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NEUROLEPTIC-LIKE EFFECTS OF THE *l*-ISOMER OF FENFLURAMINE ON STRIATAL DOPAMINE RELEASE IN FREELY MOVING RATS

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Abstract—*l*-Fenfluramine (*l*-F) was studied for its ability to release dopamine (DA) and its metabolites in freely moving rats through the trans-striatal dialysis technique. *l*-F's effect on striatal DA release was also studied in animals made tolerant to the effect of haloperidol by chronic treatment (1 mg/kg i.p. twice daily for 11 days and 48 hr wash-out) with the neuroleptic or pretreated with 300 mg/kg i.p. gamma-butyrolactone (GBL). Five and 10 mg/kg *l*-F dose-dependently increased the release of DA and its metabolites with a pattern of effects similar to that observed with neuroleptic drugs. The dose of 20 mg/kg *l*-F had the same effect as 10 mg/kg.

Repeated haloperidol treatment reduced the basal release of DA and its metabolites and a much smaller amount of DA and metabolites was released by *l*-F (10 mg/kg i.p.) and haloperidol (0.1 mg/kg i.p.) in animals treated with haloperidol than in controls.

GBL 300 mg/kg i.p. reduced basal DA release by about 50%. When 10 mg/kg *l*-F, 0.1 mg/kg haloperidol and 0.25 mg/kg *d*-amphetamine were injected i.p. 40 min after GBL, *l*-F and haloperidol did not significantly raise DA release in GBL-treated rats whereas a significant effect was observed at various times after *d*-amphetamine.

The data show that *l*-F resembles haloperidol in its ability to release DA and its metabolites from the corpus striatum of freely moving rats. The cross-tolerance between haloperidol and *l*-F for their effect on DA release suggests that a common site is involved in the mechanism of these drugs.

Fenfluramine is an anorectic agent which, unlike other phenylethylamines such as amphetamine and diethylpropion that act on brain catecholamines, reduces food intake by increasing serotonin transmission [1].

The *l*-isomer (*l*-F) of fenfluramine was recently reported to increase the metabolism of striatal dopamine (DA) in the rat brain at doses causing no changes in serotonin levels or metabolism, whereas the reverse was true for *d*-fenfluramine (*d*-F) [2]. Previous studies had shown that *l*-F inhibits amphetamine and apomorphine-induced stereotypies [3], an effect commonly attributed to these drugs' ability to activate dopamine transmission in the striatum [4, 5]. This suggests that *l*-F may have effects on central dopaminergic mechanisms similar to those of neuroleptics.

The present study was aimed at exploring further the neuroleptic-like effects of *l*-F by studying, through the trans-striatal dialysis technique, its ability to cause release of dopamine (DA) and its metabolites in naive rats and in animals tolerant to the effects of haloperidol. In one experiment the ability of gamma butyrolactone (GBL), a compound known to inhibit the impulse flow of the dopaminergic nigrostriatal system [6], to modify the effects on DA release of *l*-F, haloperidol and *d*-amphetamine was studied to gain more information on the similarities between *l*-F and neuroleptic drugs.

MATERIALS AND METHODS

Dialysis. Male CD rats (Charles River, Italy), weighing 225–250 g, were anaesthetized using 400 mg/kg chloral hydrate (Merck, Darmstadt, F.R.G.) and mounted on a Stoelting (Chicago, U.S.A.) stereotaxic apparatus for positioning the dialysis tube in the striatum. A polyacrylonitrile and sodium methallyl sulfonate copolymer dialysis tube (AN^{TM}, Hospal SpA; 0.25 mm outer diameter, with more than 15,000 molecular weight cutoff) was used. A short piece (about 5 cm) of the dialysis tube was covered with epoxy glue except for a middle zone 1 cm wide. The rat's skull was exposed and two holes were made on its lateral surface, at the level of the head of the caudate nucleus. The dialysis tube, held straight by a thin tungsten wire inside, was transversally inserted into the brain (coordinates A 1.5, V 5.1 from bregma) so that the middle glue-free zone was exactly positioned in the two caudate nuclei. The tungsten wire was removed and two steel cannulae (22 gauge inner diameter, about 1.5 mm length) were glued to the ends of the tube. These ends were bent up and fixed vertically using dental cement and modified Eppendorf (Hamburg, West Germany) tips. Finally, the skin was sutured and the rat was allowed to recover from anaesthesia and given free access to water and food for 24 hr before the experiment on output of dopamine and its metabolites.

