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BEHAVIORAL PROPERTIES OF GBR 12909, GBR 13069 AND GBR 13098: SPECIFIC INHIBITORS OF DOPAMINE UPTAKE

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Two aryl 1,4-dialkylpiperazines (GBR 12909 and GBR 13098) and one aryl 1,4-dialkenylpiperazine (GBR 13069) were very potent inhibitors of [^{3}H]dopamine uptake in vitro in tissue slices obtained from rat neostriatum (IC_{50} values between 40 and 51 nM). Each compound was considerably weaker as an inhibitor of [^{3}H]norepinephrine uptake in tissue slices obtained from rat occipital cortex (IC_{50} values between 560 and 2600 nM). These compounds thus are relatively specific inhibitors of [^{3}H]dopamine uptake in vitro. The three compounds caused ipsilateral circling in rats with unilateral lesions of the nigrostriatal pathway as well as increased locomotor activity in naive mice, both of which could be greatly attenuated by pretreatment of the rodents with the dopamine receptor antagonist haloperidol. It thus follows that the compounds have dopaminergic activity in vivo. Ex vivo experiments with GBR 13069 (drug administration in vivo, uptake in vitro) suggested that these compounds may have the same relative specificity as dopamine uptake blockers in vivo. These compounds should prove to be useful pharmacological agents.

[^{3}H]Dopamine uptake Locomotor activity Ipsilateral rotation

1. Introduction

The neuropharmacologist has within his/her complement of drugs several that are relatively specific inhibitors of norepinephrine (NE) uptake. Many available drugs, for example, are considerably better inhibitors of NE uptake than they are of either dopamine (DA) or serotonin (5-HT) uptake. A prime example of such a drug is the clinically used antidepressant desmethylimipramine (Koe, 1976). Several drugs are also available that are better inhibitors of 5-HT uptake than of DA or NE uptake (Fuller et al., 1975; Hyttel, 1978; Waldmeier et al., 1979; Wong et al., 1975, 1980a). A considerable effort has gone into the search for and the development of drugs that are specific DA uptake blockers. This effort has for the most part been unsuccessful, but there have been some notable exceptions. Recently, for example, several derivatives have been synthesized that have a relative specificity as inhibitors of DA uptake into synaptosomal preparations (Van der Zee et al., 1980; Wong and Bymaster, 1978; Wong et al., 1980b).

In the present investigation we have studied three of these analogs and confirm that they are better as inhibitors of [^{3}H]DA uptake into tissue slices from the rat neostriatum, a DA-enriched area of the brain, than they are as inhibitors of [^{3}H]NE uptake into tissue slices from the rat occipital cortex, a NE-enriched area of the brain. We will further demonstrate that the compounds have the expected dopaminergic activity in vivo in rats and mice.

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2. Materials and methods

2.1. Accumulation of [^{3}H]biogenic amines

The accumulation of [^{3}H]dopamine into rat neostriatal tissue slices or of [^{3}H]norepinephrine into rat occipital cortex tissue slices was done with previously published standard methods (Heikkila et al., 1977, 1979, 1981). Briefly, the neostriatum and occipital cortex were dissected from male Sprague-Dawley rats (150–200 g) after decapitation. The tissue was sliced with a razor blade into 1 mm sections and cross-chopped at 0.2 mm with a McIlwain-Mickle tissue chopper. The slices (1.0 $\times$ 0.2 $\times$ 0.2 mm) were dispersed into 200 vol of a modified Krebs Ringer phosphate buffer at pH 7.4 containing 5.6 mM glucose, 1.3 mM EDTA, 1.7 mM ascorbic acid and 0.08 mM pargyline hydrochloride. Ten mg of tissue contained in 2 ml of the buffer was added to 8 ml of the buffer, and the samples equilibrated at 37 °C for 5–10 min. The [^{3}H]biogenic amine (approximately 9×10^5 dpm) and the appropriate concentration of the drugs were then added simultaneously. There were 4 concentrations used for each drug; when possible, these concentrations were chosen from preliminary experiments to give a response ranging from 20% to 80% effect. The maximum concentration of drug that was used was 10^{-5} M. The samples (triplicates to quadruplicates) were gently shaken at 37 °C for 15 min, the contents rapidly filtered through 2.1 cm Whatman filter paper discs and rinsed with ice-cold saline. Radioactivity was extracted into 3 ml of absolute ethanol and measured by liquid scintillation spectrometry.

