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Action of β-phenylethylamine and related amines on nigrostriatal dopamine neurotransmission

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Abstract

The present paper describes the effect of β-phenylethylamine and its metabolites phenylethanolamine, tyramine, acetyl-phenylethylamine and phenylacetaldehyde on the dopaminergic nigrostriatal system. The rotational behavioural response to the i.v. injection of these drugs was quantified in animals with a unilateral 6-hydroxydopamine lesion of the nigrostriatal dopamine system. Only β-phenylethylamine and acetyl-phenylethylamine induced rotations ipsilateral to the side of the brain lesion. None of the compounds under study stimulated contralateral rotations. Acetyl-phenylethylamine was 90% less active than β-phenylethylamine. After β-phenylethylamine injection all animals (16/16) showed ipsilateral rotations. The dose-response curve showed that at doses as low as 1.75 mg/kg ipsilateral turns increase, with a dose-related rotational response between 1.75 mg/kg and 11.66 mg/kg, no differences being found at doses between 11.66 and 29.16 mg/kg. Rotations began a few seconds after β-phenylethylamine injection. The highest response was found 30–60 s after the injection. The duration of the response was dose-related (4 min for the 3.5 mg/kg doses). The inhibition of dopamine-β-hydroxylase activity with [1-3,5-difluorobenzyl)imidazole-2-thiol (SKF102698) did not modify the rotational response to β-phenylethylamine. The inhibition of type B monoamine oxidase activity with *l*-deprenyl induced a slight increase in the ipsilateral rotational response to β-phenylethylamine. The inhibition of tyrosine hydroxylase activity with α-methyl-*p*-tyrosine decreased the rotational response to β-phenylethylamine. The dopamine receptor antagonist, haloperidol, completely blocked the ipsilateral rotational response to β-phenylethylamine. The blocking of dopamine uptake into storage vesicles with reserpine increased the rotational action of β-phenylethylamine. Taken together, the data suggest that, at low doses, β-phenylethylamine stimulates the release of dopamine from the cytoplasmic pool and behaves as a dopamine receptor agonist with a very rapid and brief action.

Keywords: β-Phenylethylamine; Dopamine; Nigrostriatal system; Rotation

1. Introduction

β-Phenylethylamine (2-phenylethylamine) is a biogenic amine that has been found in the nervous tissue of vertebrate and invertebrate species (Durden et al., 1973; Paterson et al., 1990; Philips et al., 1978; Reynolds et al., 1980) and whose physiological function is controversial (Paterson et al., 1990). β-Phenylethylamine is synthesized in the rat brain at a rate (1.5 nmol/g/h) similar to that reported for dopamine and possesses an extremely rapid turnover with a half-life of 0.4 min (Durden and Philips, 1980). As shown in Fig. 1, β-phenylethylamine is synthesized by decarboxylation of phenylalanine (Dyck et al., 1983) and metabolized to phenylacetic acid by type B monoamine oxidase (monoamine oxidase B) or to phenylethanolamine by dopamine-β-hydroxylase. β-Phenylethylamine is probably also metabolized by other routes (to *N*-methylphenylethylamine, tyramine) that are not completely known (Saavedra, 1989; Wu and Boulton, 1975).

β-Phenylethylamine is heterogeneously distributed in the brain, with the highest concentration in the mesolimbic and caudate-putamen structures (Paterson et al., 1990; Philips et al., 1978; Reynolds et al., 1980). Striatal β-phenylethylamine is synthesized by 6-hydroxydopamine-sensitive neurons (Greenshaw et al., 1986) that arise from the substantia nigra (Juorio et al., 1991) and contain both tyrosine hydroxylase (Juorio et al., 1991) and aromatic L-amino acid decarboxylase (Dyck et al., 1983). Thus, these are probably dopaminergic neurons of the nigrostriatal system (A9; Paterson et al., 1990). Despite the suggestion that β-phenylethylamine stimulates dopamine release

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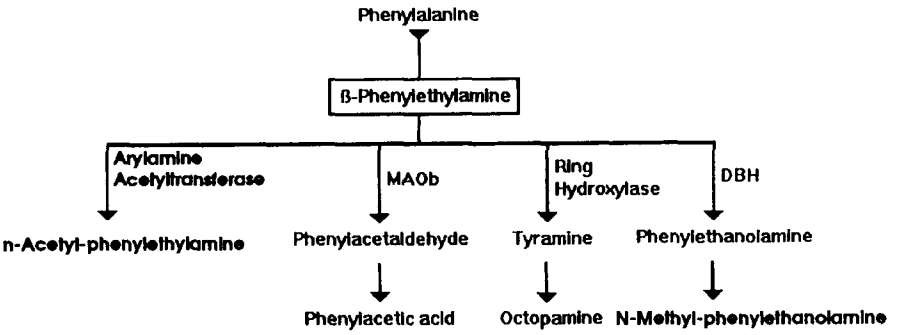

Fig. 1. β-Phenylethylamine metabolism. Type B monoamine oxidase (MAOb), dopamine-β-hydroxylase (DBH).

