shown to modulate DA-derived behavioral responses (Farzin and Attarzadeh, 2000; Huotari *et al.*, 2000). Histamine H_3 receptors are of major interest, in part, because of the following reasons:

(a) Histamine H_3 receptors are presynaptically located on dopaminergic terminals in mouse striatum, modulating DA release (Schlicker *et al.*, 1993). Histamine H_3 receptors also modulate serotonin, acetylcholine and GABA release (Arias-Montano *et al.*, 2001; Garcia *et al.*, 1997; Threlfell *et al.*, 2004). All of these neurotransmitters are intricately involved in the pathophysiological responses in PD; also implicated in L-DOPA action; and markedly dysregulated in basal ganglia of PD patients (Gibb, 1997);

(b) There is an increased number of histamine H_3 receptors in the striatum of 6-hydroxydopamine (6-OHDA) -lesioned rats, and the H_3 receptor upregulation is prevented by a DA D_1 receptor agonist (Ryu *et al.*, 1994; 1996);

(c) Histamine H_3 receptor agonists modulate D_1 receptor function, either by strongly inhibiting DA D_1 receptor-dependent release of [^{3}H]-GABA from rat striatum; or through an interaction at the terminals of GABA neurons (Arias-Montano *et al.*, 2001); or by modulating cAMP formation in striatal neurons that possess D_1 receptors (Sanchez-Lemus and Arias-Montano, 2004);

(d) Histamine H_3 receptor number is highest in the striatum and globus pallidum of human brain, exerting a regulatory effect on basal ganglia function (Martinez-Mir *et al.*, 1990);

(e) Finally, Anichtchik *et al.* (2001) have shown that histamine H_3 receptor mRNA expression in the striatum, and histamine H_3 receptor number in the substantia nigra, are substantially altered in parkinsonian versus non-parkinsonian brain.

The aforementioned findings indicate a close association between dopaminergic and histaminergic systems in brain, with the latter modulating DA synthesis/release/metabolism via H_3 receptors. In the present study we explored the possible role of histamine H_3 receptors on L-DOPA-induced behavioral effects (locomotor activity, number of rearings, stereotyped behavior and motor coordination) and L-DOPA-derived DA and metabolites in the striatum of both intact and 6-OHDA-lesioned rats. Tissue levels of DA were analyzed as well as *in vivo* microdialysate levels of DA (*i.e.*, extraneuronal origin), for a better understanding of the interplay of the two neurotransmitter systems.

MATERIALS AND METHODS

Subjects

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 12 h light:12 h dark cycle (lights on 7:00 a.m. to 7:00 p.m.), and with free access to food and water. Rat offspring were weaned at 21 days, and segregated by sex. Only male rats were used in this study. All procedures, reviewed and approved by the Local Bioethical Committee for Animal Care, are in accord with principles and guidelines described in the NIH booklet *Principles of Laboratory Animal Care.*

Neonatal Treatment

At 3 days after birth rats were pretreated with desipramine HCl (20 mg/kg, i.p., base; 1 h) (Sigma) and pargyline HCl (50 mg/kg, i.p., salt form; 0.5 h) (Sigma) and injected bilaterally i.c.v. with 6-OHDA HBr (66.7 μg base form on each side). This procedure has been described in detail (Kostrzewa and Gong, 1991). Rats were housed in the condition as above until 10 weeks for further experiments.

***In vivo* Microdialysis Procedure**

Rats were anesthetized with relanium (Polfa) (10 mg/kg, i.p.) and ketamine (Parke-Davis) (80 mg/kg, i.p.), and placed in a stereotaxic frame. The dermis overlying the skull was incised, and the dermis was retracted to expose the skull plate. A small burr hole was drilled to allow implantation of a dialysis probe with 4 mm active membrane (ID 75 μm, OD 150 μm, Polymicron Technologies, USA) into the right striatum (A +0.7, L +3.0, V -7.0 according to the Paxinos and Watson stereotaxis atlas). Two stainless steel screws were mounted near the probe and fastened to the skull with dental cement (Duracryl Plus, Spofa, Praha). 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 20 min and injected directly onto a 3 μm 150x3 mm column (MD 150/RP-18, ESA, USA), using a mobile phase consisting of 1.7 mM 1-octanesulfonic acid, 25 μM EDTA, 100 μl triethylamine/liter, 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 (E_1 +250; E_2 -175) were used for analysis, with a Coulochem (ESA, USA) data analysis system to integrate peak areas of DA, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) (Takeda *et al.*, 1990; Brus *et al.*, 2002; Nowak *et al.*, 2002). When dialysate DA levels were constant (approx. 1.5 h from the start of perfusion), rats were pretreated with the peripherally-acting DOPA decarboxylase inhibitor *S*-carbidopa (12.5 mg/kg, i.p.), 30 min before L-DOPA challenge (15 mg/kg, i.p.) (control animals). Other groups of rats were additionally injected either with imetit (5 mg/kg, i.p.) or thioperamide (5 mg/kg, i.p.), 5 min after *S*-carbidopa (*i.e.*, 25 min before L-DOPA challenge).

