Transfer of Dopamine in the Olfactory Pathway Following Nasal Administration in Mice

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Purpose. The aim of the study was to investigate whether dopamine is transferred along the olfactory pathway to the brain following nasal administration to mice.

Methods. [^{3}H]-Dopamine was administered nasally or intravenously to female mice. Brain tissue samples were excised and the radioactive content was measured. The precise localisation of dopamine radioactivity in the brain was studied using autoradiography. The presence of dopamine or its metabolites in the olfactory bulb and mucosa was ascertained using thin layer chromatography (TLC).

Results. After administration of [^{3}H]-dopamine into the right nostril, the amount of dopamine in the right bulb increased with time until, after 4 h, it was 27 times higher than in the left bulb. Among the other brain tissue samples, significantly higher amount of radioactivity was detected in the lateral olfactory tract. Radioactivity in the right olfactory bulb was shown by autoradiography to be selectively located in the peripheral layers 1 to 4 h after administration. Selective uptake of radioactivity was not seen in other regions of the brain. TLC data indicated that approximately 75% and 10% of the radioactivity in the olfactory bulb and mucosa, respectively, coeluted with dopamine.

Conclusions. The results indicate that unchanged dopamine is transferred into the olfactory bulb following nasal administration of [^{3}H]-dopamine.

KEY WORDS: nasal administration; dopamine; mice; olfactory pathway; autoradiography.

INTRODUCTION

Targeting the brain via nasal administration of drugs offers potential for drug development since the olfactory receptor cells are in direct contact with both the environment and the central nervous system (CNS). The olfactory epithelium is located in the roof of the nasal cavity adjacent to the septum and the superior turbinate. The bipolar sensory neurons, estimated to number 10 to 20 millions in humans (1), have olfactory receptors on cilia projecting into the nasal mucus. The axons of these olfactory sensory cells are grouped into small bundles, which pass through the cribriform plate of the ethmoid bone into the olfactory bulb where they synapse with second order neurons (2). The bundles of olfactory axons may thus provide a route of entry to the brain that circumvents the blood-brain barrier (BBB). The absence of a strict nose-brain barrier could, then, allow air-borne substances, viruses, metals or drugs to be delivered directly into the CNS. Thirty-five to 40 substances have been reported to reach the CNS after nasal administration in experimental animals (3). In recent studies nerve growth factor (4), local anaesthetics (5), inorganic mercury (6), taurine (7), dihydroergotamine (8), carboxylic acids (9) and 2′, 3′-didehydro-3′-deoxythymidine (10) have been transferred via the nasal route.

In fact there may be two direct pathways for transfer of substances from the olfactory mucosa into the CNS. These can be broadly classified as the olfactory nerve pathway as described above and the olfactory epithelial pathway (3). Agents able to enter the olfactory receptor cells would utilise the olfactory nerve pathway to reach the olfactory bulbs. Mouse hepatitis (11) and vesicular stomatitis viruses (12), inorganic mercury (6) and agglutinin-conjugated horseradish peroxidase (13) have been shown to enter the brain by axonal transport. Alternatively, substances entering the brain via the olfactory epithelial pathway could be absorbed by paracellular transport adjacent to the receptor cells in the nasal cavity. After entering the lamina propria these molecules could enter the perineural space and then reach the CNS (4,13,14).

Cerebrospinal fluid (CSF) drainage via the nasal route in man was demonstrated by Lowhagen *et al.* (15) and a few human studies of access to the brain after nasal administration of drugs have been published. For example, Pietrowsky *et al.* provided functional evidence for the facilitated access of vasopressin (16) and cholecystokinin (17) into the brain by this route. In another human study (18), cerebral radioactivity increased significantly after spraying a radioactive mixture of ^{99m}Tc-diethylene triaminopenta-acetic acid and hyaluronidase into the nose.

Gross *et al.* (23) made a morphometric analysis of the rodent nasal cavity and about 45% of the total nasal epithelium in mice consisted of olfactory epithelium. In human, however, the total olfactory area is limited to approximately 6% of the total surface area of the nasal cavity (24). Further, in humans, the olfactory region is located in the roof of the cavity while the olfactory area in mice is spread over a larger area in the posterior nasal cavity. It is important to take these anatomical differences between species under consideration when results are interpreted and compared.