2.2. Release of previously accumulated [^{3}H]dopamine

In release studies, the [^{3}H]DA was added and uptake was done as above. After the filtration and saline rinse, the filter paper discs with the adhering tissue slices were placed in 30 ml beakers containing 10 ml of the buffer. The drugs at appropriate concentrations were then added, the samples gently shaken for 15 min, and the radioactivity remaining in the slices was measured after filtration.

2.3. Locomotor activity in mice

For activity measurements, 4 male Swiss-Webster mice (25–30 g) were put in each cage of a Varimex Activity Chamber (Columbus Instr., Columbus, OH, U.S.A.). The mice were allowed to acclimate to their surroundings for at least 2 h. Some mice then received i.p. injections of the appropriate GBR derivative dissolved in distilled water; others received vehicle (saline) at 10 ml/kg. Activity was measured for successive 15 min periods for 1.5 h post-injection as described previously (Heikkila et al., 1979). Each experiment was repeated several times. In some experiments, the mice were pretreated with haldol dissolved in 0.5% lactic acid, 1 h prior to injection of the GBR derivative. The injection volume was 10 ml/kg.

2.4. Rotational behavior in rats with unilateral 6-hydroxydopamine lesions of the left nigrostriatal pathway

Female, Sprague-Dawley rats weighing 150–175 g were subjected to a unilateral 6-hydroxydopamine lesion of the left nigrostriatal pathway. A solution of 6-hydroxydopamine · HBr (6-OHDA) containing 12 μg in 4 μl was injected into the median forebrain bundle of rats anesthetized with methohexital sodium (Brevital, Eli Lilly, Indianapolis, IN, U.S.A.) and restrained in a David Kopf model 900 stereotaxic apparatus. Lesion coordinates from the atlas of König and Klippel (1963) were A + 4.9, L + 1.6, and DV − 2.6. The animals were subsequently tested at monthly intervals for their rotational response to amphetamine. Each animal was placed in a hemispheric plastic bowl 40 cm in diameter and the number of complete 360 ° turns was counted following the i.p. injection of d,l-amphetamine sulfate, 2.5 mg/kg as free base.

Three months after lesioning, 8 rats were selected which had attained at least 500 ipsilateral turns in 2 h following the amphetamine. These same rats rotated contralaterally following the i.p. administration of l-dopa or dopamine receptor agonists including apomorphine. The rotational response of each rat to 2.5–10 mg/kg (free base) of the GBR derivative dissolved in distilled water

was then determined. Control rats received saline at 1.0 ml/kg. The sequence of drug or saline was randomized. A rest period of 2 to 4 days separated consecutive injections. In some experiments, the rats were pretreated with haldol, dissolved in 5% lactic acid, 1 h prior to administration of the GBR derivative. The injection volume was 1 ml/kg.

2.5. Ex vivo uptake in rats

Male Sprague-Dawley rats (125–150 g) were injected i.p. with GBR 13069 at 10 or 20 mg/kg (free base) or saline vehicle. At 1 h after the injection the rats were decapitated and the brains removed. The neostriatum (left and right were pooled) and the occipital cortex were dissected out. The uptake of [^{3}H]biogenic amines was then done in tissue slices as described above.