Tissue Sample Preparation

At the end of microdialysis study (exactly 180 min after *S*-carbidopa injection) animals of all tested groups [lesioned rats treated with L-DOPA (control), lesioned rats treated with thioperamide and L-DOPA; and lesioned rats treated with imetit and L-DOPA] were decapitated. The striatum (from the left hemisphere) was rapidly dissected and placed on dry ice, weighed and stored at -70°C, pending assay. Samples were homogenized for 15-20 sec in ice-cold trichloracetic acid (0.1 M), containing 0.05 mM ascorbic acid. After centrifugation (5,000*g*, 5 min), 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 DA, DOPAC, HVA, 5-HT, 5-HIAA as well as noradrenaline (NA) were assayed by HPLC/ED (Magnusson *et al.*, 1980; Nowak *et al.*, 2002). The composition of the mobile phase was: 75 mM NaH_2PO_4 (Avocado, Research Chemicals Ltd), 1.7 mM 1-octanesulphonic acid (Avocado, Research Chemicals Ltd), 5 μM EDTA (Avocado, Research Chemicals Ltd), 100 μl triethylamine (Sigma), 9.5% acetonitrile (Lab-Scan), pH 3 adjusted with phosphoric acid (Fluka). The flow rate was maintained at 0.7 ml/min, at a temperature of 22°C, and the oxidation potential was fixed at +700 mV, 10 nA/V sensitivity. Peaks were automatically integrated by universal chromatographic interface UCI-100 (Dionex, Germany). The instrumentation included an electrochemical detector (Gilson, France) model 141 with flow cell, piston pump model 302 with head 5SC (Gilson), manometric module model 802 (Gilson), 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).

Locomotor Activity

At 16 weeks rats were acclimated for >30 min in plastic cages (48x26x18 cm) with wood chip bedding, in a quiet and well-lighted room. Thereafter rats were pretreated with the peripherally-acting

DOPA decarboxylase inhibitor *S*-carbidopa (12.5 mg/kg, i.p.), 30 min before L-DOPA challenge (15 mg/kg, i.p.) (control animals). Other groups of rats were additionally injected either with imetit (5 mg/kg, i.p.) or thioperamide (5 mg/kg, i.p.) (5 min after *S*-carbidopa; *i.e.*, 25 min before L-DOPA challenge). After the last injection rats were individually observed for locomotor activity (walking, running, grooming, sniffing, eating and digging); numbers of rearings were also counted, starting 20 min later, for 10 min periods, every 20 min until 80 min. Rats were observed for 40 min in total (Nowak *et al.*, 2005).

Stereotyped Behavior

Rats were individually placed in transparent glass cages (48x26x36 cm, on fresh wood-chip bedding, and were allowed to acclimate for 30 min. Then, all rats were injected with *S*-carbidopa (12.5 mg/kg, i.p.), 30 min before L-DOPA challenge (15 mg/kg, i.p.) (control animals). Other groups of rats were additionally injected either with imetit (5 mg/kg, i.p.) or thioperamide (5 mg/kg, i.p.) (5 min after *S*-carbidopa; *i.e.*, 25 min before L-DOPA challenge). Then, every 30 min after the last injection, and up to 90 min, the stereotyped behavior of each rats was rated by the scoring method of Creese and Iversen on a scale of 0 - 6, where a score of 6 represented no stereotyped activity (Creese and Iversen, 1975).

Locomotor Coordination (Rota rod Test)

Ten minutes after saline pretreatment (1.0 ml/kg, i.p), rats were individually placed on a wooden bar, 3 cm in diameter. The bar circulated longitudinally four times per minute, and the length of time (in sec) on the rotating bar was recorded. Any rat remaining on the bar for 300 sec, was placed back in its cage. This test was carried out twice more on each rat, with 10 min intervals between tests. The mean time was calculated for each rat. Then, rats were injected with *S*-carbidopa (12.5 mg/kg, i.p.), 30 min before L-DOPA challenge (15 mg/kg, i.p.). After 10 min, 2 and 3 h motor coordination was examined according the methods described above.

Because L-DOPA in a dose of 15 mg/kg, i.p. had an adverse effect on locomotor coordination in 6-OHDA lesioned rats, H_3 ligands were not administered as an L-DOPA pretreatment. Consequently, for assessment of locomotor coordination, imetit and thioperamide were only injected alone in rats.

Data Analysis

Group differences 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.