Dopamine is currently used to treat acute cardiovascular diseases. Because of high first pass metabolism after oral administration, the drug is usually only given by intravenous infusion. However, the nasal route has been suggested as an alternative for the administration of this drug since nasally administered dopamine has a higher bioavailability (11.7%) than dermal, buccal or rectal administration in beagle dogs. It is also possible to increase the bioavailability of dopamine after nasal administration to 20–100% by using absorption enhancers (19).

Dopamine is also an important part of the treatment of Parkinson's disease. Since dopamine does not cross the BBB in appreciable amounts, the immediate precursor, levodopa, is used to target the brain. Levodopa passes the BBB easily and is decarboxylated rapidly to dopamine within the brain. About 95% of the drug is converted to dopamine by dopa decarboxylase in the peripheral tissues and only a small proportion enters the brain. Because of this, levodopa is nearly always combined

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with a peripheral dopa decarboxylase inhibitor (20). Administration of large doses of levodopa can cause adverse effects in patients with Parkinson's disease. Physiological variables such as gastric emptying and protein-rich meals may markedly affect the amount of orally administered levodopa entering the brain and the speed with which it enters (21). Targeting the brain via nasal administration of dopamine or levodopa is a potential alternative to oral levodopa. The dose and associated adverse effects could perhaps also be decreased.

Several different methods have been used to study the uptake of substances into the brain after nasal administration. The most common methods involve sampling the CSF (5,10) or brain tissue (4,22). Autoradiography (6,7) is a useful technique for exploring the olfactory pathways into the brain but has rarely been used in nasal drug delivery experiments.

The aim of this study was to investigate whether dopamine is transferred along the olfactory pathway to the brain following nasal administration to mice, using brain tissue sampling and autoradiography.

MATERIALS AND METHODS

Drugs and Reagents

Two batches of [2, 5, 6 ^{3}H]-dopamine dissolved in 0.02 M acetic acid: ethanol (1:1) were obtained from Amersham Pharmacia Biotech, Sweden. The radiochemical purity was 99.7% and 98.5% and the specific activity was 73.3 mCi/mg and 62.8 mCi/mg for batches 1 and 2, respectively. The [^{3}H]-dopamine solution was evaporated to dryness, under a gentle stream of nitrogen at room temperature, before use. Ketamine (Ketalar® 50 mg/ml) and xylazine (Rompun® vet. 20 mg/ml) were purchased from Apoteket AB (Sweden). Hionic-Fluor™ and Soluene®-350 were purchased from the Packard Instrument Company (USA). Ultrapure deionised water (Milli Q UF Plus, Millipore, France) was used for preparation of the solutions. All other chemicals were of analytical grade.

Animals

Female NMRI mice (B&K Universal, Sweden) with a mean body weight of 22 g (range 19–25 g) were used. None of the mice showed signs of rhinitis. Animals were housed at 22°C with a 12 h light/dark cycle and given a standard pellet diet (Lactamin R 36, Ewos AB, Sweden) and tap water *ad libitum*. The study was approved (C 94/97) by the local ethical committee in Uppsala.

Animal Experiments

Mice were anaesthetised with an intraperitoneal injection of a combination of ketamine and xylazine (100 mg and 10 mg/kg body weight, respectively) and placed on a heating pad (38°C) to maintain the body temperature.

[^{3}H]-Dopamine (5 μCi; 0.08 μg) was dissolved in 0.1 M phosphate buffer (pH 7.4) and administered unilaterally to the right nostril (5 μl) using polyethylene tubes (PE-10) attached to a micropipette, or was dissolved in physiological saline and injected intravenously (25 μl) into the tail vein. The mice were then placed on their right sides on the heating pad until they woke up after approximately 1.5 h. The animals were killed at scheduled time points by exposure to gaseous CO_2 and tissues were processed as described below.

