ELSEVIER

European Journal of Pharmacology 281 (1995) 195–203

ejp

Effects of various dopamine uptake inhibitors on striatal extracellular dopamine levels and behaviours in rats

Noriyuki Nakachi [a], Yuji Kiuchi [a], Masahiro Inagaki [a], Masato Inazu [b], Yukako Yamazaki [a], Katsuji Oguchi [a,*]

[a] *Department of Pharmacology, School of Medicine, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142, Japan*
[b] *POLA R&D Laboratories, POLA Corporation, 560 Kashio, Totsuka-ku, Yokohama 244, Japan*

Received 15 March 1995; revised 20 April 1995; accepted 20 April 1995

Abstract

In vivo central effects of some dopamine uptake inhibitors were evaluated in both brain microdialysis and behavioural studies in rats, and compared with their in vitro affinities to dopamine uptake sites. IC_{50} values of GBR12909 (1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine), diclofensine, mazindol, amfonelic acid and nomifensine for inhibiting 1 nM [^{3}H]GBR12935 (1-[2-(diphenylmethoxy)ethyl]-4-(3-phenylpropyl)piperazine) binding to rat striatal membrane were 7.0, 36, 81, 187 and 290 nM, respectively. In the brain microdialysis study, dopamine levels in the striatal dialysates were increased to 16.3- (GBR12909), 14.1- (nomifensine), 4.8- (diclofensine) and 1.9-fold (amfonelic acid) the respective basal levels 40–60 min after i.p. administration (0.1 mmol/kg) and thereafter decreased slowly but remained at the elevated levels for a further 3 h, while mazindol gradually increased dopamine levels though less pronouncedly than others (1.7-fold 200 min after administration). Remarkable and comparable stereotyped behaviours (licking and forepaw treading) were continuously observed at least for 3 h after administration of GBR12909, nomifensine and amfonelic acid, while stereotypies induced by diclofensine and mazindol were moderate and marginal, respectively. In vivo potencies of dopamine uptake inhibitors to increase the extracellular dopamine levels in the striatum tended to correlate with their in vitro affinities to dopamine uptake sites except in the case of nomifensine, and correlated significantly with their potencies to induce stereotyped behaviours except in the case of amfonelic acid. Based on these findings, pharmacological characteristics of these dopamine uptake inhibitors are discussed.

Keywords: Dopamine uptake inhibitor; [^{3}H]GBR12935 binding; Brain microdialysis; Dopamine; Stereotypy

1. Introduction

Some central stimulants, such as nomifensine, diclofensine and mazindol, have been demonstrated to inhibit dopamine uptake or have relatively high and selective affinities to dopamine uptake sites in striatal preparations, and are classified as 'so-called' dopamine uptake inhibitors (Andersen, 1987; Bonnet et al., 1986; Janowsky et al., 1986). Clinically, they have been expected to have therapeutic effects on depression and Parkinson's disease (Bræstrup and Scheel-Krüger, 1976; Costall et al., 1975; Søgaard et al., 1990).

Dopamine uptake inhibitors are supposed to enhance dopaminergic neurotransmission in the central nervous system through an increase in dopamine concentration in the synaptic cleft as the result of reuptake inhibition at nerve terminals. Indeed, with a brain microdialysis technique recently developed, some dopamine uptake inhibitors have been reported to increase the extracellular concentration of dopamine in some brain regions such as the striatum and nucleus accumbens in rats (Hurd and Ungerstedt, 1989; Westerink et al., 1987). However, in vivo potencies of various dopamine uptake inhibitors to induce an elevation of the extracellular dopamine concentration have not been evaluated under the same experimental conditions, while comparative studies of their in vitro affinities to dopamine uptake sites have been reported in several articles (Andersen, 1987; Berger et al., 1985; Bonnet et al., 1986). It is possible that their in vivo potencies to inhibit dopamine uptake do not correlate with the potencies obtained from in vitro studies, due,

* Corresponding author. Tel. 03-3784-8125, fax 03-3787-4790.

Elsevier Science B.V.
SSDI 0014-2999(95)00246-4

for example, to differences in pharmacokinetic properties such as permeability of blood-brain barrier, metabolism or protein binding. Thus, comparison of in vivo effects on the dopaminergic system would bring more practical information for the assessment of dopamine uptake inhibitors.

These drugs induce behavioural hyperactivities including hyperlocomotion or several stereotypies (sniffing, oral stereotypies such as licking and biting, rearing, forepaw treading and head weaving, etc.) in rats (Bræstrup and Scheel-Krüger, 1976; Costall et al., 1975; Mueller, 1993; Westerink et al., 1987). It is speculated that these behaviours are mediated by stimulation of dopaminergic neurotransmission as the result of inhibited dopamine reuptake. However, some behaviours induced by dopamine uptake inhibitors have also been observed after stimulation and inhibition of other neurotransmitter systems such as 5-hydroxytryptamine (5-HT) (Backus et al., 1990; Pranzatelli, 1990; Pranzatelli et al., 1990) and glutamate (Löscher et al., 1993; Willins et al., 1993), respectively. Thus, it seems to be unclear whether behavioural hyperactivities produced by dopamine uptake inhibitors are really mediated by elevation of the extracellular dopamine concentration in the brain.

In this study, we measured in vitro affinities of several dopamine uptake inhibitors to dopamine uptake sites in the rat striatum, and observed changes in striatal extracellular dopamine levels and behavioural hyperactivities after administration of these drugs. Subsequently, we attempted to elucidate the correlations among these data and characterize pharmacological properties of dopamine uptake inhibitors.