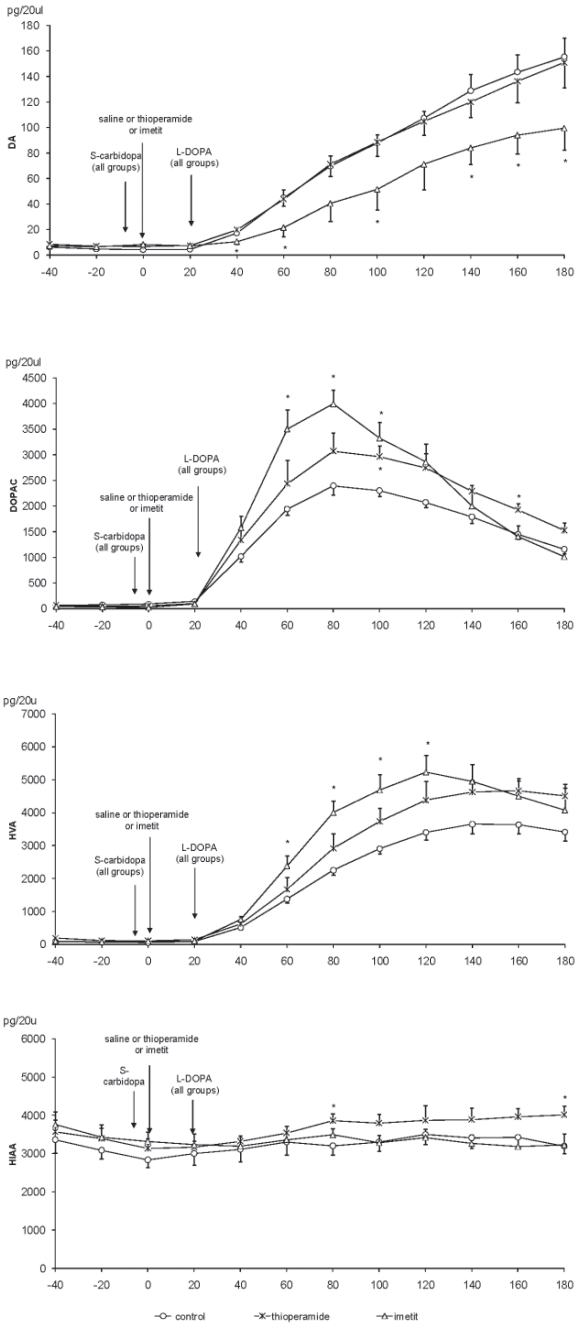

FIGURE 1 Effect of thioperamide (5.0 mg/kg) and imetit (5.0 mg/kg) on L-DOPA- (15.0 mg/kg) derived extracellular DA (a), DOPAC (b), HVA (c) and 5-HIAA (d) in neostriatum of 6-OHDA-treated rats (n=6). -o- control, -x- thioperamide, -Δ- imetit; *p <0.05.

RESULTS

In vivo Microdialysis Study

The baseline striatal extraneuronal (*i.e.*, microdialysate) level of DA in 6-OHDA lesioned rats of all examined groups varied between 3.2 - 3.6 pg/20 μl (FIG. 1a). In the control group, L-DOPA acutely produced a gradual increase in the microdialysate DA concentration, up to 160 pg/20 μl in 180 min; at this time-point, rats were sacrificed for further examination (catecholamine tissue assay). In rats pretreated with the histamine H_3 receptor agonist imetit, the L-DOPA-induced increase in microdialysate DA content was less than that seen in the control group (*i.e.*, saline in place of imetit pretreatment + L-DOPA), at 40, 60, 140-180 min (FIG. 1a). Conversely, there was no difference in the DA microdialysate concentration between control rats and the group that had been pretreated with thioperamide (histamine H_3 antagonist).

In this study the microdialysate level of DA metabolites was also analyzed. The baseline striatal extraneuronal (*i.e.*, microdialysate) level of DOPAC in 6-OHDA lesioned rats of all examined groups varied between 65.3 - 75.7 pg/20 μl (FIG. 1b). L-DOPA gradually elevated the DOPAC microdialysate concentration in all three groups, with the highest response observed at 80 min. The peak DOPAC concentration was higher in rats that had been pretreated with imetit, versus the control group (P <0.05 at 60, 80 and 100 min). Thioperamide increased the DOPAC microdialysate level to a greater extent versus controls (P <0.05 at 100 and 160 min), but to a lesser extent versus imetit (FIG. 1b). Similar data were obtained with HVA. The extraneuronal concentration of HVA in baseline samples varied between 87.4 - 98.3 pg/20 μl (FIG. 1c). L-DOPA challenge increased HVA in all groups, but to the greatest extent in rats pretreated with imetit (P <0.05 at 60 to 120 min) (FIG. 1c).The 5-HIAA microdialysate concentration did not differ between control and imetit pretreated animals (FIG. 1d). Thioperamide slightly increased the 5-HIAA level in comparison to the control (P <0.05 at 80 and 180 min).

Tissue Assay

At 150 min after L-DOPA injection, the DA striatal content was 376.9 ng/g, while DOPAC and HVA were, respectively, 155.9 and 529.6 ng/g (FIG. 2a). Imetit (5 mg/kg, i.p.), 10 minutes before L-DOPA injection, significantly reduced DA and DOPAC contents to 50-60% of control. The HVA level was also greatly diminished by imetit to 298.7 ng/g, but because of a high standard error, data were not significantly different between groups. Thioperamide was without effect (FIG. 2a).

Neither imetit nor thioperamide influenced neostriatal NA, 5-HT and 5-HIAA contents (FIG. 2b).

Locomotor Activity

Twenty min after L-DOPA injection (15 mg/kg, i.p.) locomotor activity in the control group was 119 ± 24 sec. Neither imetit nor thioperamide modified this effect; locomotor activity after imetit pretreatment was 139 ± 27 sec, and after thioperamide pretreatment 101 ± 11 sec. After 40 min, a marked increase in locomotor activity was observed in all tested groups; 509 ± 37 sec (control); 452 ± 35 sec (imetit) and 523 ± 24 sec (thioperamide), respectively. A similar effect was seen at subsequent inter-

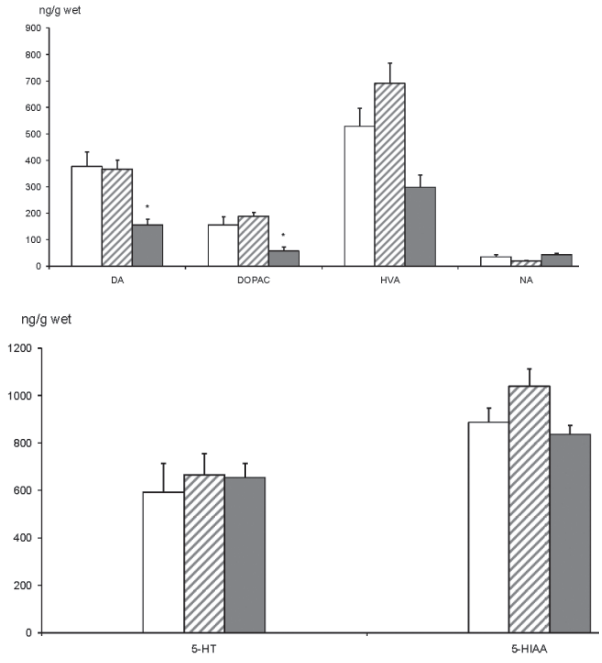

FIGURE 2 Effect of thioperamide (5.0 mg/kg) and imetit (5.0 mg/kg) on L-DOPA- (15.0 mg/kg) induced alterations in neostriatal levels of DA, DOPAC, HVA, NA (a) and 5-HT and 5-HIAA (b) in 6-OHDA-lesioned rats (n=6). (clear bar) L-DOPA, (light shaded bar) L-DOPA + thioperamide, (dark shaded bar) L-DOPA + imetit; *p <0.05.

