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Studies of four novel diphenylbutylpiperazinepyridyl derivatives on release and inhibition of reuptake of dopamine, serotonin and noradrenaline by rat brain in vitro

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Abstract

Four novel diphenylbutylpiperazinepyridyl derivatives (FG5865 (2-[4-[4,4-bis(4-fluorophenyl)butyl]-1-piperazinyl]-3-pyridinecarboxamide), FG5891 (2-[4-[4,4-bis(4-fluorophenyl)butyl]-1-piperazinyl]-*N*-methyl-3-pyridinecarboxamide), FG5893 (2-[4-[4,4-bis(4-fluorophenyl)butyl]-1-piperazinyl]-3-pyridinecarboxylic acid methyl ester) and FG5909 (2-[4-[4,4-bis(4-fluorophenyl)butyl]-1-piperazinyl]-3-hydroxypyridine) were tested concerning their effects on in vitro release and reuptake of neurotransmitters. Serotonin, noradrenaline and dopamine were those considered, with rat synaptosomes prepared respectively from frontal cortex, cortex and striatum. FG5865, FG5891, FG5893 and FG5909 were found to potently inhibit the uptake of all three neurotransmitters. In addition, FG5865, FG5891 and FG5893 increased the release of serotonin and dopamine from perfused frontal cortical and striatal tissue; FG5865 was most potent in this regard. The release induced by the FG compounds was, however, much less than that induced by e.g. fenfluramine or amphetamine. All four FG compounds were also found to inhibit glutamate-stimulated release of dopamine from striatal tissue; for FG5893 this inhibition occurred at nanomolar concentrations.

Keywords: FG5865 (2-[4-[4,4-bis(4-fluorophenyl)butyl]-1-piperazinyl]-3-pyridinecarboxamide); FG5891; (2-[4-[4,4-bis(4-fluorophenyl)butyl]-1-piperazinyl]-*N*-methyl-3-pyridinecarboxamide; FG5893 (2-[4-[4,4-bis(4-fluorophenyl)butyl]-1-piperazinyl]-3-pyridinecarboxylic acid methyl ester; FG5909 (2-[4-[4,4-bis(4-fluorophenyl)butyl]-1-piperazinyl]-3-hydroxy-pyridine; Reuptake; Release; Dopamine; 5-HT (5-hydroxytryptamine, serotonin); Noradrenaline

1. Introduction

Four novel diphenylbutylpiperazinepyridyl derivatives (FG5865, FG5891, FG5893 and FG5909) have been developed that have a high affinity for 5-HT_{2A} receptors, thus resembling amperozide, and also a high affinity for 5-HT_{1A} receptors (Pettersson et al., 1992).

Amperozide and other diphenylbutylpiperazine derivatives have previously been shown to inhibit in vitro uptake of serotonin, dopamine and noradrenaline (Eriksson, 1990; Eriksson and Christensson, 1990; Haskins et al., 1987; Hyttel, 1978). Since the four FG compounds mentioned above are diphenylbutyl derivatives, it was of interest to determine their effects on monoamine uptake. This was done in the present study, and the results show that these compounds potently inhibit in vitro uptake of monoamines.

Glutamate-stimulated release of dopamine in vitro has been reported to be inhibited by 10 μM nomifensine, an inhibitor of high-affinity dopamine uptake, and also by the NMDA receptor antagonists, AP5 (2-amino-5-phosphonopentanoic acid) and MK 801 (Lonart and Zigmond, 1990). Like nomifensine, amperozide has been shown to inhibit dopamine uptake in synaptosomes (Eriksson, 1990); in the present study FG5865, FG5891, FG5893 and FG5909 were also shown to possess such inhibitory properties. The question remained whether the FG compounds could also reduce glutamate-stimulated dopamine release, and further experimentation revealed that all four compounds, and amperozide as well, do inhibit glutamate-stimulated dopamine release.

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Several recent reports have described an increased release of striatal dopamine in the presence of glutamate (Shimizu et al., 1990; Lonart and Zigmond, 1990, 1991; Moghaddam et al., 1990). It has also been hypothesized that a deficiency in the corticostriatal glutamatergic pathway is involved in schizophrenia (Carlsson and Carlsson, 1990; Wachtel and Turski, 1990). Amperozide is a putative anti-psychotic drug currently under evaluation concerning possible use in the treatment of schizophrenia.