(Raiteri et al., 1977; Dyck, 1983; Philips and Robson, 1983; Bailey et al., 1987) and postsynaptic dopamine receptors (Antelman et al., 1977), little is known about how β-phenylethylamine affects dopaminergic transmission. We now studied, in an in vivo animal preparation, the functional role of β-phenylethylamine and related amines on dopaminergic neurotransmission of the nigrostriatal system. The aims of the study were to evaluate whether, at physiological concentrations, the effects of β-phenylethylamine on dopaminergic transmission (1) are caused by this amine or by any of its metabolites, (2) need the availability of cytoplasmic or vesicular presynaptic dopamine and (3) need the availability of dopamine receptors.

2. Materials and methods

Experiments were carried out on male Sprague-Dawley rats (Letica, Barcelona) weighing 300–350 g. The animals were housed at 22°C, two per cage, under normal laboratory conditions on a standard light-dark schedule (12:12 with lights on 3:00–15:00) and with free access to food and water.

The animals were lesioned with a procedure that induces selective destruction of more than 90% of dopaminergic nigrostriatal neurons (Castro et al., 1985). The rats received under anaesthesia (80 mg/kg ketamine + 12 mg/kg xylazine, i.p. injected), a double unilateral intracerebral injection of 6-hydroxydopamine hydrochloride (Sigma, St. Louis, MO, USA). 6-Hydroxydopamine was prepared for injection by dissolving it in a 0.9% saline solution and 0.3 μg/μl ascorbic acid to retard oxidation. The stereotaxic surgery procedure and the biochemical data used to show the extent of the lesion were described earlier (Castro et al., 1985; Burunat et al., 1988). Briefly, 6-hydroxydopamine was injected, using a stereotaxic procedure, with a 5 μl Hamilton syringe and a cannula with 0.4 mm outside diameter. Injection coordinates were 2.2 mm posterior to the bregma, 1.6 mm to the right of the midline and 8 mm below the dura for the medial forebrain bundle lesion and 2.8 mm posterior to the bregma, 2.3 mm to the right of the midline and 8 mm below the dura for the substantia nigra lesion. The infusion rate was 1 μl/min with a total dose of 8 μg of 6-hydroxydopamine in 4 μl solution for each of the double unilateral lesions. The syringe was withdrawn 3 min after the injection was terminated. Behavioural screening to detect unilateral lesioning (Castro et al., 1985) was carried out after at least 2 weeks' recovery, and animals that turned no fewer than 8 turns/min in response to an apomorphine hydrochloride (1 mg/kg i.p.; Sigma, St. Louis) challenge were included in the study. One week later, the rats were randomly allocated to the different groups of the study.

In order to facilitate the i.v. injection of drugs, one day before the test day, a silastic (Medical-grade tubing by Dow Corning with 0.020 in. ID and 0.037 in. OD) cannula was implanted in the jugular vein using the procedure reported by Harms and Ojeda (1974). On the test day, the silastic cannula was connected to a polythene tube (0.76 mm ID), the end of which was connected to a Hamilton syringe. After 15 min of adaptation to the experimental cage conditions, the rats received a 25 μl i.v. injection of a drug solution through the implanted cannula. All drugs were dissolved in saline solution 10 min before injection. After drug injection, the total number of full-body turns (complete rotations) was observed for a 20-min period. The rats were tested in cages (length, width and height, 25 cm) with a wire-netting floor and illuminated with red light (50 W lamp located 15 cm under the cage floor). The cage walls were lined with sound-attenuating foam. The behavioural test was performed over 3–8 h of the light period with a room temperature of 22°C (Castro et al., 1985; Burunat et al., 1987). The animals were tested individually since group testing has been reported to potentiate part of the behavioural effect of β-phenylethylamine (Dourish, 1982). β-Phenylethylamine hydrochloride, phenylethanolamine (L-β-hydroxyphenethylamine), tyramine hydrochloride, acetyl-phenylethylamine and phenylacetaldehyde were obtained from Sigma Chemical Company (St. Louis, MO, USA). Acetyl-phenylethylamine was synthesized in the chemical laboratories of the Natural Products Institute of La Laguna (Tenerife, Canary Islands) and was kindly supplied by Juan Trujillo.