vals, although at 90 min there was an attenuation in locomotor activity in rats injected with imetit versus controls (*i.e.*, rats injected only with L-DOPA) (FIG. 3).

To assess vertical activity of rats simultaneous with locomotor activity, the number of rearings were counted. As shown in FIG. 4, numbers of rearings in control and thioperamide groups were similar, while imetit as a pretreatment to L-DOPA enhanced vertical activity at 40, 60 and 80 min (P <0.05) (FIG. 4).

Stereotyped Behavior

Thirty min after L-DOPA, rats from the control group exhibited stereotypic reactions (*e.g.*, stereotyped walking, climbing). Then, compulsive movements (stereotyped sniffing and climbing in the same place) gradually increased, peaking at 90 min of observation. Rats injected with thioperamide and L-DOPA demonstrated a similar pattern of behavior in comparison to control animals (L-DOPA alone). In the imetit + L-DOPA group stereotyped behavior was far less pronounced (P <0.05) at all times) (FIG. 5).

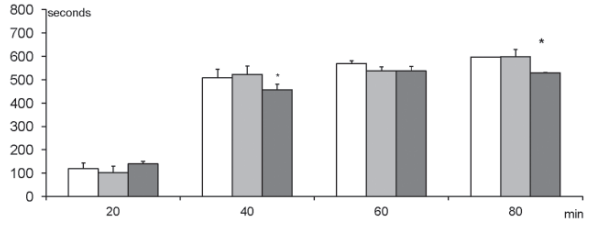

FIGURE 3 Effect of thioperamide (5.0 mg/kg) and imetit (5.0 mg/kg) on L-DOPA- (15.0 mg/kg) evoked locomotor activity in 6-OHDA-treated rats (n=8). (clear bar) L-DOPA, (light shaded bar) L-DOPA + thioperamide, (dark shaded bar) L-DOPA + imetit; *p <0.05.

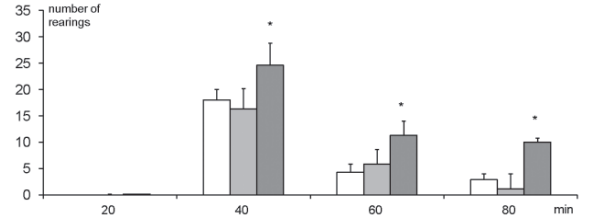

FIGURE 4 Effect of thioperamide (5.0 mg/kg) and imetit (5.0 mg/kg) on L-DOPA- (15.0 mg/kg) evoked number of rearings in 6-OHDA-treated rats (n=8). Rest of legend as in figure 3.

Motor Coordination (Rota rod Test)

Coordination time in 6-OHDA lesioned rats after saline injection was approximately 35 sec; L-DOPA (15 mg/kg i.p.) acutely shortened time on the rod in the 2nd and 3rd h after administration (P <0.05). Imetit was without effect in this regard, while thioperamide prolonged the time each rat managed to stay on the rotating bar, in comparison to saline alone (P <0.05 in 2 and 3 h) (FIG. 6).

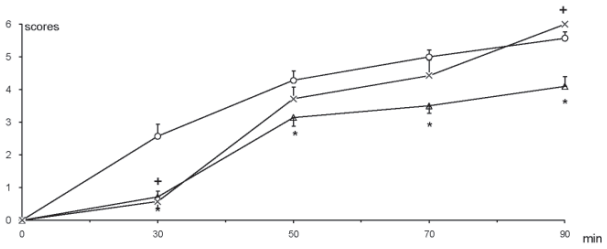

FIGURE 5 Effect of thioperamide (5.0 mg/kg) and imetit (5.0 mg/kg) on L-DOPA- (15.0 mg/kg) evoked stereotyped behavior in 6-OHDA-treated rats (n=8). -o- L-DOPA, -x- L-DOPA + thioperamide, -Δ- L-DOPA + imetit; *p <0.05 (L-DOPA / L-DOPA+imetit); ^{+}p <0.05 (L-DOPA / L-DOPA + thioperamide).

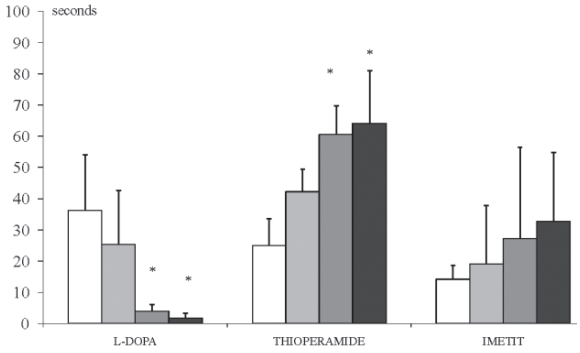

FIGURE 6 Effect of L-DOPA (15.0 mg/kg), thioperamide (5.0 mg/kg) and imetit (5.0 mg/kg) on motor coordination in 6-OHDA-treated rats (n=6). (clear bar) Motor coordination after saline injection; (light shaded bar) Motor coordination 10 min after L-DOPA, thioperamide, or imetit; (mid shaded bar) Motor coordination 2 h after L-DOPA, thioperamide, or imetit; (dark shaded bar) Motor coordination 3 h after L-DOPA, thioperamide, or imetit; *p <0.05.