The results from clinical trials are promising, with effects on both positive and negative symptoms (Axelsson et al., 1991; Björk et al., 1992; Mertens et al., 1989). Amperozide is a 5-HT_{2A} receptor antagonist which preferentially affects emotional behaviour in animal tests (Svartengren and Simonsson, 1990; Gustafsson and Christensson, 1990). Furthermore, the drug has been shown to increase the basal release of dopamine and inhibit amphetamine-stimulated release of dopamine in vitro (Eriksson and Christensson, 1990; Eriksson, 1990) and in vivo (Ichikawa and Meltzer, 1992). In view of the suggested connection between schizophrenia and glutamate-stimulated release of dopamine, the second aim of the present in vitro study was to determine the effect of amperozide and related derivatives on such dopamine release in perfused striatal tissue.

Ethanol self-administration and reinforcing effects thereof have also been suggested to be regulated by dopamine and glutamate neurotransmission (Rassnick et al., 1992). Thus, compounds affecting glutamate-stimulated release of dopamine might be useful in the treatment of drug abuse.

In the absence of Mg^{2+}, low concentrations of glutamate (0.01–1 mM) have been shown to stimulate dopamine release in vitro (Lonart and Zigmond, 1991). However, in the same study, when physiological concentrations of Mg^{2+} were used, higher concentrations of glutamate were required to stimulate the release of dopamine. Based on this knowledge, when a concentration of 1.15 mM Mg^{2+} was used in the present study, a relatively high concentration of glutamate (12 mM) was also chosen.

2. Materials and methods

2.1. Uptake in synaptosomes

Experiments on uptake in synaptosomes were performed according to the method described by Shank et al. (1987). In short, striatal, cortical or frontal cortical tissue was removed from 200–300 g male Sprague-Dawley rats and put in cold (4°C) Tris buffer (composition: 134 mM NaCl, 4.1 mM KCl, 1.1 mM KH_2PO_4, 1.2 mM $MgCl_2$, 5.5 mM glucose, 23.6 mM Tris, 1.3 mM $CaCl_2$, 0.03 mM disodium EDTA (ethylenediaminetetraacetic acid), 0.1 mM ascorbic acid, 0.01 mM pargyline; pH 7.4 adjusted with HCl).

The tissue was homogenized in 1 ml (striatal and frontal cortical tissue) or 2 ml (cortical tissue) of 0.32 M sucrose by applying a motor-driven Teflon pestle 5 times. The homogenate was centrifuged for 10 min at $1000 \times g$ at 4°C and the pellet was discarded. The supernatant was recentrifuged for 20 min at $10\,000 \times g$ at 4°C, and the resulting pellet was suspended in 4 ml (striatal and frontal cortical tissue) or 8 ml (cortical tissue) of Tris buffer at 4°C. For each experiment, a mixture of 0.7 ml of Tris buffer, 0.1 ml of synaptosomal suspension and 0.1 ml of test compound solution was incubated for 10 min at 37°C. Subsequently, 0.1 ml of [7-^{3}H]dopamine (New England Nuclear, specific activity 30 Ci/mmol), L-[7-^{3}H]noradrenaline (New England Nuclear, specific activity 15 Ci/mmol) or 5-[1,2-^{3}H]serotonin creatinine sulphate (New England Nuclear, specific activity 30 Ci/mmol) was added (final concentration 7, 14 and 8 nM; respectively) and the incubation was continued for an additional 5 min (dopamine and serotonin) or 10 min (noradrenaline). The uptake was terminated by adding 4 ml of ice-cold Tris buffer. The sample was then collected on a Millipore filter (0.45 μm) and transferred to a scintillation vial containing 4 ml of Ready Protein scintillation liquid, and the radioactivity was determined.