For the experiment the rat was put in a cage and one of the two 22 gauge steel cannulae was connected, by polythene tubing, to a 2.5 cm^3 syringe containing Kreb's solution (147.2 mM NaCl, 4.0 mM KCl, 3.4 mM $CaCl_2$ in bidistilled water, pH 6.1). The flow of Kreb's solution was maintained constant at 2 μl/min using a Model 355 Syringe Pump (Sage Instruments, Cambridge, MA). Samples of 40 μl of

the perfusate, enriched with brain dopamine and its metabolites, were collected every 20 min in polypropylene mini-vials containing 10 μl of 1 N $HClO_4$, and directly assayed by high performance liquid chromatography using a reverse-phase column and coulochemical detector. Before any drug injection at least four to five samples of constant basal dopamine release were collected.

Analytical procedure. Dopamine (DA), dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were separated through a reverse-phase column (Bondapak C^{18}, Waters, Mildford, MA) using a mobile phase of 0.1 M sodium acetate, 14% CH_3OH, 0.8 mM 1-heptanesulfonic acid, 0.1 mM Na_2 EDTA, pH 4.1. A flow rate of 0.85 ml/min was maintained by a dual piston Constametric III pump (LCD/Milton Roy, Riviera Beach, FL).

DA, DOPAC and HVA were measured using a coulochemical detector (Model 5100 A, Coulochem detector, ESA, Bedford, MA) coupled with a Model 5011 ESA analytical cell with two in-series electrodes. The first electrode was used to oxidize DA, DOPAC, HVA at +0.35 V, the second to reduce the substances at −0.15 V. DOPAC and HVA were read as first electrode output signal, DA as second output signal. The sensitivity of the method was sufficient to detect about 0.025 pM of DA.

Histological examinations. After completion of the experiments, animals were killed by decapitation, brains were immediately frozen in minced dry ice and 40 μm sections cut in a cryostat. Only data from animals in which the dialysis tube was exactly located in the head of caudate nuclei were considered in the results.

Drugs. Drugs used were: *l*-fenfluramine (Servier, Neuilly-sur-Seine, France), haloperidol (Lusofarmaco, Milan, Italy), *d*-amphetamine sulphate (Recordati, Milan, Italy), gamma butyrolactone (Janssen, Beerse, Belgium). Haloperidol was dissolved in a small volume of HCl which was diluted with water and the pH brought to about 5.0 with NaOH. All other drugs were dissolved in saline. All drugs were injected intraperitoneally.

Drug treatment. All the drugs were injected during the phase of stable output of DA and its metabolites. In one experiment, the animals received various doses of *l*-F (5, 10 and 20 mg/kg i.p.) and the release of DA, HVA and DOPAC was measured for 180 min after injection. In another experiment, the effects of 10 mg/kg *l*-F or 0.1 mg/kg i.p. haloperidol were studied in animals which had received 1 mg/kg i.p. haloperidol or vehicle twice daily for 11 days followed by a 48 hr wash-out period. Finally, the effect of intraperitoneal injection of 10 mg/kg *l*-F, 0.1 mg/kg haloperidol and 0.25 mg/kg *d*-amphetamine on the release of DA was studied in animals which had received 300 mg/kg i.p. GBL 40 min before.

Measurement of l-*F and* l-*norfenfluramine (*l-*NF) levels in the brain of rats chronically treated with haloperidol.* Animals which had received 1 mg/kg i.p. haloperidol or vehicle twice daily for 11 days were treated intraperitoneally with 10 mg/kg *l*-F 48 hr after the last injection of haloperidol or vehicle. One hour after *l*-F treatment the animals were killed by decapitation and the brains rapidly removed for the assay of *l*-F and *l*-NF.