2. Materials and methods

2.1. Animals

Male Wistar rats (6–7 weeks) from Sankyo Labo Service Co. (Tokyo, Japan) were used throughout in vitro and in vivo experiments.

2.2. Chemicals

[^{3}H]GBR12935 (1-[2-(diphenylmethoxy)ethyl]-4-(3-phenylpropyl)piperazine) (53 Ci/mmol) was purchased from Dupont-NEN (Boston, MA, USA). GBR12909 hydrochloride (1-[2-[bis(4-fluorophenyl) methoxy]ethyl]-4-(3-phenylpropyl)piperazine dihydrochloride), amfonelic acid, fluspirilene, flunarizine dihydrochloride and bupropion hydrochloride were purchased from Research Biochemicals (Natick, MA, USA). Pimozide and *d*-methamphetamine were purchased from Wako Pure Chemical Industries (Osaka, Japan) and Dainihon Pharmaceutical (Osaka, Japan), respectively. Cocaine hydrochloride was obtained from Takeda Chemical Industries (Osaka, Japan). Diclofensine hydrochloride, mazindol and nomifensine maleate were kindly donated by Hoffmann-La Roche (Basel, Switzerland), Sandoz Pharmaceuticals (Basel, Switzerland) and Hoechst Japan (Tokyo, Japan), respectively. All other chemicals used were obtained from commercial sources.

2.3. [^{3}H]GBR12935 binding studies

[^{3}H]GBR12935 binding to the rat striatal membrane was evaluated according to the method of Andersen (1987) with minor modifications. Rats were decapitated and the brains were immediately removed. The striatal tissue was dissected on ice and homogenized in 5 ml of ice-cold 50 mM Tris-citrate (pH 7.4 at 4°C) buffer containing 120 mM NaCl and 4 mM $MgCl_2$ using a Physcotron (Nichione-Irika, Tokyo, Japan). The homogenate was centrifuged at $48000 \times g$ for 20 min at 4°C. The pellet was resuspended and recentrifuged. The final pellet was resuspended in 1000 volumes (original tissue wet weight) of the above buffer. The assay sample contained 800 μl of membrane preparation (100–150 μg protein) and 100 μl of the buffer with or without tested agents (final concentration range: 10^{-11}–10^{-3} M), some of which were diluted from dimethyl sulfoxide solution (final dimethyl sulfoxide concentration was less than 0.1%). The binding reaction was started by the addition of 100 μl aliquots of [^{3}H]GBR12935 (1 nM final concentration, diluted from 500 nM stock solution in ethanol) in a total volume of 1 ml. Incubation was for 60 min at 4°C and was terminated by rapid vacuum filtration with a Brandel Cell Harvester (Gaithersberg, MD, USA) over Whatman GF/B glass-fiber filters presoaked with 0.1% bovine serum albumin solution. The filters were washed 3 times with 3 ml of ice-cold 0.9% NaCl and transferred to scintillation vials. The radioactivity remaining in the filters was determined with a Packard Tri-Carb Liquid Scintillation Counter (Meriden, CT, USA) in 4 ml Aquasol-2 with 50–54% counting efficiency. Nonspecific binding was defined as the binding in the presence of 30 μM GBR12909. The IC_{50} value and Hill coefficient for each drug were calculated by nonlinear regression fitting.

2.4. In vivo brain microdialysis

Two or three days before the experiment, the skull of a rat was exposed under anaesthesia with pentobarbital sodium (40 mg/kg i.p.) and a guide cannula (G-5, Eicom, Kyoto, Japan) was implanted above the striatum (A: 0.5 mm, L: 2.5 mm, V: 3.0 mm from bregma) and fixed with dental cement (G-C Dental Industrial Corp., Tokyo, Japan). On the day before the experiment, an I-shaped microdialysis probe (mem-

brane length: 3 mm, diameter: 200 μm, Eicom) filled with Ringer's solution (147 mM NaCl, 4 mM KCl, 2.3 mM $CaCl_2$ and 1 mM sodium phosphate, pH 7.4) was inserted into the guide. On the day of the experiment, each rat was placed in an individual plastic test cage (30 × 30 × 35 cm) with a thin layer of sawdust. The microdialysis probe was perfused at a flow rate of 1 μl/min with Ringer's solution. Following 2-h perfusion for stabilization, dialysates were collected every 20 min, and an aliquot (20 μl) was mixed with 20 μl of 0.02 M acetic acid and directly injected into a high performance liquid chromatography (HPLC) system. After the collection of four basal dialysates, drug solutions (GBR12909, nomifensine, diclofensine, mazindol and amfonelic acid) with the same dose of 0.1 mmol/kg or saline (control) were i.p. administered. Drugs were dissolved or suspended in water immediately before administration. Thereafter, dialysates were collected for a further 4 h. The dopamine level in acidified dialysates was determined by HPLC with electrochemical and coulometric detection as previously described (Matsumoto et al., 1993). The data were expressed as percentages of the basal concentration, which was the mean of dopamine concentrations in four basal dialysates.