2.2. Release of [^{3}H]dopamine and [^{3}H]serotonin

Release was studied according to a slightly modified version of the method presented by Niddam et al. (1985). Male Sprague-Dawley rats weighing about 200 g were decapitated, the corpus striata or frontal cortex was dissected free, cut into approximately 1 mm^3 pieces with a scalpel and put into a Krebs-Ringer buffer (KRB) solution containing 118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.15 mM $MgSO_4$, 10 mM glucose, 1.15 mM KH_2PO_4, 25 mM $NaHCO_3$, 1 mM ascorbic acid and 0.01 mM pargyline; the buffer solution was equilibrated with 95% O_2/5% CO_2 and had a pH = 7.4. After preincubation of the tissue samples for 15 min in KRB at 37°C, 5 μCi [7-^{3}H]dopamine (New England Nuclear, specific activity 30 Ci/mmol) or 20 μCi 5-[1,2-^{3}H]serotonin creatinine sulphate (New England Nuclear, specific activity 30 Ci/mmol) was added (final concentration 0.13 μM), and the incubation was continued for 30 min. The pieces of tissue were then washed 3 times with KRB, placed in columns and continuously perfused at 37°C with KRB at a flow of 1 ml/min. The time when the tissue samples were placed in the columns was considered zero; perfusion was performed without disturbance during the first 60 min of an experiment, and 4-min fractions were collected during the remainder of the procedure (i.e. a period of

100 min). Release of ^3H was determined according to the following schedule: basal release was measured during the 60–80 min interval; during the 80–100 min interval, a drug was continually added with the perfusion buffer and ^3H was measured; for the remainder of the experiment, normal perfusion (i.e. without the addition of a drug) was performed and ^3H was monitored. In other experiments, stimulation with L-glutamic acid for a total of 8 min was performed starting at 76 min (S_1) and repeated at 136 min (S_2). At 104 min, drugs were added to the column and allowed to remain until the end of the experiment. Excess of radioactivity released during the stimulations was determined by subtracting the basal release of ^3H from the stimulated release and then calculating the ratio S_2/S_1.

2.3. Drugs

Amperozide hydrochloride (synthesized by KABI Pharmacia, Sweden), nomifensine-hydrogen-maleate (Hoechst), (+) MK801 (RBI), citalopram (Lundbeck), *p*-chloro-amphetamine, *d*-amphetamine, amitriptyline (Lundbeck), fluoxetine (Lilly), 8-OH-DPAT 8-hydroxy-2-(di-*n*-propylamino)tetralin (RBI), fenfluramine (A. Benzon), zimelidine (Astra), FG5865, FG5891 and FG5909 were dissolved in KRB or distilled water. Clozapine (Sandoz), GBR 12909 (RBI) and FG5893 were first dissolved in a few drops of HCl or acetic acid and then diluted with KRB.

2.4. Statistics

Statistical analysis was performed using the analysis of variance followed by Student's *t*-test.

3. Results

3.1. Uptake inhibition

Table 1 illustrates the effect of compounds on the in vitro uptake of serotonin, dopamine and noradrenaline by synaptosomes from frontal cortex, striatum and cortex. As indicated, FG5865, FG5893 and FG5909 inhibited the uptake of serotonin, dopamine and noradrenaline. These three compounds were more potent than zimelidine and slightly less potent than fluoxetine to inhibit the uptake of serotonin. FG5891 was a less potent serotonin uptake inhibitor than the other three FG compounds.

All four FG compounds were more potent than amperozide and less potent than nomifensine to inhibit the uptake of dopamine.

3.2. Basal release of [^3H]serotonin and [^3H]dopamine

The effect of the four FG compounds (concentration 5 μM) on the in vitro release of serotonin and dopamine is shown in Tables 2 and 3. As seen in Table 2, FG5865, FG5891 and FG5893 stimulated the release of serotonin from perfused frontal cortical tissue, and FG5865 was the most potent in this regard; FG5909 had no significant effect on this release. The release induced by the four FG compounds was small compared to that induced by the more effective serotonin-releasers, *p*-chloro-amphetamine and fenfluramine (Table 2). FG5865 induced a serotonin release which was comparable to that induced by *d*-amphetamine and 8-OH-DPAT (Table 2). The four FG compounds were relatively more potent inhibitors of serotonin reuptake than inducers of serotonin release, thus re-

Table 1
Uptake of [^3H]serotonin, [^3H]dopamine and [^3H]noradrenaline by synaptosomes from rat frontal cortex, striatum and cortex