Brain concentrations of *l*-F and *l*-NF were determined using a modification of the electron capture gas–liquid chromatographic method described by Belvedere *et al.* [7]. Briefly, brains were homogenized (10 ml/g) in 0.1 N HCl and centrifuged. Aliquots of 1–2 ml were made alkaline with sodium hydroxide and extracted with benzene after the addition of amphetamine as internal standard. After centrifugation, the benzene extract was concentrated to 0.5 ml and derivativized with trichloroacetyl chloride solution. One to two microlitres of the benzine phase were injected into the chromatographic column, which was a glass tube (2 m × 3 mm i.d.) packed with 80–100 mesh Supelcoport with 3% 0V-17 (Supelchem, Milan, Italy) as the stationary phase. All calibration curves were run every day during the experiments in drug-free rat brain homogenate. The method had a sensitivity limit of 0.5 nmol/g or better for *l*-F and its metabolite. The coefficient of variation for identical samples containing 0.5–5 nmol/g was 5–10%.

Statistics. Data were expressed as percentage of basal release (mean of the last 4–5 samples of stabilized pretreatment release) for each 20 min sample, except for the experiment with chronic haloperidol in which data were expressed as the difference between accumulated picomoles of monoamines over the 120 min post-drug period and those accumulated in the 120 min before drug injection (this value was extrapolated if the pre-drug collection period was shorter than 120 min). When data were expressed as a percentage of basal release, statistical analysis was done by the non-parametric Kruskall–Wallis test; actual data were analyzed by Student's *t*-test.

RESULTS

In agreement with recent findings [8], after an initial tendency for DA output to decrease and HVA and DOPAC outputs to increase, about 2 hr after the start of the experiment the output of DA, HVA and DOPAC reached a plateau which was maintained for at least 8 hr. Values for controls and the various treatments were obtained only during this phase of stable output of DA and its metabolites.

As shown in Fig. 1(a), 5 and 10 mg/kg *l*-F dose-dependently increased the release of striatal DA but no further increase was observed with 20 mg/kg. As recently shown for various neuroleptics [9], the pattern of effects was similar on the release of HVA and DOPAC, although with 5 mg/kg *l*-F the increases never reached statistical significance (Figs 1b and c).

The temporal pattern of outputs for DA, HVA and DOPAC was similar to that previously described for neuroleptic drugs [7]. The increase of DA output was significant 20 min after injection and reached its maximum at 40 min, then tended to drop though remaining significantly higher than controls at 180 min after injection of 10 and 20 mg/kg *l*-F. A similar pattern was observed for DOPAC, but the peak time was 80 min after injection. HVA output rose linearly until 100 min after injection and was