2.5. Behavioural observations

Behaviours of rats during microdialysis experiments were monitored with a video camera set up outside the plastic cage for 160 min after drug administration. The videotapes were analyzed by two observers who were blind to the administered drugs. The global stereotyped behaviours were scored every 20 min, based on the first 3-min observation according to the rating scale of Costall et al. (1972) as follows: 0 = the appearance of the animals is the same as that of saline-treated rats, 1 = discontinuous sniffing, constant exploratory activity, 2 = continuous sniffing, periodic exploratory activity, 3 = continuous sniffing, discontinuous biting, gnawing or licking, very brief periods of locomotor activity, 4 = continuous biting, gnawing or licking, no exploratory activity. Scores over the 160-min periods were summed to give the total score. In addition to oral stereotypies included in the above-mentioned rating scale, several behavioural components observed after drug administration were also evaluated. Forepaw treading (piano playing) was rated using a graded scoring method according to Pranzatelli (1990) with a slight modification, where 0 = absent, 1 = mild or infrequent, 2 = moderate or intermediate, 3 = severe or continuous. This behaviour was also assessed every 20 min after a 3-min observation and the total behavioural scores over the 160-min observation periods were calculated. The number of line crossings (lines divided the floor into 9 parts), rearing (forepaw off cage floor), skin jerks (paraspinal muscle contraction) and shaking behaviours (wet-dog shakes and head shakes) were counted for 160 min after drug administration.

2.6. Statistics

The significance of statistical differences of dopamine levels in striatal dialysates was assessed with a two-way analysis of variance followed by Newman-Keuls test. Total scores or counts over 160 min of each behavioural measurement were analyzed with the Kruskal-Wallis test followed by the Tukey-type non-

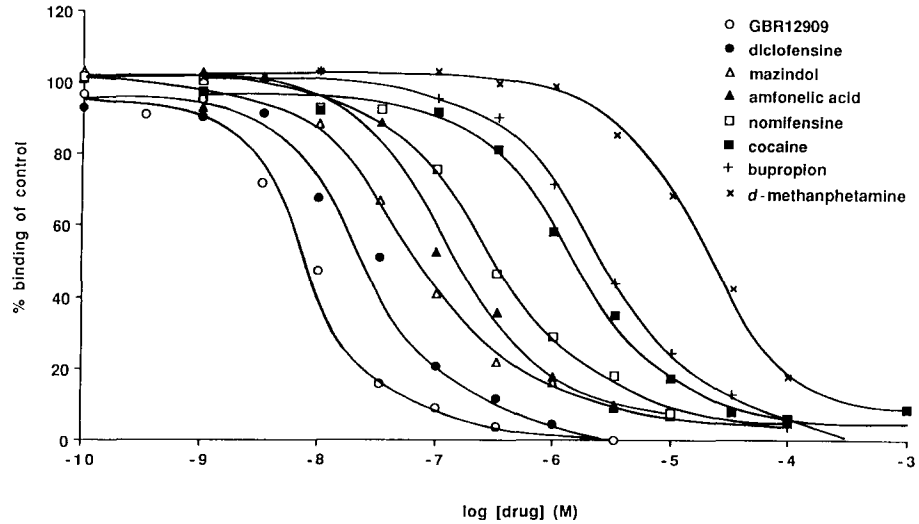

Fig. 1. Displacement curves of dopamine uptake inhibitors and related agents against [^{3}H]GBR12935 binding to the striatal membrane. 1 nM [^{3}H]GBR12935 and the membrane suspension were incubated with increasing concentrations of agents at 4°C for 60 min. Non-specific binding was defined in the presence of 30 μM GBR12909. Incubation was terminated by filtration of samples. Each curve is based on data obtained from a single experiment, which was repeated with similar results.

parametric test. Correlations were analyzed with Spearman's rank correlation test.

3. Results

3.1. [^{3}H]GBR12935 binding studies

Various agents including dopamine uptake inhibitors were tested for their ability to inhibit the binding of 1 nM [^{3}H]GBR12935 to the striatal membrane (Table 1). Representative displacement curves for several agents are shown in Fig. 1. All agents tested displaced [^{3}H]GBR12935 binding and GBR12909 was found to be the most potent with an IC_{50} value of 7.0 nM. The potency order of dopamine uptake inhibitors which were used in in vivo experiments was GBR12909 > diclofensine > mazindol > amfonelic acid > nomifensine. Pimozide, fluspirilene and flunarizine, which have structural similarity with GBR12909, showed lower potencies to inhibit [^{3}H]GBR12935 binding than the above-mentioned dopamine uptake inhibitors, but their IC_{50} values were less then 1 μM. Cocaine, bupropion and *d*-methamphetamine were much less effective (IC_{50} values > 1 μM). Hill coefficients of dopamine uptake inhibitors determined from displacement curves were relatively low (< 0.8) except for GBR12909.

3.2. In vivo brain microdialysis

Changes in dopamine levels in the striatal dialysates after i.p. administration (0.1 mmol/kg) of dopamine uptake inhibitors are shown in Fig. 2. All drugs induced significant and sustained increases in dopamine levels throughout the experimental periods for 4 h. The maximum increase was obtained within 60 min after administration of GBR12909 (60 min), nomifensine, diclofensine and amfonelic acid (40 min). The maximum increases in dopamine levels were greatest for GBR12909 followed by nomifensine, diclofensine and amfonelic acid, which were 16.3-, 14.1-, 4.8- and 1.9-fold the basal dopamine levels, respectively. Thereafter, the dopamine levels gradually (relatively steeper in the case of nomifensine) decreased but still remained at elevated levels of 10.9- (GBR12909), 3.8- (nomifensine), 2.8- (diclofensine) and 1.5-fold (amfonelic acid) the basal levels at 240 min after administration. On the other hand, mazindol increased dopamine levels gradually and significantly from 60 min after administration. However, the elevation of dopamine levels by mazindol was less pronounced than with the other drugs and the maximum increase obtained at 200 min was 1.7-fold the basal level.