The statistical analyses were performed with the Statistic-SX program (NH Analytical Software). Because we could not assume that an interval scale can be made with

the number of rotations, non parametric statistics were used (Kruskal-Wallis one-way non-parametric analysis of variance, Mann-Whitney two-unpaired samples non-parametric U-test and Wilcoxon two-paired samples non-parametric test).

2.1. Action of β-phenylethylamine and β-phenylethylamine metabolites on dopaminergic neurotransmission

Fifty-two 6-hydroxydopamine-lesioned rats were i.v. injected with saline solution or phenylethanolamine, tyramine, acetyl-phenylethylamine, phenylacetaldehyde or phenylethylamine. In order to study the dose-response and time course of the rotational response to β-phenylethylamine, another group of 59 lesioned rats were i.v. injected with different doses of β-phenylethylamine (from 0.29 to 29.16 mg/kg). The total number of full-body turns was observed over 20 min after each drug was administered.

2.2. The effect of blocking dopamine-β-hydroxylase, type B monoamine oxidase or tyrosine hydroxylase activities on the rotational response to β-phenylethylamine

All these studies were carried out on 6-hydroxydopamine unilaterally lesioned rats. Dopamine-β-hydroxylase was inhibited with SKF102698 (100 mg/kg; [1-(3,5-difluorobenzyl)imidazole-2-thiol], synthesized and kindly supplied by Smith Kline Beecham Pharmaceuticals). This dose induced a marked and selective decrease in dopamine-β-hydroxylase activity in the brain (Ohlstein et al., 1988). SKF102698 was prepared daily by dissolving in a final concentration of 5% polyethylene glycol 400 in 1% methocel. The rotational action of β-phenylethylamine was quantified 1 h before and 6 h after SKF102698 administration. Type B monoamine oxidase was inhibited by R(−)-deprenyl hydrochloride (0.625 mg/kg; Research Biochemicals; Wu and Boulton, 1975; Dyck et al., 1983). Before deprenyl injection the rats were i.v. injected with saline solution. The rotational action of β-phenylethylamine was quantified 1 h before and 6 h after deprenyl injection. Tyrosine hydroxylase was inhibited with α-methyl-*p*-tyrosine (two doses of 100 mg/kg with a time interval of 4 h; DL-α-methyltyrosin-methylester hydrochloride; Aldrich-Chemie, West Germany). In this case the action of β-phenylethylamine was evaluated 1 h before and 2 h after α-methyl-*p*-tyrosine administration. For all these studies the effect of β-phenylethylamine was quantified during the 20 min that followed each β-phenylethylamine (3.50 mg/kg) i.v. injection.

2.3. The effect of blocking the dopamine receptors on the rotational response to β-phenylethylamine

6-Hydroxydopamine-lesioned rats were i.v. injected with β-phenylethylamine (3.50 mg/kg) 1 h before and 30 min after haloperidol (0.3 mg/kg; Syntex Latino, Madrid) i.p.