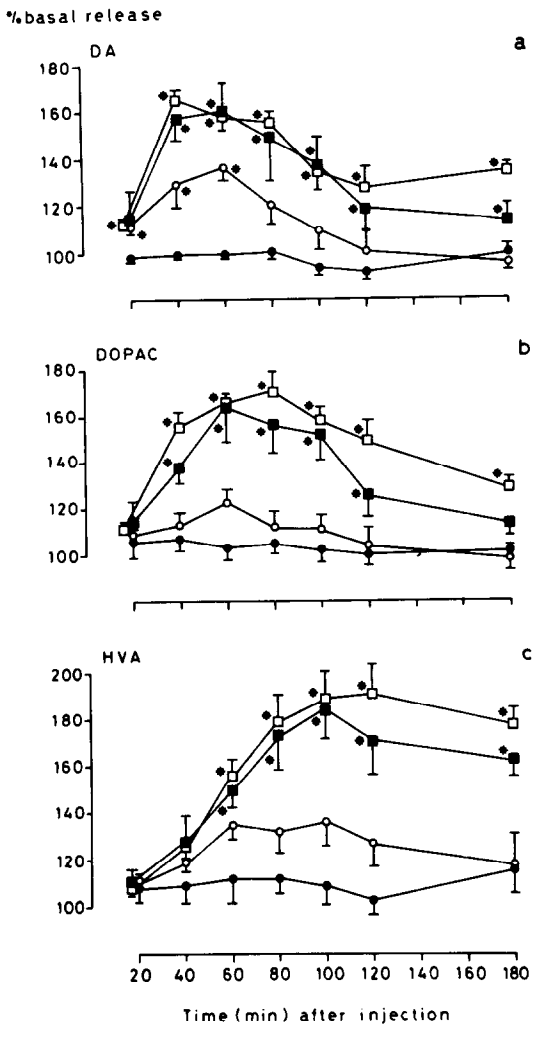

Fig. 1. Time course of the effects of *l*-fenfluramine 5 (○-○-○), 10 (■-■-■), 20 mg/kg (□-□-□) and saline (●-●-●) on the release of DA (a), DOPAC (b) and HVA (c). Basal output values (mean ± SEM of three 20 min periods before drug treatment) for DA, DOPAC and HVA were 0.727 ± 0.08; 53.2 ± 4.4 and 40.5 ± 3.2 pmoles. At least 4 animals per group were used. * $P < 0.05$ vs saline (Kruskall–Wallis test).

Table 1. Output of DA, DOPAC and HVA in rats chronically treated with haloperidol

Treatment	pmoles/120 min DA	DOPAC	HVA
Vehicle	5.51 ± 0.79	460 ± 31	311 ± 28
Haloperidol	2.81 ± 0.42†	384 ± 28	211 ± 19*

Rats received vehicle or 1 mg/kg haloperidol intraperitoneally twice daily for 11 days. Release was studied 48 hr after the last injection.

Each value is the mean ± SEM of 11–12 animals.

* $P < 0.05$; † $P < 0.01$ vs vehicle (Student's *t*-test).

still much higher than controls at the end of the experiments (3 hr after drug injection).

Tables 1 and 2 illustrate the effects of *l*-F and haloperidol in animals chronically treated with haloperidol. Repeated treatment with haloperidol significantly reduced the basal release of DA and HVA with no effect on DOPAC (Table 1). Considering the net effects of challenged doses of *l*-F and haloperidol (differences between accumulated picomoles of DA, HVA and DOPAC over 120 min before and after drug injection) it was found that a much smaller amount of DA and metabolites was released by *l*-F and haloperidol in animals treated with haloperidol than in vehicle treated rats (Table 2).

l-NF levels were not significantly different in animals treated chronically with haloperidol and vehicle while the concentrations of *l*-F were significantly higher in haloperidol-treated animals. Striatal levels (nmoles/g ± SE) were: vehicle 23.4 ± 3.8, haloperidol 55.1 ± 10.0 ($P < 0.05$ vs vehicle) for *l*-F; vehicle 52.5 ± 1 and haloperidol 41.2 for *l*-NF.

Figures 2(a) and (b) show the effects on DA release of selected doses of *l*-F, haloperidol and *d*-amphetamine in controls and in animals which had received 300 mg/kg i.p. GBL 40 min before. Only DA release was studied in these experiments since a previous study showed that GBL counteracted the effect of haloperidol on the release of DA but not HVA and DOPAC [9]. In controls, the selected doses of the various drugs produced similar but not identical increases in DA release. *l*-F and *d*-amphetamine showed peak effect 40 min after injection

Table 2. Effects of *l*-fenfluramine and haloperidol on the output of DA, DOPAC and HVA in rats chronically treated with haloperidol

Treatment	Release in 120 min[a] DA	DOPAC	HVA
Vehicle[b]			
l-Fenfluramine	2.21 ± 0.22	147 ± 12	174 ± 18
haloperidol	3.62 ± 0.44	431 ± 93	327 ± 26
Haloperidol[b]			
l-Fenfluramine	0.46 ± 0.17†	57 ± 18†	64 ± 15†
haloperidol	2.43 ± 0.32*	227 ± 25†	184 ± 30†

[a] Differences between accumulated pmoles of DA, DOPAC or HVA over the 120 min post-drug period and those accumulated in the 120 min before drug injection. Each value is the mean ± SEM of 5–6 animals.

[b] Rats received vehicle or 1 mg/kg haloperidol intraperitoneally twice daily for 11 days. Forty-eight hours after the last injection they were given 10 mg/kg *l*-fenfluramine or 0.1 mg/kg haloperidol i.p.

* $P < 0.05$; † $P < 0.01$; (Student's *t*-test).