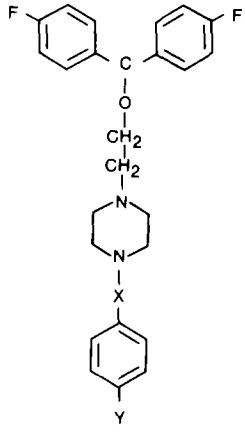
2.6. Sources of drugs

[^{3}H]Dopamine ([^{3}H]DA, 30.4 Ci/mmol) and [^{3}H]norepinephrine ([^{3}H]NE 11.8 Ci/mmol) were obtained from New England Nuclear (Boston, MA, U.S.A.); GBR 12909 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-[3-phenyl-propyl]piperazine dihydrochloride, GBR 13069 [1-[2-[bis(4-fluorophenyl) methoxy]ethyl]-4-(3-phenyl-2-propenyl) piperazine dimethane sulfonate] and GBR 13098 1-[2-[bis(4-fluorophenyl) methoxy]ethyl]-4-[3-(4-fluorophenyl) propyl] piperazine dimethane sulfonate were kindly supplied by Dr. W. Hespe (Gist-Brocades, Haarlem, The Netherlands); amfonelic acid (7-benzyl-1-ethyl-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid), Win 35,065-2 (methyl(-)-3β-phenyl-1 αH, 5 αH-tropane 2β-carboxylate 1,5-naphthalene disulfonate), and Win 35,428 (methyl (−)-3 β-(p-fluorophenyl)-1 αH, 5 αH-tropane-2 β-carboxylate 1,5-naphthalene disulfonate) were kindly supplied by Dr. Robert Clarke (Sterling-Winthrop Research Institute, Renssalaer, NY, U.S.A.); mazindol (5-hydroxy-5-(4′-chlorophenyl)-2,3-dihydro-5H-imidazo (2,1-a) isoindole) was kindly supplied by Dr. W. Houlihan (Sandoz Pharmaceuticals, E. Hanover, NJ, U.S.A.); CDCI (N-cyclopropylmethyl-10,11-dihydro-5H-dibenzo-[a,d] cyclohepten-5-imine) was kindly supplied by Dr. W.F. Herblin (Dupont, Wilmington, DE, U.S.A.) Desipramine hydrochloride (DMI) was obtained from U.S.V. (Scarsdale, NY., U.S.A.). Haldol (haloperidol) was from McNeil Pharmaceuticals (Spring House, PA, U.S.A.). The haldol was dissolved in either 5% lactic acid (rotation) or 0.5% lactic acid (locomotor activity) as described above. The mazindol and CDCI were dissolved in 100 μl of 1 N HCl and 9.9 ml distilled water, the amfonelic acid was dissolved in 100 μl of 1 N NaOH and 9.9 ml distilled water; all other drugs were dissolved in distilled water alone.

3. Results

3.1. Uptake and release experiments

All three GBR derivatives (fig. 1) were very potent inhibitors of [^{3}H]DA accumulation into rat brain neostriatal tissue slices, with IC_{50} values ranging between 40 and 51 nM (table 1). In contrast, all 3 compounds were very weak as releasing agents for [^{3}H]DA. At a concentration of 1000 nM, all 3 GBR derivatives inhibited [^{3}H]DA accu-



		X	Y
GBR	12909	$-CH_2-CH_2-CH_2$	H
GBR	13069	$-CH_2-CH=CH-$	H
GBR	13098	$-CH_2-CH_2-CH_2$	F

Fig. 1. The structures of the three GBR derivatives used in the present study.

TABLE 1

IC_{50} values for GBR 13069, GBR 13098 and GBR 12909 as uptake inhibitors for [^{3}H]DA into rat neostriatal tissue slices and for [^{3}H]NE into rat occipital cortex tissue slices; a comparison with the values for several other uptake inhibitors. Data represent the mean IC_{50} value in nM ± S.D. for at least 3 separate experiments. Potency ratios represent the IC_{50} value for each drug against [^{3}H]NE divided by its IC_{50} value against [^{3}H]DA. A ratio greater than 1 thus indicates the compound is more potent against [^{3}H]DA than against [^{3}H]NE. [a] Heikkila et al. (1977); [b] Heikkila et al. (1979); [c] Heikkila et al. (1976).