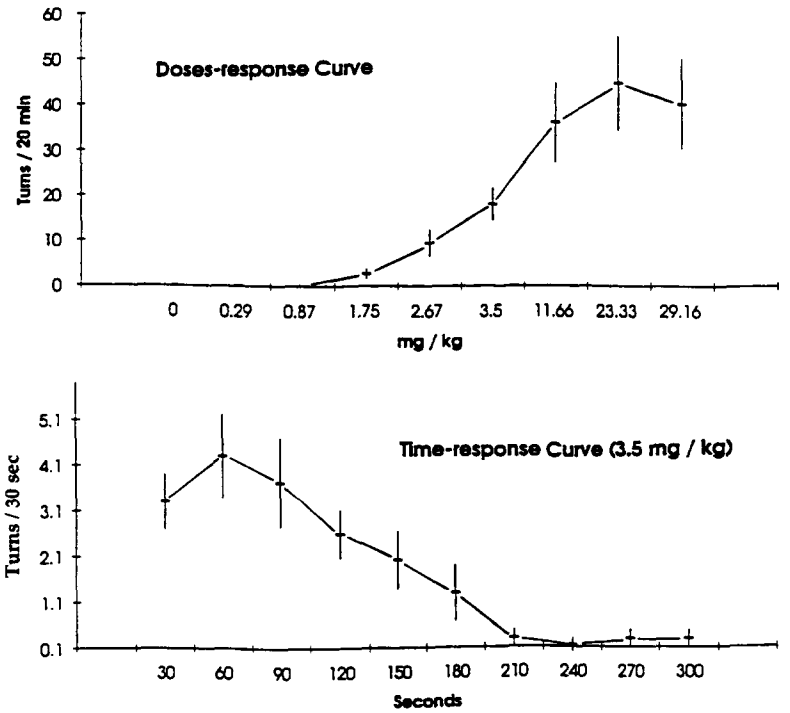

Fig. 2. Doses-response and time-response curves for β-phenylethylamine rotational effect in 6-hydroxydopamine unilaterally lesioned rats. In order to obtain the dose-response curve a group of lesioned rats were i.v. injected with saline solution ($n = 7$) or β-phenylethylamine 0.29 mg/kg ($n = 7$), 0.87 mg/kg ($n = 7$), 1.75 mg/kg ($n = 7$), 2.62 mg/kg ($n = 7$), 3.50 mg/kg ($n = 7$), 11.16 mg/kg ($n = 7$), 23.33 mg/kg ($n = 7$) or 29.16 mg/kg ($n = 3$). The study of the time-response curve was made with lesioned rats ($n = 7$) i.v. injected with 3.50 mg/kg of β-phenylethylamine. Values are mean ± S.E.M. of turns ipsilateral to the lesioned side.

administration. The number of full-body turns was observed during the 20 min that followed each β-phenylethylamine injection.

2.4. The effect of blocking vesicular monoamine uptake on the rotational response to β-phenylethylamine

6-Hydroxydopamine-lesioned rats were i.v. injected with β-phenylethylamine (3.50 mg/kg) 16 h after the reserpine (5 mg/kg prepared by dissolving in a minimal amount of glacial acetic acid and further diluted to 2.5 mg/ml with 5% ethanol/H_2O; Sigma, St. Louis) or reserpine-vehicle injection and the number of full-body turns was observed for a 20-min period.

3. Results

3.1. Effect of β-phenylethylamine and β-phenylethylamine metabolites on dopaminergic neurotransmission

Fig. 2 shows the ipsilateral turns induced by different doses of β-phenylethylamine. The analysis of variance showed statistically significant differences between doses ($P < 0.0001$). The 0.29 mg/kg ($P = 0.94$ vs. saline control) and 0.87 mg/kg ($P = 0.70$ vs. saline control) doses did not stimulate ipsilateral turns. The 1.75 mg/kg dose induced an increase in ipsilateral turns ($P = 0.02$ vs. saline control). A dose-dependent response was observed between 1.75 mg/kg and 11.66 mg/kg ($P = 0.008$, 2.67 mg/kg vs. saline control; $P = 0.002$, 3.50 mg/kg vs. saline control; $P = 0.002$, 11.66 mg/kg vs. saline control; $P = 0.002$, 23.33 mg/kg vs. saline control). No differences were found between the 23.33 and 29.16 mg/kg doses. As the time course response for the 3.5 mg/kg dose shows (Fig. 2), rotations began a few seconds after β-phenylethylamine injection, the highest response being observed after 30–60 s. The duration of response was also dose-related. With the 3.5 mg/kg dose, the ipsilateral turns decreased to low values 4 min after injection. At the highest doses the response was present for 12–15 min. The selectivity of this action was evaluated by injecting a similar dose (3.5 mg/kg) of the main metabolites. β-Phenylethylamine and acetyl-phenylethylamine but not phenylethanolamine ($n = 6$), tyramine ($n = 6$) or phenylacetaldehyde ($n = 8$) induced rotations. Acetyl-phenylethylamine (1.3 ± 0.9 turns/20 min; $n = 7$) was 90% less active than β-phenylethylamine (10.6 ± 1.6 turns/20 min; $n = 17$). After β-phenylethylamine injection all animals (16/16) showed ipsilateral rotations ($P = 0.0001$ contralateral turns in β-phenylethylamine-rats vs. saline control rats) and none showed contralateral rotations during the test session.

3.2. The effect of blocking dopamine-β-hydroxylase, type B monoamine oxidase or tyrosine hydroxylase activities on the rotational response to β-phenylethylamine

The dopamine-β-hydroxylase inhibitor, SKF102698, failed to affect the ipsilateral rotational response to β-phenylethylamine. Thus, no significant differences were found for the β-phenylethylamine response before and after administration of the inhibitor either as to the time course or as to the total rotational response ($P > 0.05$