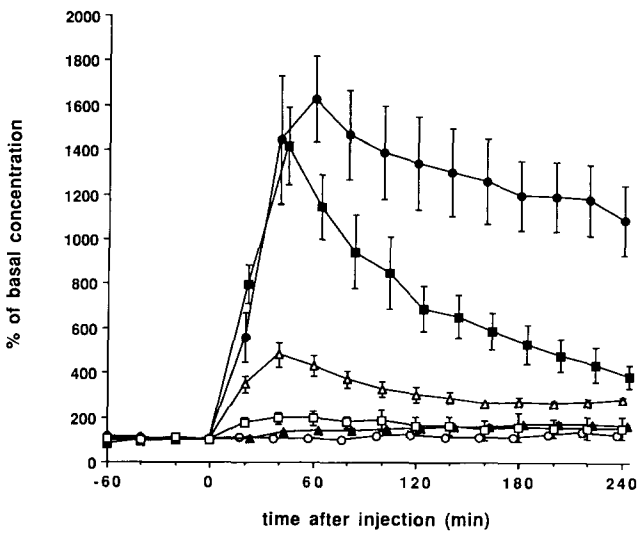

Fig. 2. Effect of dopamine uptake inhibitors on dopamine levels in dialysates collected from the rat striatum. For stabilization, Ringer's solution was perfused (2 μl/min) for 2 h and four consecutive dialysate samples were collected for detection of basal dopamine levels. Thereafter, saline (control, ○), 0.1 mmol/kg of GBR12909 (●), diclofensine (△), mazindol (▲), amfonelic acid (□) or nomifensine (■) was administered i.p. and dialysates were collected for a further 4 h. Changes in the dopamine level were expressed as percentages of basal values. Each point represents the mean ± S.E. for 4–5 rats. Increases in dopamine levels were significant after administration of GBR12909, nomifensine and diclofensine (20–240 min, $P < 0.01$), amfonelic acid (20–80j min, $P < 0.05$) and mazindol (60, 80, 200 and 240 min, $P < 0.05$; 140–180 min, $P < 0.01$) compared with the level of the corresponding control.

Table 1
Inhibitory potencies of various agents on [^{3}H]GBR12935 binding in the striatal membrane

Drug	IC_{50} (nM)	n_H
GBR12909	7.0 ± 0.8	0.9
Diclofensine	36 ± 2	0.7
Mazindol	81 ± 6	0.5
Amfonelic acid	187 ± 13	0.6
Nomifensine	290 ± 33	0.7
Pimozide	366 ± 20	0.9
Fluspirilene	777 ± 36	1.1
Flunarizine	1150 ± 70	1.7
Cocaine	1790 ± 90	0.8
Bupropion	4250 ± 1530	0.8
d-Methamphetamine	19000 ± 1400	0.9

The displacement of [^{3}H]GBR12935 binding by various agents was evaluated as described in the legend of Fig. 1. IC_{50} values represent the concentration inhibiting 50% of specific binding and were calculated by non-linear regression fitting. Values are means ± S.E. of 3 experiments conducted in duplicate. n_H represents a mean value of the Hill coefficient.

3.3. Behavioural observations

Results of behavioural observations after i.p. administration of dopamine uptake inhibitors are shown in

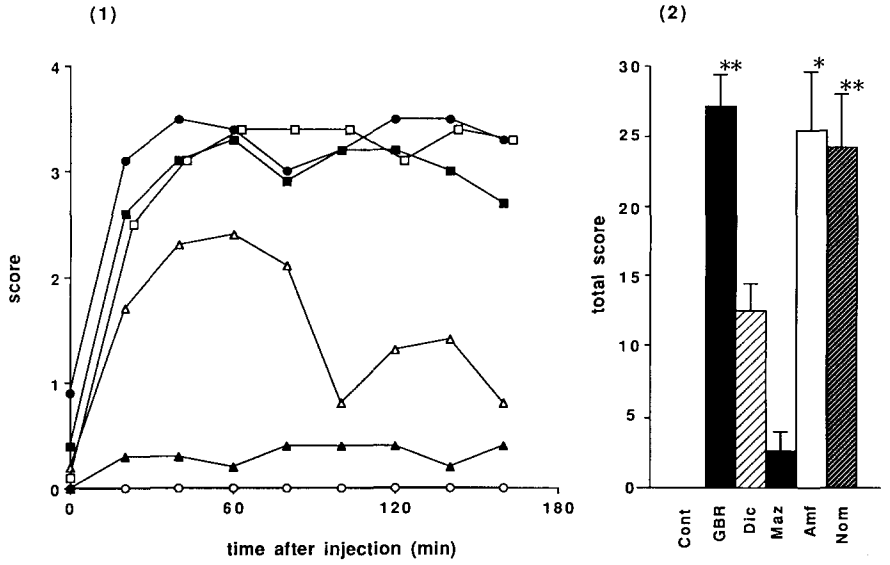

Fig. 3. Global stereotyped behaviours induced by dopamine uptake inhibitors. Saline (control, ○), 0.1 mmol/kg of GBR12909 (●), diclofensine (△), mazindol (▲), amfonelic acid (□) or nomifensine (■) was administered i.p. and behaviours were scored every 20 min based on the first 3-min observation by the rating scale of Costall et al. (1972), which is graded mainly based on the intensity of oral stereotypies as described in the text. (1) Time course of stereotyped behaviours up to 160 min after administration. Each point represents the mean score of 4–5 rats. (2) Total score (mean ± S.E.) over the 9 observation periods (0–160 min). Cont: control, GBR: GBR12909, Dic: diclofensine, Maz: mazindol, Amf: amfonelic acid, Nom: nomifensine. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with control.