Drug	IC_{50} value (nM) vs.		Potency ratio
	[^{3}H]DA	[^{3}H]NE	
GBR 13069	40 ± 14	800 ± 200	20
GBR 13098	43 ± 21	560 ± 180	13
GBR 12909	51 ± 10	2600 ± 1200	51
Amfonelic acid	170 ± 90	280 ± 50	1.6
Mazindol	280 ± 20 [a]	1.5 ± 0.8	0.005
Win 35,428	320 ± 70 [b]	89 ± 21	0.28
Win 35,065-2	740 ± 310 [b]	140 ± 30	0.19
Nomifensine	780 ± 120	12 ± 1	0.015
CDCI	3800 ± 1100	17000 ± 3000	4.5
Desipramine	≫ 10000	18 ± 10 [c]	≪ 0.002

mulation by over 90% but released less than 25% of the previously accumulated [^{3}H]DA from rat neostriatal tissue slices (data not shown). Higher concentrations of the GBR derivatives were not tested for their capacity to induce release. The observed inhibition of [^{3}H]DA accumulation is thus a true inhibition of uptake and not an apparent inhibition of uptake due to the releasing properties of the compounds (for discussion of this concept, see Heikkila et al., 1977). All 3 GBR derivatives were weaker inhibitors of [^{3}H]NE uptake into rat occipital cortex with IC_{50} values between 560 and 2600 nM (table 1). The compounds ranged from being 51 times more potent against [^{3}H]DA than against [^{3}H]NE (GBR 12909) to 13 times more potent (GBR 13098).

The 3 GBR compounds were considerably more potent inhibitors of [^{3}H]DA uptake into neostriatal tissue slices than several other known inhibitors including amfonelic acid, mazindol, Win 35,428 and Win 35,065-2 and nomifensine (table 1). In contrast, these same 5 compounds were all considerably better than the GBR derivatives as inhibitors of [^{3}H]NE uptake into rat occipital cortex tissue slices (table 1). The potency ratios for the 3 GBR compunds and these 5 drugs ranged from 51 to 0.005 (table 1). CDCI with a potency ratio of 4.5 was more potent as an inhibitor of neostriatal [^{3}H]DA uptake than of cortical [^{3}H]NE uptake (table 1). However, CDCI was a weak inhibitor of both [^{3}H]DA and [^{3}H]NE uptake. Desipramine (DMI) differed from the other compounds (table 1), in that it was a potent inhibitor of [^{3}H]NE uptake but a very weak inhibitor of [^{3}H]DA uptake.

3.2. Rotation in 6-OHDA lesioned rats

The 8 rats studied exhibited random turning during a 10 min preliminary period used to acquaint them with the hemisphere and rotational harness. But within several minutes of injection of GBR 13069 or GBR 13098, the rats commenced vigorous ipsilateral (left) turns, especially at the 5.0 and 10.0 mg/kg doses (tables 2, 3, fig. 2). The

TABLE 2

Cumulative 6 h ipsilateral rotation for data shown in fig. 2. Data represent mean 6 h rotations/rat ± S.E.M. for the 8 rats.

Drug	Dose (mg/kg)	Ipsilateral rotations
GBR 13069	2.5	404 ± 123
	5.0	845 ± 151
	10.0	2,229 ± 335
GBR 13098	2.5	211 ± 51
	5.0	459 ± 115
	10.0	1,683 ± 317

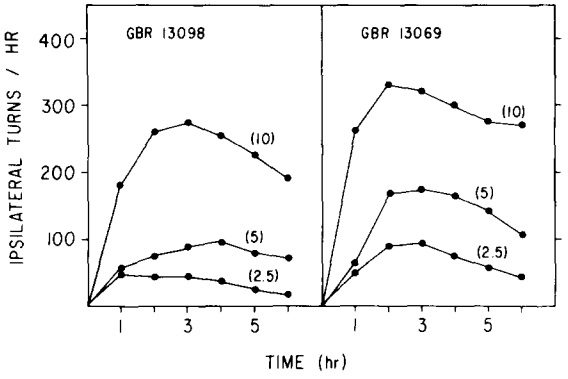


Fig. 2. Ipsilateral rotational behavior after the i.p. injection of GBR 13098 and GBR 13069. Doses of drugs in parentheses are in mg/kg free base (see text for details). Results are the mean value for 8 rats. The 1 h point represents the interval from the point of injection to 1 h, and so on.

compounds had a rather long duration of action; at the two higher doses the rats were still rotating vigorously 6 h after injection (fig. 2). While there was no dramatic difference in potency, at each dose the cumulative 6 h totals were greater for GBR 13069 than for the GBR 13098 (table 2). In contrast to the vigorous rotation seen with the GBR derivatives, saline injected 6-OHDA-lesioned animals rotated only 80-100 total turns in 6 h (data not shown). GBR 12909 also caused an intense rotational behavior (table 3). This ipsilateral rotation to the GBR analogs was greatly attenuated in rats that had been pretreated with the DA receptor antagonist haloperidol at 0.05 or 0.25 mg/kg. For data with GBR 12909 and GBR 13069, see table 3.