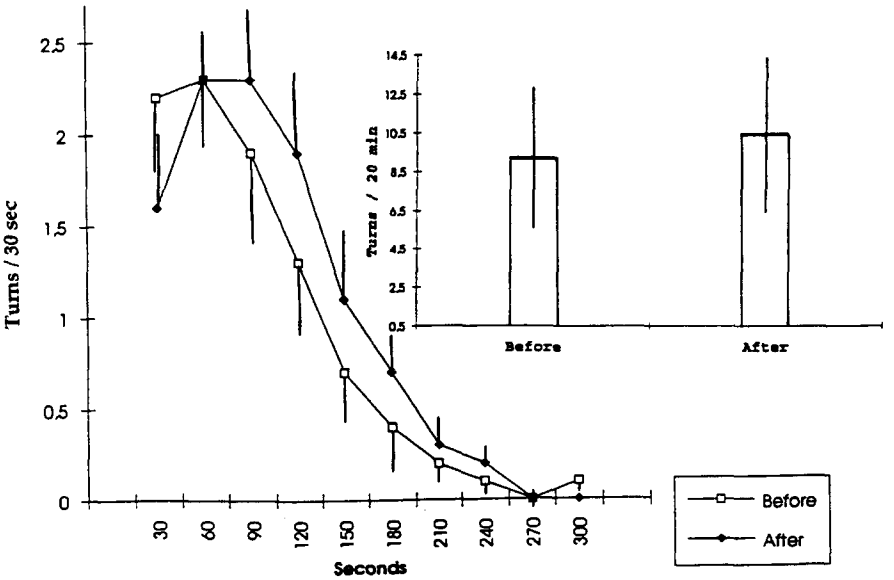

Fig. 3. Time-response curve and total response for β-phenylethylamine rotational effects before and after dopamine-β-hydroxylase (DBH) inhibition. Lesioned rats ($n = 10$) were i.v. injected with β-phenylethylamine 3.50 mg/kg before and 6 h after the inhibition of dopamine-β-hydroxylase (100 mg/kg p.o. of SKF102698). After each β-phenylethylamine injection the total number of full-body turns per side was observed for a 20-min period. Values are mean − S.E.M. (before DBH), mean + S.E.M. (after DBH) or mean ± S.E.M. (total response) of turns ipsilateral to the lesioned side.

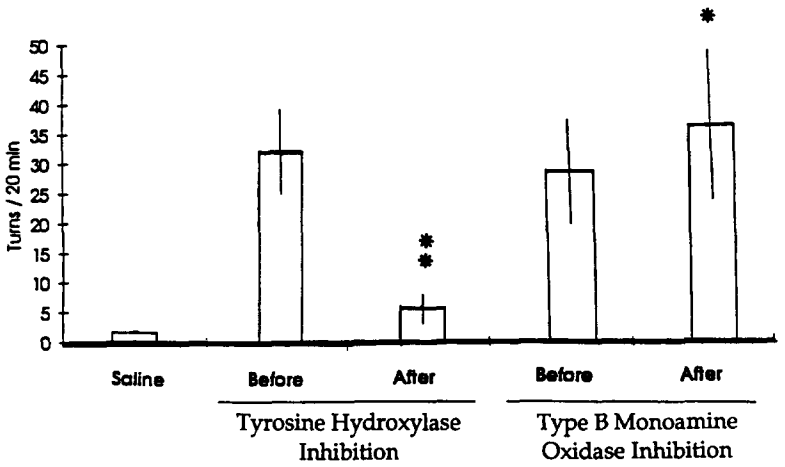

Fig. 4. Rotational response to saline or β-phenylethylamine administration before and after tyrosine hydroxylase or type B monoamine oxidase inhibition. One hour before the first β-phenylethylamine administration, rats were i.v. injected with saline solution. In order to study the effect of tyrosine hydroxylase inhibition on the response to β-phenylethylamine, a group ($n = 7$) was i.v. injected with β-phenylethylamine (3.50 mg/kg) before and 2 h after tyrosine hydroxylase inhibition (100 mg/kg of DL-α-methyltyrosin-methylester hydrochloride i.p. injected 1 and 5 h after the first β-phenylethylamine injection). In the study of type B monoamine oxidase inhibition, β-phenylethylamine (3.50 mg/kg, i.v.) was administered ($n = 7$) before and 2 h after the inhibition of this enzyme with $R(-)$-deprenyl hydrochloride (0.625 mg/kg i.p. injected 1 h after the first β-phenylethylamine administration). After saline or β-phenylethylamine injection the total number of full-body turns was quantified for a 20-min period. Values are mean ± S.E.M. of turns ipsilateral to the lesioned side. * $P < 0.05$ β-phenylethylamine response after vs. before monoamine oxidase inhibition. ** $P < 0.001$ β-phenylethylamine response after vs. before tyrosine hydroxylase inhibition.

before vs. after SKF102698 administration; Fig. 3). The type B monoamine oxidase inhibitor, *l*-deprenyl, induced a slight increase in the ipsilateral rotational response to β-phenylethylamine. Fig. 4 shows that β-phenylethylamine increased rotations when compaired to saline injection (analysis of variance $P = 0.0003$). The statistical test for paired data also showed a different response to β-phenylethylamine before and after *l*-deprenyl treatment. The rotational response to β-phenylethylamine increased after administration of the inhibitor (the two-tailed P value for Wilcoxon's signed rank test was $P = 0.022$ before vs. after *l*-deprenyl administration). The tyrosine hydroxylase inhibitor, α-methyl-*p*-tyrosine, decreased the ipsilateral rotational response to β-phenylethylamine. Thus, statistically significant differences were found for the β-phenylethylamine response before and after administration of the inhibitor regarding the total rotational response ($P = 0.007$ before vs. after α-methyl-*p*-tyrosine administration; Fig. 4). A positive correlation was found for β-phenylethylamine-induced rotations before and after inhibitor injection (Spearman correlation $r = 0.85$, $P = 0.014$).