DISCUSSION

The present study demonstrates that the histamine H_3 agonist imetit but not the histamine H_3 antagonist thioperamide, attenuates an L-DOPA-induced increase in extracellular DA in the neostriatum of 6-OHDA lesioned animals. However, both histamine H_3 ligands (thioperamide to a far less extent) increased DOPAC and HVA microdialysate concentrations, probably by enhancing intraneuronal DA utilization. In behavioral tests we demonstrated that imetit diminished stereotyped behavior but increased vertical activity in rats, being without effect on motor coordination. Conversely, thioperamide did not affect locomotor "components" but markedly improved motor coordination.

Methodological Consideration

In the present work, two highly selective H_3 ligands were used, imetit which is a full agonist, with approximately 60-times greater *in vivo* and *in vitro* potency than histamine and with 4-times greater potency than (*R*)-α-methylhistamine (Garbarg *et al.*, 1992); and thioperamide, a potent and specific histamine H_3 receptor antagonist/inverse agonist (Ganellin *et al.*, 1995; Wieland *et al.*, 2001). Based on earlier findings, both H_3 ligands (in a dose range of 0.2-10 mg/kg) influence behavioral responses evoked by dopamine receptor stimulation in rats and mice (Clapham and Kilpatrick, 1994; Perez-Garcia *et al.*, 1999; Farzin and Attarzadeh, 2000; Garcia-Ramirez *et al.*, 2004). In the present study we tested an intermediate dose (5 mg/kg) of each ligand.

Among the manifold Parkinson's disease animal models that have been introduced (Moore *et al.*, 2005; Ossowska *et al.*, 2005), the neonatal 6-OHDA-lesioned rat in the present study is posed as a near-ideal model of severe Parkinson's disease. This is due to the non-lethality of the procedure, profound destruction of nigrostriatal dopaminergic fibers, near-total DA-denervation of striatum, and reproducibility. As previously demonstrated, ontogenetic 6-OHDA treatment is associated with reliably marked reduction in adulthood levels of striatal DA (~99%), DOPAC (~98%) and HVA (~98%) (Kostrzewa *et al.*, 2006).

Motor Coordination and pro-Dyskinetic Effect of Histamine H_3 Receptor Stimulation in 6-OHDA-Lesioned Rats

Because neither histamine H_3 receptor agonism nor antagonism directly affected locomotor activity in the present study, histaminergic H_3 receptors appear to influence locomotor activity primarily via their modulatory effect on dopaminergic system function (Perez-Garcia *et al.*, 1999). In this vein, Clapham and Kilpatric (1994) showed that thioperamide reduced amphetamine-induced locomotor activity in the mouse. This observation is in agreement with others (Toyota *et al.*, 2002). However, in the present study L-DOPA-evoked locomotor activity was not affected by either imetit or thioperamide pretreatments. This apparent discrepancy may be related to use of L-DOPA (DA precursor) which enhances DA synthesis, in contrast to amphetamine which evokes DA release. Also, in the present study a rat model of severe parkinsonism was engaged. The important finding is that imetit, injected prior to L-DOPA, significantly enhanced vertical activity in 40, 60 and 80 min of observation (FIG. 4). Johnston *et al.* (2005) demonstrated that drugs that have previously been found to reduce L-DOPA induced dyskinesia in parkinsonian primates and PD patients without compromising the anti-parkinsonian efficacy of L-DOPA, dose-dependently reduce vertical components of activity when co-administered with L-DOPA in reserpine-treated rats (DA depleted animals). Our findings coupled with these latter findings, imply that histamine H_3 receptors are likely to augment motor dyskinesia in L-DOPA-treated PD patients. This observation is also in agreement with our recently published data (Nowak *et al.*, 2006a).

The histamine H_3 receptor antagonist thioperamide alone improved motor coordination in the rota-rod test while imetit was without effect (FIG. 6). Moreover, the high dose of L-DOPA (15 mg/kg, i.p.) in the current study perturbed motor coordination of rats, probably by evoking stereotyped behavior. Interestingly, Song *et al.* (2006) demonstrated that microinjection of histamine into the cerebellar interpositus nucleus remarkably increased time that animals balanced steadily on the rota-rod. In addition, administration of the selective histamine H_2 receptor antagonist ranitidine considerably decreased animal endurance on the rota-rod, but the

selective histamine H_1 receptor antagonist triprolidine had no effect. Although these results are seemingly inconsistent with our data, the above authors i) did not investigate histamine H_3 receptor ligands, ii) did not employ parkinsonian rat, and iii) focally injected into the cerebellum.

Stereotyped Behavior in 6-OHDA-Lesioned Rats

Farzin and Attarzadeh (2000) found that imetit diminished while thioperamide potentiated apomorphine-induced licking behavior in mice. There are only sparse data concerning a role for histamine H_3 receptors in L-DOPA effects. Huotari *et al.* (2000) found that the histamine H_3 receptor agonist, *R*-α-methylhistamine, reduced contralateral L-DOPA-induced circling in rats with a unilateral lesion of nigrostriatal fibers (a model of hemiparkinsonism). However, less clear was the effect of thioperamide on rat behavior. This is in line with our data, as we demonstrated imetit significantly attenuated stereotyped behavior evoked by L-DOPA administration; the thioperamide effect was also less clear, but in general this substance did not affect the examined parameter (FIG. 5).