TABLE 3

The effects of haldol pretreatment on the ipsilateral rotation induced by GBR 12909 or GBR 13069. Rats were injected with haldol dissolved in 5% lactic acid or the lactic acid alone. One h later they received the GBR derivative at 7.5 mg/kg (free base), and rotation measured. The data represent the mean 6 h ipsilateral rotations/rat ± S.E.M. for 4 rats.

Drug	Haldol (mg/kg)	Ipsilateral rotations	% Inhibition
GBR 12909	–	1400 ± 178	–
	0.05	920 ± 453	34
	0.25	21 ± 5	98
GBR 13069	–	1804 ± 340	–
	0.05	1261 ± 404	30
	0.25	2 ± 1	100

3.3. Locomotor activity

GBR 13069 caused large and dose-dependent increases in motor activity in mice at doses of 5, 10 and 20 mg/kg (fig. 3). Injection of the the drug brought about a quick onset of activity which was still vigorous at 1.5 h when the experiment was ceased. The dose-dependent effect was much clearer with the GBR 13069 than with GBR 13098. The 5 and 10 mg/kg doses of GBR 13098 caused a slight increase while the 20 mg/kg dose caused a large increase in activity (fig. 3). Saline-injected animals showed only an initial increase in activity which quickly decreased to below 50 counts/15 min. At each dose GBR 13069 appeared to be considerably more potent than GBR 13098 in increasing locomotor activity. GBR 12909 also caused large increases in locomotor activity, particularly at 20 mg/kg (table 4). The increase in activity induced by the GBR derivatives was greatly attenuated when mice were pretreated with haloperidol (0.05–1.0 mg/kg). (For data with GBR 12909 and GBR 13069, see table 4.)

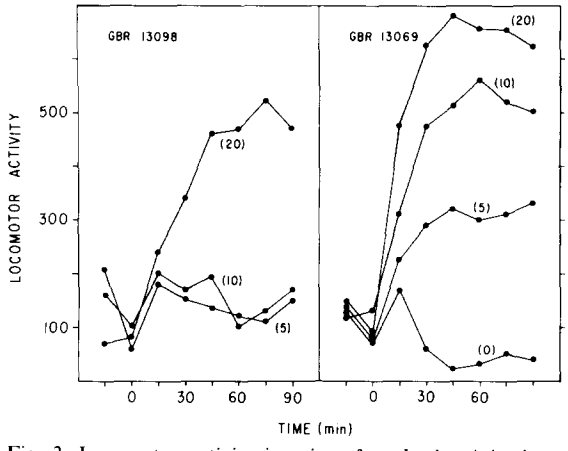


Fig. 3. Locomotor activity in mice after the i.p. injection of GBR 13098 and GBR 13069. Doses of the drugs in parentheses are in mg/kg salt form (see text for details). Each curve represents the mean of from 3–5 separate experiments, no animal was used more than one time. There were 4 mice per chamber in each experiment. Note that the data are for 15 min intervals. Controls (0) were given saline.

TABLE 4

The effect of haldol pretreatment on locomotor activity induced by GBR 12909 (20 mg/kg) or GBR 13069 (10 mg/kg). Mice (4 per chamber) were acclimated to the activity chamber and then injected with haldol dissolved in 0.5% lactic acid or the lactic acid alone. After 1 h they received the GBR derivative and locomotor activity measured for 1.5 h. Data represent the mean locomotor activity ± S.E.M. for 4–6 separate experiments at each dose of haldol.