Compounds	IC_{50} (μM)		
	Serotonin	Dopamine	Noradrenaline
FG5865	0.043 ± 0.002	0.25 ± 0.01	0.22 ± 0.015
FG5891	1.35 ± 0.1	0.41 ± 0.01	0.72 ± 0.02
FG5893	0.08 ± 0.003	0.35 ± 0.01	0.48 ± 0.02
FG5909	0.072 ± 0.008	0.31 ± 0.02	0.63 ± 0.05
Amperozide	0.32 ± 0.02	1.05 ± 0.05	0.78 ± 0.02
Amitriptyline	0.11 ± 0.01	9.0 ± 1.0	0.05 ± 0.002
d-Amphetamine	4.9 ± 0.1	0.18 ± 0.01	0.076 ± 0.004
Citalopram	< 0.01	> 10	7.6
Fenfluramine	0.22 ± 0.01	10.2 ± 0.5	1.2 ± 0.05
Fluoxetine	0.016 ± 0.001	3.1 ± 0.1	0.48 ± 0.03
GBR 12909	0.060 ± 0.001	0.002 ± 0.0001	0.021 ± 0.0005
Nomifensine	2.6 ± 0.2	0.19 ± 0.05	0.043 ± 0.007
8-OH-DPAT	0.37 ± 0.05	34 ± 1	22 ± 3
Zimelidine	0.12 ± 0.05	10.65 ± 1.5	3.4 ± 0.2

IC_{50} is the drug concentration which inhibited uptake by 50%.

Table 2
Release of [^{3}H]serotonin from in vitro perfused frontal cortical tissue after addition of 5 μM test compound

Compound	Peak effect for the %5-HT release	Duration of the increase (min)
FG5865	273 ± 45 [b]	80
FG5891	148 ± 5 [b]	80
FG5893	159 ± 1 [b]	60
FG5909	119 ± 16 [ns]	–
p-Chloro-amphetamine	647 ± 19 [b]	60
Fenfluramine	529 ± 38 [b]	40
Citalopram	129 ± 6 [a]	20
Amperozide	176 ± 24 [b]	40
d-Amphetamine	241 ± 10 [b]	20
8-OH-DPAT	269 ± 16 [b]	40

Levels of significance as compared to controls (Student's *t*-test) are denoted [a] $P < 0.05$ and [b] $P < 0.01$.

semble citalopram more closely than they do fenfluramine.

Considering dopamine, FG5865, FG5891 and FG5893 were also found to induce release from perfused striatal tissue, but the release was slight compared to that induced by a potent dopamine releaser such as *d*-amphetamine; FG5909 had no significant release-inducing effect (Table 3). Also concerning dopamine, the four FG compounds were more potent to inhibit reuptake than induce release.

3.3. Glutamate-stimulated release of [^{3}H]dopamine

Basal release of [^{3}H]dopamine from striatal tissue could be stimulated an average of 6-fold by the addition of 12 mM glutamate. Using this concentration, a stable ratio between two consecutive stimulations, S_1 and S_2, was obtained ($S_2/S_1 = 1.27 \pm 0.14$, $n = 15$).

Drugs were introduced into the in vitro system at a time point between the two glutamate stimulations and were subsequently kept present during the rest of the experiment. FG5893 and amperozide attenuated the glutamate-stimulated release of [^{3}H]dopamine in a concentration-dependent way (Table 4); significant inhibition of release was seen at an amperozide concentration as low as 10 nM. The dopamine-uptake inhibitor nomifensine caused similar attenuation (Table 4), whereas the NMDA receptor antagonist, MK801, was a comparatively less potent inhibitor (Table 4). When FG5865, FG5891 and FG5909 were tested at a concentration of 1 μM, they were all found to inhibit glutamate-stimulated release of dopamine (Table 4).

Table 3
Release of [^{3}H]dopamine from in vitro perfused striatal tissue after addition of 5 μM test compound

Compound	Peak effect for the % dopamine release	Duration of the increase (min)
FG5865	166 ± 3 [a]	40
FG5891	178 ± 6 [b]	40
FG5893	146 ± 3 [b]	40
FG5909	111 ± 3 [ns]	–
d-Amphetamine	1324 ± 27 [b]	60
Amperozide	159 ± 6 [a]	20

Levels of significance as compared to controls (Student's *t*-test) are denoted [a] $P < 0.05$ and [b] $P < 0.01$.