Garcia-Ramirez *et al.* (2004) also determined that in rats with a 6-OHDA lesion of either substantia nigra pars compacta or medial forebrain bundle, apomorphine-induced contralateral turning was reduced by intranigral imetit, an effect prevented by systemic thioperamide administration. It is commonly accepted that drugs that diminished stereotyped behavior (evoked by dopaminomimetics) do so by reducing dopaminergic transmission. Therefore, based on the above data and our own observation it is reasonable to conclude that histamine H_3 receptor stimulation may counteract the effectiveness of L-DOPA therapy.

Histamine H_3 Receptor Ligands and Striatal DA, DOPA and HVA. Microdialysis Study.

Behavioral findings are in agreement with the microdialysis data presented in this paper, showing that histamine H_3 receptor agonism reduces L-DOPA-derived DA in extraneurnonal striatum and in striatal tissue.

Maeda *et al.* (1999) showed that the DA D_2 agonist quinpirole dose-dependently (0.01-3 mg/kg s.c.) suppressed DA release both in the intact and DA-denervated striatum. However, in denervated striatum, L-DOPA-derived DA release was not affected by quinpirole pretreatment - suggesting that in the largely DA-denervated striatum, regulation by presynaptic DA D_2 receptors is still operative on endogenous DA release but not on release of DA derived from exogenously administered L-DOPA. If DA D_2 autoreceptors have minimal modulatory influence over L-DOPA-associated DA release - the cardinal mechanism regulating this process - it would be rather doubtful that diminished DA release into an extraneuronal space following histamine H_3 agonist treatment is mediated by presynaptic histamine H_3 receptors located on the near-totally destroyed DA terminals. Additionally, in other recently published studies (Nowak *et al.*, 2006a) we showed that neither activation nor block of histamine H_3 receptors modifies endogenous DA release in the neostriatum of 6-OHDA lesioned animals. Nevertheless, one must be cognizant that *in vivo* metabolism of L-DOPA to DA may occur in non-dopaminergic neurons. Based on our previous data, it appeared that 5-HT neuronal phenotypes could be involved in L-DOPA metabolism to DA (Johnston *et al.*, 2005; Nowak *et al.*, 2006b; Kostrzewa *et al.*, 2007). Also, Tanaka *et al.* (1999) showed that exogenously administered L-DOPA is converted into DA in serotonergic terminals and thereafter released into the extracellular space. Furthermore, Kannari *et al.* (2001) established that such release is controlled by 5-HT_{1A} but not 5-HT_{1B} receptors. 5-HT_{1A} receptor activation attenuates L-DOPA-derived DA in the microdialysate. According to our results, neither imetit nor thioperamide influenced 5-HT and 5-HIAA tissue levels as well as microdialysate 5-HIAA level in the neostriatum. Unfortunately we were not able to determine 5-HT content in the striatal microdialysate. Threlfell *et al.* (2004), however, did report that the histamine H_3 agonist imepip inhibited evoked 5-HT release by up to 60% in substantia nigra pars reticulate - in intact rats. Although 5-HT and 5-HIAA were not affected by histamine H_3 ligands (in our study) we can not discount the possibility that serotoninergic nerves are associated with H_3 receptor-mediated effects. Further studies with a 5-HT_{1A} antagonist would help to elucidate this problem.

Histamine H_3 Receptor Ligands and Striatal DA, DOPAC and HVA Level. Tissue Study.

In the present study, only imetit suppressed DA, DOPAC and HVA striatal tissue levels of rats acutely treated with L-DOPA. Conversely, an increase in DOPAC and HVA levels was observed in microdialysates after imetit pretreatment. The increase in intraneuronal DA metabolism induced by imetit pretreatment (and assessed by DOPAC and HVA microdialysate concentrations) may reflect "a wash out" effect.

CONCLUSION

The present study is believed to be the first to show that histamine H_3 receptor modulation substantially influences neostriatal DA metabolism after L-DOPA treatment of parkinsonian rats. Furthermore, behavioral data suggest that histamine H_3 receptor stimulation may augment motor dyskinesia (an occurrence often seen in L-DOPA treated patients) and simultaneously reduce effectiveness of L-DOPA therapy. In contrast, histamine H_3 receptor blockade may contribute to improvement of some motor complication occurring in PD patients.

Summing up, the present results provide insight into the association between histamine H_3 receptors in L-DOPA effects in PD, and implicate H_3 receptors as a therapeutic target in the PD.

References

Anichtchik OV, N Peitsaro, JO Rinne, H Kalimo and P Panula (2001) Distribution and modulation of histamine H_3 receptors in basal ganglia and frontal cortex of healthy controls and patients with Parkinson's disease. *Neurobiol. Dis.* **8**, 707-716.

Arias-Monatano JA, B Floran, M Garcia, J Aceves and JM Young (2001) Histamine H_3 receptor-mediated inhibition of depolarization-induced, dopamine D_1 receptor-dependent release of [^{3}H]-γ-aminobutryic acid from rat striatal slices. *Br. J. Pharmacol.* **133**, 165-171.

Arrang JM, B Devaux, JP Chodkiewicz and JC Schwartz (1988) H_3-receptors control histamine release in human brain. *J. Neurochem.* **51**, 105-108.

Brown RE, DR Stevens and HL Haas (2001) The physiology of brain histamine. *Prog. Neurobiol.* **63**, 637-672.