Fig. 3 and Fig. 4. GBR12909, amfonelic acid and nomifensine induced marked and continuous oral stereotypies (mainly licking), which was reflected in high behavioural scores evaluated by the rating scale of Costall et al. (1972). They appeared within 20 min after administration and, in most cases, lasted up to the end

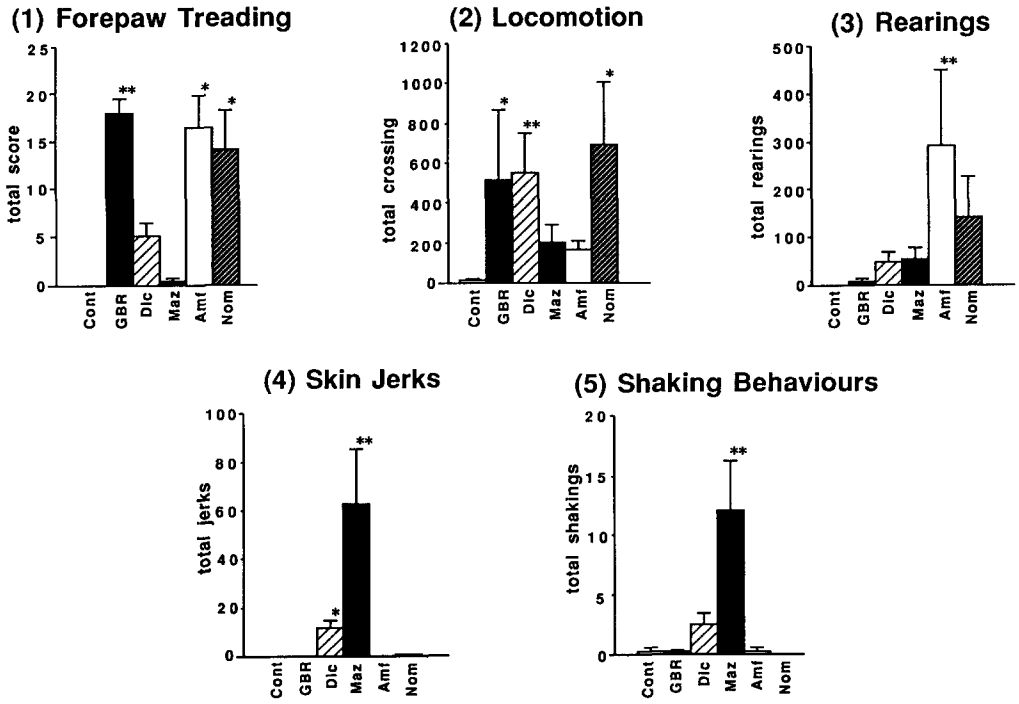

Fig. 4. Behavioural components induced by dopamine uptake inhibitors. The drugs (0.1 mmol/kg) and saline (control) were administered i.p. (1) Forepaw treading: total score over the 9 observation periods (0–160 min), (2) locomotion: number of line crossing for 160 min, (3) rearings, (4) skin jerks and (5) shakings behaviours: the total number of respective behaviours over 160 min. Each point represents the mean ± S.E. for 4–5 rats. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with control. See the legend of Fig. 3 for details.

of the observation period of 160 min (Fig. 3). The oral stereotypies induced by diclofensine were less pronounced (mainly sniffing) than those induced by the above three drugs and disappeared gradually after the peak appearance around 60 min. On the other hand, mazindol rarely induced oral stereotypies throughout the experiment. Accordingly, total behavioural scores (0–160 min) for GBR12909, amfonelic acid and nomifensine were similarly high, followed by those for diclofensine and mazindol. Reciprocal forepaw treading was evoked concomitantly, but not always, with licking. Thus, this stereotypy was predominantly observed after administration of GBR12909, amfonelic acid and nomifensine (Fig. 4). On the other hand, rearings were markedly induced by amfonelic acid and nomifensine, mainly within the first 20 min after administration (Fig. 4). In contrast to the above-mentioned stereotypies, shaking behaviour and skin jerks were specifically induced by mazindol (Fig. 4). These two behaviours were observed throughout the experimental period but the frequency was slightly higher 60–100 min after administration of mazindol. All drugs increased locomotion as assessed by the number of line crossings compared with the control (Fig. 4). However, there was a marked inter-individual difference in locomotion among rats treated with GBR12909 and nomifensine: locomotion in rats which showed continuous licking was strongly suppressed, whereas a few rats in these two groups which showed less pronounced stereotypy, mainly sniffing, had a greatly increased locomotion. In contrast, the other three drugs increased locomotion with less inter-individual differences, with the order diclofensine > mazindol > amfonelic acid.

3.4. Correlations among in vitro and in vivo potencies of dopamine uptake inhibitors

The potencies of the dopamine uptake inhibitors to increase extracellular dopamine levels in the striatum were compared with their IC_{50} values for inhibition of [^{3}H]GBR12935 binding (Fig. 5) or the score of stereotyped behaviours observed simultaneously (Fig. 6). Maximum changes in dopamine levels after administration of drugs tended to correlate, but not significantly, with their IC_{50} values, with the exception of nomifensine. In spite of having the highest IC_{50} value, nomifensine increased the dopamine level to a greater extent than diclofensine, mazindol and amfonelic acid. The average change in dopamine levels after drug administration, defined as the mean value of changes in 60–160 min samples, correlated significantly with the total score of stereotyped behaviours for the corresponding observation periods, with the exception of amfonelic acid. The total score for amfonelic acid was as high as that for GBR12909 and nomifensine in spite of its lower potency to increase dopamine levels.