Drug	Haldol (mg/kg)	Locomotor activity (counts/1.5 h)	% Inhibition
GBR 12909	–	2937 ± 317	–
	0.05	2812 ± 409	4
	0.25	821 ± 334	72
	0.50	625 ± 325	79
	1.0	484 ± 198	84
GBR 13069	–	1747 ± 190	–
	0.05	1525 ± 158	13
	0.25	349 ± 39	80
	0.50	312 ± 153	82
	1.0	150 ± 65	91

3.4. *Ex vivo experiments*

After GBR 13069 was administered to rats at 10 mg/kg free base, [^{3}H]DA uptake into neostriatal tissue slices was diminished by 39% at 1 h (61% of control). In contrast, GBR administration at 10 mg/kg, had no effect on the uptake of [^{3}H]NE into the occipital cortex slices (table 5). Similar effects were obtained with 20 mg/kg GBR 13069 (table 5).

TABLE 5

Ex vivo experiments; the effect of GBR 13069 on [^{3}H]DA and [^{3}H]NE uptake. GBR 13069 (10 mg/kg or 20 mg/kg as free base) or saline were injected into rats. One h later, the rats were sacrificed and neostriatum and occipital cortex removed. Data are expressed as a percent of the mean control ± S.E.M. for 10 control rats and 5 rats at each dose of GBR 13069. The control [^{3}H]DA uptake in the neostriatum was 89721 dpm/10 mg of tissue and the control [^{3}H]NE uptake in the occipital cortex was 8364 dpm/10 mg of tissue. * $P < 0.001$ compared to control. ** Not significant compared to control.

GBR 13069	[^{3}H]DA uptake (neostriatum)	[^{3}H]NE uptake (occipital cortex)
–	100 ± 4%	100 ± 6
10 mg/kg	61 ± 3% *	103 ± 12 **
20 mg/kg	58 ± 9% *	90 ± 11 **

4. Discussion

4.1. *Uptake and release experiments*

As pointed out, GBR 13069, GBR 13098 and GBR 12909 were very potent inhibitors of [^{3}H]DA uptake into rat neostriatal tissue slices (table 1). In fact, these compounds were considerably better as inhibitors of [^{3}H]DA uptake than were amfonelic acid, mazindol, nomifensine and two cocaine derivatives (Win 35,065-2 and Win 35,428), which are widely recognized as being among the most potent inhibitors of DA uptake known (Koe, 1976; Heikkila, 1981; Ross and Kelder, 1979). Thus, in vitro at least, the three GBR compounds can safely be classified as being among the most potent known inhibitors of DA uptake. For this reason alone, these compounds may serve as useful pharmacological tools.

Not only were these three GBR derivatives extremely potent inhibitors of [^{3}H]DA uptake, they were furthermore relatively selective in this regard: the three compounds were considerably better in inhibiting [^{3}H]DA uptake into rat neostriatal tissue slices than they were in inhibiting [^{3}H]NE uptake into rat occipital cortex tissue slices (table 1). The other five compounds in table 1 which were potent inhibitors of [^{3}H]DA uptake (amfonelic acid, mazindol, Win 35,065-2, Win 35,428 and nomifensine) ranged from being virtually equipotent in inhibiting [^{3}H]DA and [^{3}H]NE uptake into the appropriate brain area (amfonelic acid) to being very much better in inhibiting [^{3}H]NE uptake (mazindol). CDCI was more potent as an inhibitor of [^{3}H]DA uptake than of [^{3}H]NE uptake but in contrast to the three GBR derivatives, CDCI was extremely weak as an inhibitor of [^{3}H]DA uptake.