3.3. The effect of blocking the dopamine receptors on the rotational response to β-phenylethylamine

After haloperidol administration (0.3 mg/kg), the animals remained awake and responded to the stimulus from handling by the experimenter. The dopamine receptor antagonist, haloperidol, completely blocked the ipsilateral rotational response to β-phenylethylamine. Thus, statisti-

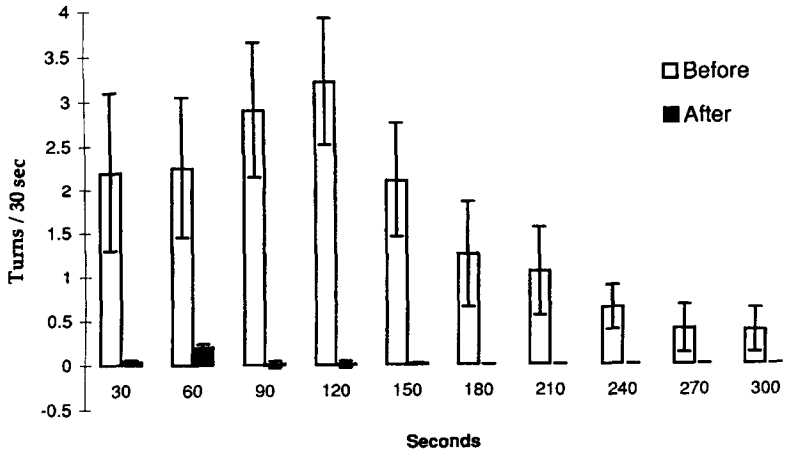

Fig. 5. Rotational response to β-phenylethylamine before and after dopamine receptor blockade. Rats ($n = 11$) were i.v. injected with β-phenylethylamine (3.50 mg/kg) before and 1 h after the dopamine receptor blockade (0.3 mg/kg of haloperidol i.p. injected 1 h after the first β-phenylethylamine injection). Values are mean ± S.E.M. of turns ipsilateral to the lesioned side.

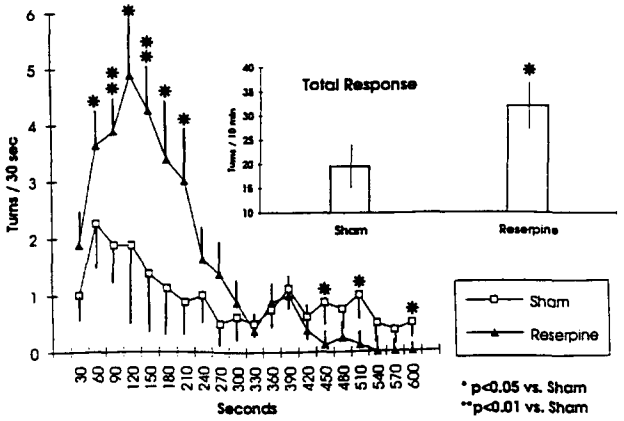

Fig. 6. Rotational response for β-phenylethylamine before and after block of the vesicular monoamine uptake with reserpine. Rats were i.v. injected with β-phenylethylamine (3.50 mg/kg) 16 h after reserpine (5 mg/kg ip; $n = 10$) or reserpine-vehicle ($n = 8$) administration. Values are mean + S.E.M. (reserpine + β-phenylethylamine), mean − S.E.M. (reserpine-vehicle + β-phenylethylamine) or mean ± S.E.M. (total response) of turns ipsilateral to the lesioned side during the 10 min that followed β-phenylethylamine administration.

cally significant differences were found for the β-phenylethylamine response before and after administration of haloperidol at all the 30 s intervals of the 5 min that followed β-phenylethylamine administration ($P = 0.001$ before vs. after haloperidol administration; Fig. 5).