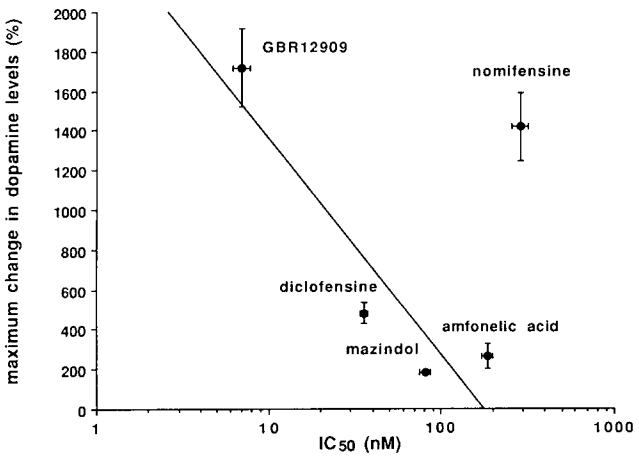

Fig. 5. Correlation between IC_{50} values of dopamine uptake inhibitors against [^{3}H]GBR12935 binding to the striatum and their in vivo potencies to increase dopamine levels in striatal dialysates. Maximum changes in dopamine levels after i.p. administration were expressed as percentages of basal levels. Values were taken from Table 1 and Fig. 2. When nomifensine was excluded, IC_{50} and in vivo potency of each agent tended to show correlation ($r = 0.925$, $P < 0.1$).

4. Discussion

All drugs used in this study (GBR12909, nomifensine, diclofensine, amfonelic acid and mazindol) were

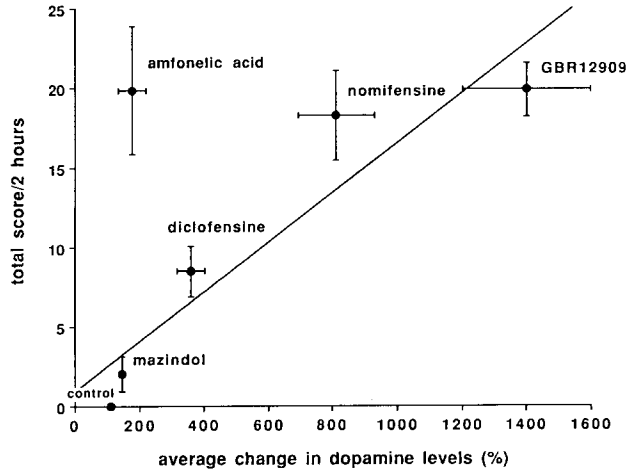

Fig. 6. Correlation between changes in dopamine levels in striatal dialysate and stereotyped behaviours induced by dopamine uptake inhibitors. Dopamine levels in dialysates obtained 60–160 min after i.p. administration were averaged and expressed as percentages of basal levels. Dopamine levels were relatively stable during this period as shown in Fig. 2. Also, the score of global stereotyped behaviours was summed up over the corresponding observation period. When amfonelic acid was excluded, dopamine level changes and stereotyped behaviours induced by each drug showed a significant correlation ($r = 0.933$, $P < 0.05$).

found to increase the in vivo extracellular levels of dopamine. The increase of dopamine is probably a reflection of their potent in vivo inhibitory effects on dopamine reuptake in the striatum since these agents have high in vitro inhibitory potencies against [^{3}H]GBR12935 binding which is specific to dopamine uptake sites (Andersen, 1987). In addition, the increase of extracellular dopamine levels after i.p. administration of these dopamine uptake inhibitors lasted longer (at least 4 h) than that with dopamine releasers such as amphetamine and β-phenylethylamine (Becker and Cha, 1989; Hurd and Ungerstedt, 1989; Kuroki et al., 1990). The long-lasting effect of these drugs on dopamine levels suggests a sustained increase in the drug concentration in the brain. In fact, Menacherry and Justice (1990) observed a sustained maximum concentration of GBR12909 in the dialysates from rat nucleus accumbens between 1 and 3 h after i.p. administration (100 mg/kg).

It was suspected that in vitro potencies of dopamine uptake inhibitors are not always reflected in their in vivo ones due to possible differences in their pharmacokinetics. For example, when administered to rats, cocaine has been reported to show highly enhanced in vivo potency relative to its in vitro potency compared with GBR12909, and this inconsistency between in vivo and in vitro potencies was partially accounted for by a much higher brain concentration of cocaine than of GBR12909 (Menacherry and Justice, 1990). However, in the present study, the IC_{50} value of each drug against [^{3}H]GBR12935 binding tended to correlate with the extent of the maximum increase in extracellular dopamine levels after i.p. administration with the exception of nomifensine. Therefore, maximum drug concentrations in the rat brain after i.p. administration (0.1 mmol/kg) were supposed to be comparable except for that of nomifensine.