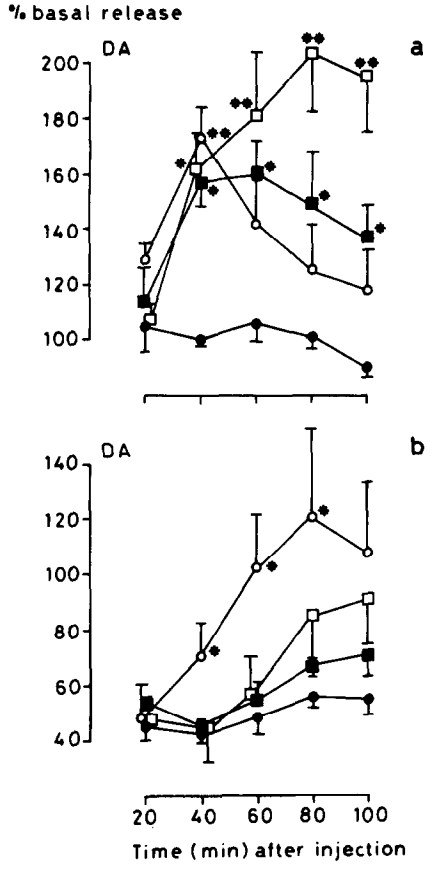

Fig. 2. (a) Effects of 10 mg/kg i.p. *l*-F (□-□-□), 0.1 mg/kg i.p. haloperidol (■-■-■), 0.25 mg/kg i.p. *d*-amphetamine (○-○-○) and saline (●-●-●) on the release of DA in control animals. Basal DA output values (mean ± SE of three 20-min periods before drug treatment) were 0.645 ± 0.05 pmoles. At least four animals per group were used. (b) Effects of 10 mg/kg i.p. *l*-F (□-□-□), 0.1 mg/kg i.p. haloperidol (■-■-■), 0.25 mg/kg *d*-amphetamine (○-○-○) or saline (●-●-●) on DA release in animals which had received 300 mg/kg i.p. GBL 40 min before. Basal output levels (mean ± SEM of three 20 min periods before drug treatment) were 0.537 ± 0.5 pmoles. Four animals per group were used.
** $P < 0.01$; * $P < 0.05$ vs GBL (Kruskall–Wallis test).

while the haloperidol peak occurred at 80 min (Fig. 2a).

As shown in Fig. 2(b), 300 mg/kg i.p. GBL reducted basal DA release by about 50% 60 min after injection and this effect was maintained for another 80 min. When 10 mg/kg *l*-F, 0.1 mg/kg haloperidol and 0.25 mg/kg *d*-amphetamine were injected i.p. to rats which had received GBL 40 min before, *l*-F and haloperidol were unable to increase DA release, but amphetamine had a significant effect at various times.

Histological examination after completion of the various experiments indicated that in most animals the dialysis tube was located in the head of the caudate nuclei (see in Fig. 3 a photo of a representative location of the dialysis tube). The data from the few animals in which the dialysis tube was not appropriately placed were not included in the results.

DISCUSSION

Although *l*-F is structurally related to *d*-amphetamine, it releases DA from rat corpus striatum by a different mechanism. In fact, *d*-amphetamine has been reported to increase the release of dopamine and decrease that of DA metabolites [8, 10] while *l*-F dose-dependently increases the release of DA, HVA and DOPAC. The pattern of effects of *l*-F was similar to that reported for various neuroleptics supporting the hypothesis that *l*-F may act as a central DA receptor blocker in the rat brain [3, 11]. This is also suggested by the finding that the ability of *l*-F to release DA was markedly reduced in animals repeatedly treated with haloperidol.

The activation of DA containing neurons by neuroleptics is attributed to a feedback mechanism consequent to their blockade of DA receptors [12] and there is evidence that repeated treatment with neuroleptics induces hypersensitivity of striatal DA postsynaptic receptors with consequent development of tolerance to the effect of these drugs on striatal DA turnover [13–15]. The development of cross-tolerance between haloperidol and *l*-F suggests that they act on a common site to increase striatal DA release. The reduced effect of *l*-F in animals chronically treated with haloperidol cannot be attributed to changes in *l*-F metabolism or reduced entry into the brain since the levels of *l*-F 1 hr after 10 mg/kg i.p. were even higher in haloperidol-treated animals than in those given vehicle, whereas the levels of its metabolite *l*-NF were not significantly changed.