Brus R, P Nowak, A Sokola, RM Kotrzewa and J Shani (2002) Behavioral and biochemical effects of new central dopamine D_3 and D_4 receptor antagonists in rats. *Pharmacol. Rev. Comm.* **12**, 39-59.

Brus R, RM Kostrzewa, P Nowak, KW Perry and JP Kostrzewa (2003) Ontogenetic quinpirole treatments fail to prime for D_2 agonist-enhancement of locomotor activity in 6-hydroxydopamine-lesioned rats. *Neurotox. Res.* **5**, 329-338.

Clapham J and GJ Kilpatrick (1994) Thioperamide, the selective histamine H_3 receptor antagonist, attenuates stimulant-induced locomotor activity in the mouse. *Eur. J. Pharmacol.* **259**, 107-114.

Creese I and SD Iversen (1975) Behavioral sequel of dopaminergic degeneration: post-synaptic supersensitivity, In: *Modern Pharmacology - Toxicology* (Ellsodin JR and JR Buney, Eds.) (Marcel Dekker Publ.) **3**, 171-190.

Cumming P, C Shaw and SR Vincent (1991) High affinity histamine binding site is the H_3 receptor: characterization and autoradiographic localization in rat brain. *Synapse* **8**, 144-151.

Farzin D and M Attarzadeh (2000) Influence of different histamine receptor agonists and antagonists on apomorphine-induced licking behavior in rat. *Eur. J. Pharmacol.* **404**, 169-174.

Ganellin CR, SK Hosseini, YS Khalaf, W Tertiuk, JM Arrang, M Garbarg, X Ligneau and JC Schwartz (1995) Design of potent non-thiourea H_3-receptor histamine antagonists. *J. Med. Chem.* **38**, 3342-3350.

Garbarg M, JM Arrang, A Rouleau, X Ligneau, MD Tuong, JC Schwartz and CR Ganellin (1992) *S*-[2-(4-imidazolyl) ethyl] isothiourea, a highly specific and potent histamine H_3 receptor agonist. *J. Pharmacol. Exp. Ther.* **263**, 304-310.

Garcia M, B Floran, JA rias-Monatano, JM Young and Aceves (1997) Histamine H_3 receptor activation selectively inhibits dopamine D_1 receptor-dependent [^{3}H]GABA release from depolarization-stimulated slices of rat substantia nigra pars reticulata. *Neuroscience* **80**, 241-249.

Garcia-Ramirez M, J Aceves and JA Arias-Montano (2004) Intranigral injection of the H_3 agonist immepip and systemic apomorphine elicit ipsilateral turning behaviour in naive rats, but reduce contralateral turning in hemiparkinsonian rats. *Behav. Brain Res.* **154**, 409-415.

Gibb WR (1997) Functional neuropathology in Parkinson's disease. *Eur. Neurol.* **38**, 21-25.

Huotari M, K Kukkonen, N Liikka, T Potasev, A Raasmaja and PT Mannisto (2000) Effects of histamine H_3-ligands on the levodopa-induced turning behavior of hemiparkinsonian rats. *Parkinsonism Relat. Disord.* **6**, 159-164.

Johnston TH, J Lee, J Gomez-Ramirez, SH Fox and JM Brotchie (2005) A simple rodent assay for the *in vivo* identification of agents with potential to reduce levodopa-induced dyskinesia in Parkinson's disease. *Exp. Neurol.* **191**, 243-250.

Kannari K, H Yamato, H Shen, M Tomiyama, T Suda and M Matsunaga (2001) Activation of 5-HT_{1A} but not 5-HT_{1B} receptors attenuates an increase in extracellular dopamine derived from exogenously administered L-DOPA in the striatum with nigrostriatal denervation. *J. Neurochem.* **76**, 1346-1353.

Kostrzewa RM and L Gong (1991) Supersensitized D_1 receptors mediate enhanced oral activity after neonatal 6-OHDA. *Pharmacol. Biochem. Behav.* **39**, 677-682.

Kostrzewa RM, JP Kostrzewa, P Nowak, RA Kostrzewa and R Brus (2004) Dopamine D_2 agonist priming in intact and

dopamine-lesioned rats. *Neurotox. Res.* **6**, 457-462.

Kostrzewa RM, P Nowak, JP Kostrzewa, RA Kostrzewa and R Brus (2005) Peculiarities of L-DOPA treatment of Parkinson's disease. *Amino Acids* **28**, 157-164.

Kostrzewa RM, JP Kostrzewa, R Brus, RA Kostrzewa and P Nowak (2006) Proposed animal model of severe Parkinson's disease: neonatal 6-hydroxydopamine lesion of dopaminergic innervation of striatum. *J. Neural. Transm.* (Suppl.) **70**, 277-279.

Kostrzewa RM, NY Huang, JP Kostrzewa, P Nowak and R Brus (2007) Modeling tardive dyskinesia: predicitive 5-HT_{2C} receptor antagonist treatment. *Neurotox. Res.* **11**, 41-50.

Maeda T, K Kannari, T Suda and M Matsunaga (1999) Loss of regulation by presynaptic dopamine D_2 receptors of exogenous L-DOPA-derived dopamine release in the dopaminergic denervated striatum. *Brain Res.* **817**, 185-191.

Magnusson O, LB Nilsson and D Westerlund (1980) Simultaneous determination of dopamine, DOPAC and homovanillic acid. Direct injection of supernatants from brain tissue homogenates in a liquid chromatography--electrochemical detection system. *J. Chromatogr.* **221**, 237-247.