Mazindol was found to need a longer time (200 min) to induce the maximum dopamine level after administration than other drugs (40–60 min). Thus, there is a possibility that transport or distribution of mazindol into the brain might be slower than that of other drugs. The reason why nomifensine induced a marked dopamine increase similarly to GBR12909 in spite of its relatively high IC_{50} value is not clear. Possible differences in pharmacokinetics between nomifensine and other drugs, such as a difference in permeability from blood into the brain, may have induced a higher brain concentration of nomifensine than of other drugs. In fact, Kellner et al. (1977) demonstrated rapid and extensive distribution of nomifensine in the rat brain, as early as 5 min after i.v. administration, and Ings (1979) showed that the concentration of nomifensine in the rat brain was approximately 3 times higher than its plasma concentration at 60 min after oral administration (8 mg/kg). On the other hand, the concentration of mazindol in the rat brain was shown to be approximately half of its plasma concentration 2 h after oral administration (1 mg/kg) (personal communication from Sandoz Pharmaceuticals). Alternatively, nomifensine might have a stimulatory effect on dopamine release in addition to the uptake inhibition since the dopamine level after its administration showed a steeper initial increase followed by a steeper decrease compared with the other drugs. This sharp change in dopamine overflow induced by nomifensine seems to be similar to that observed after administration of amphetamine (Hurd and Ungerstedt, 1989). However, there are no data which show that nomifensine induces the release of dopamine directly.

In the present study, a significant positive correlation was also found between the increase in extracellular dopamine levels after dopamine uptake inhibitors and the intensity of concomitantly evoked stereotyped behaviours (mainly oral stereotypies characterized by licking or biting), except in the case of amfonelic acid. Agents which are known in vitro to enhance dopaminergic transmission through stimulation of release (e.g. cocaine and amphetamine) or inhibition of uptake (e.g. nomifensine and GBR12909) have been demonstrated to induce oral stereotypies in rats and it has been speculated that these behaviours are mediated by increased dopamine activity (Bræstrup and Scheel-Krüger, 1976; Costall et al., 1972, 1975; Pani et al., 1990; Westerink et al., 1987). Our study showed that this is likely to be the case, at least with dopamine uptake inhibitors used in this study except for amfonelic acid.

Amfonelic acid has been suggested to induce stereotypies by stimulating dopaminergic transmission through the inhibition of dopamine reuptake or increasing the release of dopamine in rats (McMillen et al., 1991; Rivest and Marsden, 1992). However, in the present study, amfonelic acid was shown to evoke much less increase in extracellular dopamine levels in spite of remarkable stereotypies indistinguishable from those evoked by nomifensine and GBR12909. This finding is consistent with the previous report of Westerink et al. (1987). Therefore, it is possible to suspect that the uptake inhibition or the increase in the release of dopamine might not play a major role in the behavioural stimulatory effect, and that amfonelic acid might enhance dopaminergic transmission through other mechanisms, for example, through direct stimulation of dopamine receptors. However, the precise mechanisms remain to be clarified.

Forepaw treading, which is a component of the 5-HT syndrome observed after administration of 5-HT receptor agonists such as 5-hydroxytryptophan, 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) or 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) (Backus et al., 1990; Pranzatelli et al., 1990; Przegalin-

ski et al., 1990), was also induced by GBR12909, nomifensine and amfonelic acid. These findings suggest that this stereotyped behaviour could be evoked by stimulation not only of 5-HT receptors but also of dopamine receptors or that it might be a non-specific behaviour induced concomitantly with other predominant stereotypies.

On the other hand, mazindol which is known to be a representative dopamine uptake inhibitor induced specifically skin jerks and shaking behaviours, while oral stereotypies were observed only infrequently. It is reported that skin jerks and shaking behaviours are induced by stimulation of 5-HT_2 receptors (Pranzatelli, 1990) and mazindol has been demonstrated to have relatively high potency for inhibition of in vitro 5-HT and norepinephrine uptake (Wong and Bymaster, 1978). Therefore, it seems likely that the extracellular 5-HT level in the brain is increased due to 5-HT uptake inhibition, leading to stimulation of 5-HT_2 receptors. However, the effect of mazindol on the extracellular 5-HT level should be verified using in vivo brain microdialysis.

In summary, these findings indicate that studies with in vivo microdialysis in combination with simultaneous observation of behaviours would be very informative in the evaluation of in vivo potencies of agents which act on dopaminergic nerve terminals and affect dopaminergic neurotransmission.

References

Andersen, P.H., 1987, Biochemical and pharmacological characterization of [^{3}H]GBR12935 binding in vitro to rat striatal membranes: labeling of the dopamine uptake complex, J. Neurochem. 48, 1887.

Backus, L.I., T. Sharp and D.G. Grahame-Smith, 1990, Behavioural evidence for a functional interaction between central 5-HT_2 and 5-HT_{1A} receptors, Br. J. Pharmacol. 100, 793.

Becker, J.B. and J.-H. Cha, 1989, Estrous cycle-dependent variation in amphetamine-induced behaviors and striatal dopamine release assessed with microdialysis, Behav. Brain Res. 35, 117.

Berger, P., A. Janowsky, F. Vocci, P. Skolnick, M.M. Schweri and S.M. Paul, 1985, [^{3}H]GBR-12935: a specific high affinity ligand for labeling the dopamine transport complex, Eur. J. Pharmacol. 107, 289.

Bonnet, J.-J., P. Protais, A. Chagraoui and J. Costentin, 1986, High-affinity [^{3}H]GBR 12783 binding to a specific site associated with the neuronal dopamine uptake complex in the central nervous system, Eur. J. Pharmacol. 126, 211.

Bræstrup, C. and J. Scheel-Krüger, 1976, Methylphenidate-like effects of the new antidepressant drug nomifensine (HOE 984), Eur. J. Pharmacol. 38, 305.