Table 4
Glutamate-stimulated release of [^{3}H]dopamine from in vitro perfused striatal tissue in the presence of different concentrations of compounds

Compound	Concentration of compound		
	10 nM	100 nM	1 μM
FG5893	53 ± 9 [a]	24 ± 6 [c]	12 ± 1 [b]
Amperozide	64 ± 3 [b]	47 ± 8 [b]	9 ± 2 [c]
Nomifensine	48 ± 5 [b]	29 ± 5 [c]	14 ± 3 [c]
MK 801		71 ± 10	57 ± 2 [a]
FG5865			5 [a]
FG5891			5 [b]
FG5909			7 [b]

Glutamate was used at a concentration of 12 mM. For controls the ratio, S_2/S_1, between two stimulations with glutamate, was 1.27 ± 0.14 ($n = 15$). The effect of drugs on S_2/S_1 is expressed as percentage of control S_2/S_1 values. Levels of significance as compared to controls (Student's *t*-test) are denoted [a] $P < 0.05$, [b] $P < 0.01$ and [c] $P < 0.001$.

4. Discussion

In the present study all four diphenylbutylpiperazinepyridyl derivatives were found to potently inhibit the reuptake of serotonin, dopamine and noradrenaline. Considering serotonin reuptake, the IC_{50} values of FG5865, FG5893 and FG5909 were intermediate to those of fluoxetine and zimelidine; FG5891 was less potent.

Perhaps future work on the FG compounds could also include the nucleus of raphe, since this brain region is known to have a high density of serotonin uptake sites (Plenge et al., 1990). Other inhibitors of serotonin uptake, e.g. fluvoxamine and clomipramine, have been shown to preferentially increase extracellular serotonin in the raphe nuclei (Bel and Artigas, 1992).

In analogy with amperozide (a diphenylbutylpiperazine carboxamide) and diphenybutylpiperidines, all four of the diphenylbutylpiperazinepyridyl derivatives tested in the present study inhibited the reuptake of dopamine and noradrenaline (Eriksson, 1990; Hyttel, 1978). However, FG5865, FG5893 and FG5909 were 4–9 times more potent to inhibit the uptake of serotonin than that of dopamine and noradrenaline. In contrast, FG5891 inhibited dopamine and noradrenaline uptake more potently than serotonin uptake.

FG5865, FG5891 and FG5893 all increased the release of serotonin and dopamine, but the level of release was small compared to the release induced by

e.g. fenfluramine (serotonin release) or *d*-amphetamine (dopamine release). FG5909 had no effect on serotonin or dopamine release. The four FG compounds were relatively more potent to inhibit the reuptake of serotonin, as compared to inducing serotonin release, and in this regard they resemble citalopram more closely than they do fenfluramine. The FG compounds were also relatively more potent inhibitors of dopamine reuptake than inducers of dopamine release.

All four FG compounds potently inhibited the glutamate-stimulated release of dopamine, and FG5893 and amperozide did so at concentrations as low as 10 nM. Since amperozide has no affinity for NMDA receptors (J. Svartengren, personal communication), the observed inhibition of glutamate-stimulated release of dopamine is supposed to be due to the previously demonstrated dopamine uptake-inhibiting properties of amperozide (Eriksson and Christensson, 1990; Eriksson, 1990). However, in these studies, when amperozide and nomifensine were used to inhibit dopamine uptake in chopped tissue, the IC_{50} values obtained were in μM rather than nM concentrations, although these levels could probably be lowered by optimizing the experimental conditions. The connection between inhibition of dopamine uptake and inhibition of glutamate-stimulated dopamine release suggested by Lonart and Zigmond (1990) does seem to exist.

It has been proposed that neuroleptics exert their antipsychotic action by strengthening glutamatergic activity (Wachtel and Turski, 1990). If this is true, amperozide should not inhibit glutamate-stimulated release of dopamine. However, high concentrations of amperozide (10 μM) have been found to slightly increase dopamine release (Eriksson, 1990) and to inhibit both glutamate- (present results) and amphetamine-stimulated dopamine release (Eriksson, 1990). These findings suggest a modulatory role of amperozide in dopamine release. Not only does amperozide reduce the excessive dopamine release induced by glutamate or amphetamine treatment, but it also increases release when levels are low (basal).

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