Martinez-Mir MI, H Pollard, J Moreau, JM Arrang, M Ruat, E Traiffort, JC Schwartz and JM Palacios (1990) Three histamine receptors (H_1, H_2 and H_3) visualized in the brain of human and non-human primates. *Brain Res.* **526**, 322-327.

Moore DJ, AB West, VL Dawson and TM Dawson (2005) Molecular pathophysiology of Parkinson's disease. *Annu. Rev. Neurosci.* **28**, 57-87.

Nowak P, R Brus, J Oswiecimska, A Sokola and RM Kostrzewa (2002) 7-Nitroindazole enhances amphetamine-evoked dopamine release in rat striatum. An *in vivo* microdialysis and voltammetric study. *J. Physiol. Pharmacol.* **53**, 251-263.

Nowak P, RM Kostrzewa, A Kwiecinski, A Bortel, L Labus and R Brus (2005) Neurotoxic action of 6-hydroxydopamine on the nigrostriatal dopaminergic pathway in rats sensitized with D-amphetamine. *J. Physiol. Pharmacol.* **56**, 325-333.

Nowak P, J Dabrowska, G Szczerbak, I Biedka, R Brus and RM Kostrzewa (2006a) Effect of the central H_3 histamine receptor agonist and antagonist on D_1 evoked behavioral responses in rats with dopaminergic system denervation. *Eur. J. Pharmacol.* **552**, 46-54.

Nowak P, G Szczerbak, I Biedka, M Drosik, RM Kostrzewa and R Brus (2006b) Effect of ketanserin and amphetamine on nigrostriatal neurotransmission and reactive oxygen species in Parkinsonian rats. *In vivo* microdialysis study. *J. Physiol. Pharmacol.* **57**, 583-597.

Ossowska K, J Wardas, M Smialowska, K Kuter, T Lenda, JM Wieronska, B Zieba, P Nowak, J Dabrowska, A Bortel, A Kwiecinski and S Wolfarth (2005) A slowly developing dysfunction of dopaminergic nigrostriatal neurons induced by long-term paraquat administration in rats: an animal model of preclinical stages of Parkinson's disease? *Eur. J. Neurosci.* **22**, 1294-1304.

Perez-Garcia C, L Morales, MV Cano, I Sancho and LF Alguacil (1999) Effects of histamine H_3 receptor ligands in experimental models of anxiety and depression. *Psychopharmacology (Berl.)* **142**, 215-220.

Pollard H, J Moreau, JM Arrang and JC Schwartz (1993) A detailed autoradiographic mapping of histamine H_3 receptors in rat brain areas. *Neuroscience* **52**, 169-189.

Ryu JH, K Yanai and T Watanabe (1994) Marked increase in histamine H_3 receptors in the striatum and substantia nigra after 6-hydroxydopamine-induced denervation of dopaminergic neurons: an autoradiographic study. *Neurosci. Lett.* **178**, 19-22.

Ryu JH, K Yanai, XL Zhao and T Watanabe (1996) The effect of dopamine D_1 receptor stimulation on the up-regulation of histamine H_3-receptors following destruction of the ascending dopaminergic neurones. *Br. J. Pharmacol.* **118**(3), 585-592.

Sanchez-Lemus E and JA Arias-Montano (2004) Histamine H_3 receptor activation inhibits dopamine D_1 receptor-induced cAMP accumulation in rat striatal slices. *Neurosci. Lett.* **364**, 179-184.

Schlicker E, K Fink, M Detzner and M Gothert (1993) Histamine inhibits dopamine release in the mouse striatum via presynaptic H_3 receptors. *J. Neural. Transm. Gen. Sect.* **93**, 1-10.

Song YN, HZ Li, JN Zhu, CL Guo and JJ Wang (2006) Histamine improves rat rota-rod and balance beam performances through H_2 receptors in the cerebellar interpositus nucleus. *Neuroscience* **140**, 33-43.

Takeda H, T Matsumiya and T Shibuya (1990) Detection and identification modes for the highly sensitive and simultaneous determination of various biogenic amines by coulometric high-performance liquid chromatography. *J. Chromatogr.* **515**, 265-278.

Tanaka H, K Kannari, T Maeda, M Tomiyama, T Suda and M Matsunaga (1999) Role of serotonergic neurons in L-DOPA-derived extracellular dopamine in the striatum of 6-OHDA-lesioned rats. *Neuroreport* **10**, 631-634.

Threlfell S, SJ Cragg, I Kallo, GF Turi, CW Coen and SA Greenfield (2004) Histamine H_3 receptors inhibit serotonin release in substantia nigra pars reticulata. *J. Neurosci.* **24**, 8704-8710.

Toyota H, C Dugovic, M Koehl, AD Laposky, C Weber, K Ngo, Y Wu, DH Lee, K Yanai, E Sakurai, T Watanabe, C Liu, J Chen, AJ Barbier, FW Turek, WP Fung-Leung and TW Lovenberg (2002) Behavioral characterization of mice lacking histamine H_3 receptors. *Mol. Pharmacol.* **62**, 389-397.

Widnell K (2005) Pathophysiology of motor fluctuations in Parkinson's disease. *Mov. Disord.* **20**, S17-S22.

Wieland K, G Bongers, Y Yamamoto, T Hashimoto, A Yamatodani, WM Menge, H Timmerman, TW Lovenberg and R Leurs (2001) Constitutive activity of histamine H_3 receptors stably expressed in SK-N-MC cells: display of agonism and inverse agonism by H_3 antagonists. *J. Pharmacol. Exp. Ther.* **299**, 908-914.