Costall, B., R.J. Naylor and J.E. Olley, 1972, The substantia nigra and stereotyped behaviour, Eur. J. Pharmacol. 18, 95.

Costall, B., D.M. Kelly and R.J. Naylor, 1975, Nomifensine: a potent dopaminergic agonist of antiparkinson potential, Psychopharmacologia 41, 153.

Hurd, Y.L. and U. Ungerstedt, 1989, In vivo neurochemical profile of dopamine uptake inhibitors and releasers in rat caudate-putamen, Eur. J. Pharmacol. 166, 251.

Ings, R.M.J., 1979, The comparative disposition of nomifensine (Merital) in the pregnant and non-pregnant rat, Arch. Int. Pharmacodyn. Ther. 242, 180.

Janowsky, A., P. Berger, F. Vocci, R. Labarca, P. Skolnick and S.M. Paul, 1986, Characterization of sodium-dependent [^{3}H]GBR-12935 binding in brain: a radioligand for selective labelling of the dopamine transport complex, J. Neurochem. 46, 1272.

Kellner, H.-M., C. Baeder, O. Christ, W. Heptner, I. Hornke and R.M.J. Ings, 1977, Kinetics and metabolism of nomifensine in animals, Br. J. Clin. Pharmacol. 4, 109S.

Kuroki, T., T. Tsutsumi, M. Hirano, T. Matsumoto, Y. Tatebayashi, K. Nishiyama, H. Uchimura, A. Shiraishi, T. Nakahara and K. Nakamura, 1990, Behavioral sensitization to beta-phenylethylamine (PEA): enduring modifications of specific dopaminergic neuron systems in rat, Psychopharmacologia 102, 5.

Löscher, W., R. Annies and D. Hönack, 1993, Comparison of competitive and uncompetitive NMDA receptor antagonists with regard to monoaminergic neuronal activity and behavioural effects in rats, Eur. J. Pharmacol. 242, 263.

Matsumoto, M., M. Inagaki, Y. Kiuchi, J. Izumi, Y. Yamazaki and K. Oguchi, 1993, Role of calcium ions in dopamine release induced by sodium cyanide perfusion in rat striatum, Neuropharmacology 32, 681.

McMillen, B.A., S.M. Scott and H.L. Williams, 1991, Effects of subchronic amphetamine or amfonelic acid on rat brain dopaminergic and serotonergic function, J. Neural Transm. 83, 55.

Menacherry, S.D. and J.B. Justice, Jr., 1990, In vivo microdialysis and thermospray tandem mass spectrometry of the dopamine uptake blocker 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)-piperazine (GBR-12909), Anal. Chem. 62, 597.

Mueller, K., 1993, Locomotor stereotypy is produced by methylphenidate and amfonelic acid and reduced by haloperidol but not clozapine or thioridazine, Pharmacol. Biochem. Behav. 45, 71.

Pani, L., A. Kuzmin, M. Diana, G.D. Montis, G.L. Gessa and Z.L. Rossetti, 1990, Calcium receptor antagonists modify cocaine effects in the central nervous system differently, Eur. J. Pharmacol. 190, 217.

Pranzatelli, M.R., 1990, Evidence for involvement of 5-HT_2 and 5-HT_{1C} receptors in the behavioral effects of the 5-HT agonist 1-(2,5-dimethoxy-4-iodophenyl aminopropane)-2 (DOI), Neurosci. Lett. 115, 74.

Pranzatelli, M.R., A.M. Dollison and Y.-Y. Huang, 1990, The functional significance of neonatal 5,7-dihydroxytryptamine lesions in the rat: response to selective 5-HT_{1A} and 5-$HT_{2,1C}$ agonists, Brain Res. Bull. 24, 747.

Przegalinski, E., A.M. Ismaiel, E.Chojnacka-Wójcik, B. Budziszewska, E. Tatarczynska and E. Blaszczynska, 1990, The behavioural, but not the hypothermic or corticosterone, response to 8-hydroxy-2-(di-*n*-propylamino)-tetralin, is antagonized by NAN-190 in the rat, Neuropharmacology 29, 521.

Rivest, R. and C.A. Marsden, 1992, Differential effects of amfonelic acid on the haloperidol-and clozapine-induced increase in extracellular DOPAC in the nucleus accumbens and the striatum, Synapse 10, 71.

Søgaard, U., J. Michalow, B. Butler, A.L. Laursen, S.H. Ingersen, B.K. Skrumsager and O.J. Rafaelsen, 1990, A tolerance study of single and multiple dosing of the selective dopamine uptake inhibitor GBR12909 in healthy subjects, Int. Clin. Psychopharmacol. 5, 237.

Westerink, B.H.C., G. Damsma, J.B. De Vries and H. Koning, 1987, Dopamine re-uptake inhibitors show inconsistent effects on the

in vivo release of dopamine as measured by intracerebral dialysis in the rat, Eur. J. Pharmacol. 135, 123.

Willins, D.L., S. Naraynan, L.J. Wallace and N.J. Uretsky, 1993, The role of dopamine and AMPA/kainate receptors in the nucleus accumbens in the hypermotility response to MK801, Pharmacol. Biochem. Behav. 46, 881.

Wong, D.T. and F.P. Bymaster, 1978, An inhibitor of dopamine uptake, LR5182, *cis*-3-(3,4-dichlorophenyl)-2-*N*,*N*-dimethyl-aminomethyl-bicyclo-[2,2,2]-octane, hydrochloride, Life Sci. 23, 1041.