Further evidence that *l*-F releases DA by a mechanism different from that of *d*-amphetamine and similar to that of neuroleptics derives from the experiments with GBL, a compound which inhibits the firing activity of nigrostriatal DA neurons [6] and reduces the release of DA in freely moving rats [8]. A recent study showed that GBL blocks the stimulation of DA release caused by neuroleptics, suggesting that the effect of these substances on DA release depends on their ability to increase the firing activity of DA neurons [9]. The release of biogenic amines upon nerve stimulation is mediated by exocytosis and is absolutely Ca^{2+}-dependent [16]. In addition, noradrenaline and DA may be released by a Ca^{2+}-independent mechanism which is the reversal of the catecholamine uptake carrier system [17, 18]. A recent study showed that amphetamine uses this mechanism to release DA from the corpus striatum [19]. It was therefore of interest to study the effect of GBL on DA release caused by *l*-F in comparison with haloperidol and *d*-amphetamine.

As previously reported [9], GBL treatment significantly reduced the ability of haloperidol to increase DA release. In addition we found that *d*-amphetamine could still significantly increase DA release in GBL-treated rats. The fact that the effect of *l*-F was completely blocked by GBL again suggests that this compound, in spite of its phenylethylamine structure, resembles neuroleptics in its ability to release DA from the corpus striatum of freely moving rats.

In conclusion, *l*-F appears to resemble haloperidol in its ability to release DA and its metabolites from the corpus striatum of freely moving rats. *l*-F may

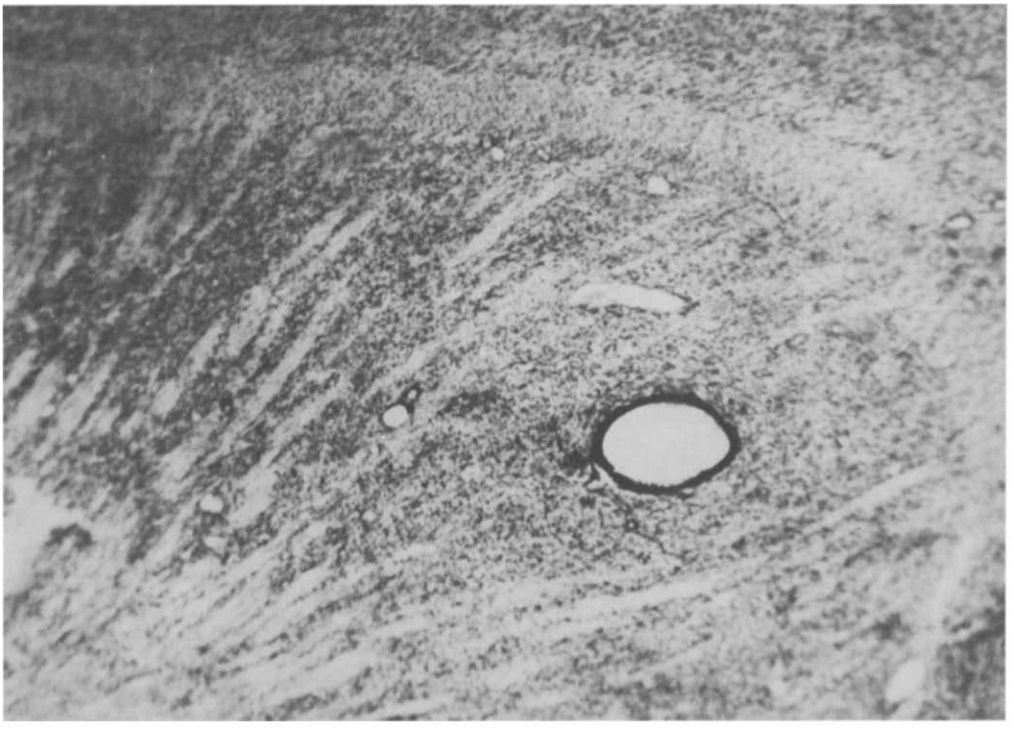

Fig. 3. Photo of a representative placement of the dialysis tube in the head of the caudate nucleus.

therefore constitute a novel DA blocking agent with potential antipsychotic activity in humans.

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