3.4. The effect of blocking vesicular monoamine uptake on the rotational response to β-phenylethylamine

The ipsilateral rotational response to β-phenylethylamine increased after reserpine block of the uptake of dopamine into storage vesicles (Fig. 6). Thus, significant differences were found for the β-phenylethylamine effect when comparing the total response of reserpine-pretreated and reserpine-vehicle-pretreated rats ($P = 0.02$ sham vs. reserpine-pretreated rats). In addition, the time course of the β-phenylethylamine rotational effect shows that reserpine pretreatment also decreased the duration of the β-phenylethylamine response. Thus, the reserpine-pretreated rats showed an increase between 60-210 s ($P = 0.02$ sham vs. reserpine groups 30–60 s; $P = 0.003$ sham vs. reserpine groups 60–90 s; $P = 0.03$ sham vs. reserpine groups 90–120 s; $P = 0.0008$ sham vs. reserpine groups 120–150 s; $P = 0.04$ sham vs. reserpine groups 150–180 s; $P = 0.03$ sham vs. reserpine groups 180–210 s) and a decrease between 450–600 s ($P = 0.01$ sham vs. reserpine groups 430–450 s; $P = 0.04$ sham vs. reserpine groups 480–510 s; $P = 0.03$ sham vs. reserpine groups 570–600 s for the rotational response to β-phenylethylamine).

4. Discussion

Both its low concentration in the brain and its relatively low potency to modify behaviour have taken to suggest that β-phenylethylamine is merely a metabolic by-product with no physiological role (Paterson et al., 1990). The present study supplied evidence that β-phenylethylamine can induce a selective modification of behaviour when administered at low doses. β-Phenylethylamine has a high affinity for non-neural tissues (lung, liver, kidney) and only less than 1% of i.v. injected β-phenylethylamine reaches the brain (Wu and Boulton, 1975). After its peripheral injection, its distribution in the brain is not homogeneous and less than 20% of the β-phenylethylamine that reaches the brain is taken up by the striatum. Thus, only a small portion of the administered β-phenylethylamine reaches the dopaminergic synapses of the nigrostriatal system. This distribution and the relatively low potency previously found for other behavioural patterns (Paterson et al., 1990) have led to the use of doses higher than 20 mg/kg (Antelman et al., 1977; Dourish and Cooper, 1984a, b; Ortmann et al., 1984; Dourish et al., 1983; Dourish, 1982a, b; Jackson and Smythe, 1973). As Fig. 3 shows, a rotational response was now found for a dose as low as 1.75 mg/kg. This is a dose 10–60 times lower than that reported previously for other behavioural stimulation and cannot be considered to be a high dose. In addition, none of the main β-phenylethylamine metabolites (phenylethanolamine, tyramine, acetylphenylethylamine or phenylacetaldehyde) induced a rotational response similar to that found with β-phenylethylamine (Fig. 1). Pharmacological block (Figs. 3 and 4) of the main metabolic routes for β-phenylethylamine (dopamine-β-hydroxylase and type B monoamine oxidase) does not decrease its behavioural effects. Thus, in the present study β-phenylethylamine behaves as a direct and selective drug and not as a simple precursor of a rotationally active metabolite. Apomorphine has been considered to be the dopamine receptor agonist with the shortest half-life. Its rotational response begins 3-4 min after injection and persists for more than 45 min (Castro et al., 1985). The rotational response to β-phenylethylamine began a few seconds after its i.v. injection and persisted only for a few minutes (Fig. 2). Thus, β-phenylethylamine is probably the dopamine receptor agonist with the fastest and briefest action since it readily crosses the blood-brain barrier and has a very high turnover rate.

It has been suggested that β-phenylethylamine stimulates postsynaptic dopamine or β-phenylethylamine receptors (Antelman et al., 1977). However, a postsynaptic effect was reported only for high β-phenylethylamine doses (100 mg/kg i.p. injected, Antelman et al., 1977 or 80 μg injected in the striatum, Nguyen et al., 1986). Using lower doses we have not found evidence to support this postsynaptic action. The unilateral lesion of the nigrostriatal system induces, in the ipsilateral striatum, a decrease in dopaminergic terminals and an increase in dopamine postsynaptic receptors (Seeman, 1981; Castro et al., 1985; Burunat et al., 1987, 1988). Because β-phenylethylamine induced ipsilateral rotations in all the animals (Fig. 2), the present data suggest that this drug is more active in the

unlesioned brain side. Both its higher effectiveness in the side with the lower number of dopamine receptors and the blocking effect (Fig. 5) of haloperidol (neuroleptic drugs are not capable of displacing [^{3}H] β-phenylethylamine from its specific binding sites; Hauger et al., 1982) suggest that the rotational action of β-phenylethylamine is not induced by its direct action on postsynaptic dopamine or β-phenylethylamine receptors.