To our knowledge, very few other compounds are known which are better inhibitors of DA uptake into dopaminergic brain areas than of NE uptake into noradrenergic brain areas. One such compound, LR 5182 (cis-3-(3,4-dichlorophenyl)-2-N,N,-dimethylaminomethyl-bicyclo-[2,2,2]-octane), was found (Wong and Bymaster, 1978; Wong et al., 1980b) to be approximately 10–20-fold more potent as an inhibitor of DA uptake into a neostriatal synaptosomal preparation (K_i value of

3-6 nM) than of NE uptake into a hypothalamic synaptosomal preparation (K_i value of 58 nM). Other structural analogs of LR 5182 were also more potent as inhibitors of DA uptake, with potency ratios between 3 and 10 (Wong et al., 1980b). LR 5182, however, while being more potent in vitro as a DA uptake inhibitor than as a NE uptake inhibitor, for some reason did not show the same relative specificity in vivo (Wong and Bymaster, 1978). Many investigators mistakenly believe that benztropine and nomifensine are "specific" inhibitors of DA uptake. However, considerable evidence exists to show that this is not the case (see nomifensine data in table 1, as well as Wong and Bymaster, 1978; Van der Zee et al., 1980; Koe, 1976; Petrali, 1980). In all of these studies, it is clearly shown that nomifensine and/or benztropine are not specific inhibitors of DA uptake. And in fact, some of these studies seem to indicate that if anything, these two "DA uptake inhibitors", are more potent inhibitors of NE uptake than of DA uptake.

The in vitro uptake data from the present study confirm the results of Van der Zee et al. (1980), obtained in synaptosomes, who showed that these GBR derivatives were relatively specific inhibitors of DA uptake. These authors found GBR 12909 to be 49-fold more potent as an inhibitor of DA uptake into a neostriatal synaptosomal preparation than of NE uptake into a hypothalamic synaptosomal preparation. GBR 13069 and GBR 13098 were found to be 47- and 30-fold more potent respectively, as inhibitors of DA uptake. These ratios are close to the values obtained in the present study (table 1). Van der Zee et al. (1980) also found that mazindol and nomifensine, as well as benztropine, while being quite good inhibitors of DA uptake, were all better as inhibitors of NE uptake. The potency ratios obtained by these authors were 0.037 for mazindol, 0.078 for nomifensine and 0.41 for benztropine. One point of difference between the present study and that of Van der Zee et al. (1980) was that IC_{50} values in synaptosomes (Van der Zee et al., 1980) were somewhat lower than IC_{50} values obtained in tissue slices (present study). In separate experiments, we have confirmed this observation.

4.2. In vivo experiments

The three GBR derivatives had the expected dopaminergic activity in vivo. Their administration led to increased locomotor activity in mice (fig. 3, table 4) as well as ipsilateral rotation in rats with unilateral lesions of the nigrostriatal pathway (fig. 2, tables 2, 3). Both the increased locomotor activity as well as the ipsilateral circling induced by the GBR derivatives could be greatly attenuated if the rodents were pretreated with dopamine receptor antagonists such as haldol (tables 3, 4). It thus follows that these compounds, which are extremely active in vitro, can also block the uptake of synaptically released DA in vivo. The data in table 5 indicate that the compounds not only are active in vivo, but that they also have a relative specificity in vivo.

4.3. General considerations

GBR 13069 and its two close structural analogs should serve as useful pharmacological tools in vitro. These compounds clearly are much more potent as inhibitors of [^{3}H]DA uptake than of [^{3}H]NE uptake (table 1, also Van der Zee et al., 1980). These latter authors also pointed out that GBR 13069 and its two analogs were quite weak as inhibitors of 5-HT uptake. These compounds can therefore be used by those interested in a relatively specific inhibitor of DA uptake much as compounds such as DMI or nisoxitine have been used as relatively specific inhibitors of NE uptake, and fluoxitine and several other compounds have been utilized as relatively specific inhibitors of 5-HT uptake.

The data in table 5 seem to indicate that these compounds also may have a relative specificity as DA uptake inhibitors in vivo. One possible use of these compounds will be through the combined treatment of an animal with GBR 13069 and 6-hydroxydopamine, to create an animal which has a severe lesioning of its noradrenergic system with little or no lesioning in its dopaminergic system. This follows in that GBR 13069 should prevent the uptake of 6-OHDA into dopaminergic terminals and thus allow more 6-OHDA to enter into noradrenergic terminals. These experiments

with GBR 13069 and 6-OHDA are currently in progress. Clearly there are many other potential uses, both experimental as well as clinical for compounds that have a relatively pure dopaminergic activity.

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