It has been previously reported that β-phenylethylamine stimulates the release of dopamine from synaptosomes (Raiteri et al., 1977) and striatal brain slices (Dyck, 1983). In vivo studies (Philips, 1986; Philips and Robson, 1983; Bailey et al., 1987) have shown a biochemical action of β-phenylethylamine on striatal dopamine release at doses higher than 25 mg/kg (Jackson and Smythe, 1973; Philips, 1986). The present data show a rotational action for doses lower than 25 mg/kg and suggest that, at low doses, β-phenylethylamine also increases dopamine release. The rotational response to β-phenylethylamine (3.5 mg/kg) decreased after blocking of the endogenous synthesis of dopamine (Fig. 4). The tyrosine hydroxylase inhibition induced by α-methyl-*p*-tyrosine injection decreased the rotational response to β-phenylethylamine to 20% of the initial response. In addition, the dopamine receptor blockade with haloperidol induced a decrease in rotational response to β-phenylethylamine (Fig. 5). Thus, both dopamine and dopamine receptor availability are necessary for the rotational action of low doses of β-phenylethylamine. We are of the opinion that β-phenylethylamine increases dopamine release and that the interaction of released dopamine with postsynaptic dopamine receptors is the sole final pathway for the rotational action of low doses of β-phenylethylamine. Evidence has been reported that, in addition to the depolarization-evoked vesicular exocytosis, the cytoplasmic monoamines may also be released from nerve endings by a Ca^{2+}-independent carrier-mediated process, which is a reversal of the amine uptake carrier systems (Moore, 1977; McMillen, 1983; Cantrill et al., 1983; Liang and Rutledge, 1982). This is probably a carrier-mediated facilitated diffusion that is resistant to reserpine action (Liang and Rutledge, 1982; Parker and Cubeddu, 1986) and is involved in the action of amphetamine on dopamine release (Moore, 1977; McMillen, 1983; Parker and Cubeddu, 1986). Experiment 4 showed that reserpine pretreatment, rather than decreasing, increased the rotational action of β-phenylethylamine (Fig. 6). Reserpine blocks dopamine uptake into storage vesicles and increases the percentage of cytoplasmic dopamine. Thus, the present data suggest that, like amphetamine, β-phenylethylamine stimulates the release of dopamine from the cytoplasmic pool.

In summary, the dopamine receptor agonist, β-phenylethylamine, is more active in the brain side with both the higher number of dopaminergic synapses and the lower density of dopaminergic receptors. Its rotational activity is decreased by reduction of the dopamine synthesis rate, completely inhibited by the blockade of dopamine receptors and facilitated by the blockade of dopamine uptake into storage vesicles. Taken together, the present data suggest that low doses of β-phenylethylamine facilitate cytoplasmic dopamine release from nigrostriatal neurons. Because the entire rotational response to β-phenylethylamine was blocked by haloperidol, the present data also suggest that any rotational response to β-phenylethylamine is dopamine-release-related. Striatal β-phenylethylamine is probably synthesized and released by nigrostriatal dopaminergic neurons (Greenshaw et al., 1986; Juorio et al., 1991; Dyck et al., 1983). The mechanism for β-phenylethylamine release is still unknown. Because (1) reserpine produces no changes in brain β-phenylethylamine levels (Boulton et al., 1977), (2) high K^+ concentrations do not stimulate the release of β-phenylethylamine (Henry et al., 1988) and (3) β-phenylethylamine is released at a rate dependent on the amount remaining in the tissue (Dyck et al., 1983), it has been suggested that β-phenylethylamine diffuses out of dopamine neurons at a rate dependent on the rate of synthesis (Paterson et al., 1990; Juorio et al., 1991). Taking together the results of the present and previous studies, we suggest that the rate of synthesis for endogenous β-phenylethylamine could be a main factor in the control of cytoplasmic dopamine release from nigrostriatal neurons. Both dopamine and β-phenylethylamine have been related to depression (Sandler et al., 1979; Sabelli et al., 1983; Nakagawara, 1992), schizophrenia (Potkin et al., 1979; Szymanski et al., 1987) and aggressive disorders (Boulton et al., 1983; Yu et al., 1983). The rotational procedure of Ungerstedt used here has been much used as an animal model of Parkinson's disease (Ungerstedt, 1971; Castro et al., 1985; Burunat et al., 1987; Burunat et al., 1988). Parkinsonian patients present a progressive degeneration of the nigrostriatal cells that, as remarked above, synthesize both dopamine and β-phenylethylamine. Perhaps disturbances of β-phenylethylamine release can also be related to some problems such as the 'on-off' phenomena observed in the management of patients with Parkinson's disease (Marsden, 1980; Obeso et al., 1989). We are now testing this hypothesis. Since β-phenylethylamine behaves as a dopamine receptor agonist with a very fast and brief action, its continuous administration with implanted pumps could be useful for the accurate control of a number of neurological disturbances that have been related to both dopamine and β-phenylethylamine.

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