Thin Layer Chromatography

Two hours after nasal administration of dopamine (batch 2), the animals were killed (n = 2) as described above. The olfactory bulb and olfactory mucosa were dissected and immediately homogenised in ice-cold tubes. The homogenisation medium (100 μl) consisted of dopamine hydrochloride (0.5 mg/ml), 3, 4-dihydroxyphenylacetic acid (DOPAC) (0.5 mg/ml) and sodium metabisulfite (10 mg/ml) in 0.1 M perchloric acid. The homogenates were centrifuged (10 000 g, 4°C) for 10 min and 20 μl of each supernatant was applied in a 2 cm line on the thin layer chromatography (TLC) plate (Silica gel 60 F_{254} on plastic sheet, Merck). The plate was air dried 90 min before development in n-butanol: acetic acid: water (4:1:1) for 3 h (25). After air drying overnight, the plates were illuminated with UV light, and the bands were located. The plates were cut into strips corresponding to the various bands and placed in scintillation vials with 1 ml sodium metabisulfite (10 mg/ml) in perchloric acid (0.1 M) overnight. A liquid scintillation cocktail (Hionic-Fluor™) was added and the tubes were placed in the dark at room temperature overnight before counting in a liquid scintillator analyser (Tri-Carb 1900CA, Packard Instruments Company, USA).

Brain Tissue Sampling

Dopamine (batch 1) was administered nasally to 30 mice, divided into 5 groups, who were killed 0.5, 1, 2, 4 or 8 h post-dose, as described above. Three animals received intravenous dopamine (batch 2) and these were killed 30 min after the injection. After decapitation the skull was cut open and the olfactory bulbs, lateral olfactory tract, anterior and posterior portions of cortex and the remaining brain were carefully excised. The oesophagus and trachea were also dissected. All samples were weighed and dissolved in 1 ml tissue solubiliser (Soluene®-350) and 10 ml of the scintillation cocktail (Hionic-Fluor™) was added to each sample. The radioactivity was measured for 10 min/sample in the liquid scintillator analyser.

Tape Section Autoradiography

Two mice were killed 1, 2, 4, or 24 h after nasal administration of labelled dopamine (batch 1 or 2). The autoradiography experiments were performed as originally described by Ullberg (26). In short, the heads or whole bodies were embedded in aqueous carboxymethyl cellulose and frozen in a cyclohexane dry-ice bath. Series of transversal (head only) or sagittal (whole body) sections (20 μm; Jung Cryomacrocut, Leica) were collected on tape at various levels and processed for autoradiography using Hyperfilm-[^{3}H] (CEA for Amersham, Sweden). The exposure of the film was performed at −20°C.

Statistics

Results are presented as mean values ± standard deviations (S.D.). When comparison involved more than two mean values, a one-way analysis of variance (ANOVA) was used followed by the Fisher PLSD tests for comparison between individual means. The paired Student's t-test (two tailed) was used to test the significance between two means. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Thin Layer Chromatography

The profiles of the radioactivity in each segment of the TLC plate after application of the extract from the homogenates of the right olfactory bulb or mucosa are shown in Fig. 1. About 75% of the radioactivity in the olfactory bulb coeluted with dopamine (Fig. 1 a). In the olfactory mucosa, conversely, only about 10% coeluted with dopamine (Fig. 1 b).

Brain Tissue Sampling

The radioactivity of the brain tissue samples is illustrated in Fig. 2. The radioactive content of the right olfactory bulbs was significantly higher than that of the left bulbs up to 4 h after administration (Fig 2 a). However, at the last sampling point (8 h), the radioactivity concentration in the right bulbs had decreased to almost the same level as in the left bulbs, i.e., no statistically significant difference between the bulbs was detected. The highest level of radioactivity (967 ± 672 dpm/mg) in the right bulbs appeared 4 h after the unilateral nasal administration of [^{3}H]-dopamine. The average value of the radioactivity in the brain (including the olfactory bulbs) at that time was 0.12% of the given dose of [^{3}H]-dopamine. Low levels of radioactivity were detected in the other regions of the brain (Fig. 2 b). The radioactivity in the lateral olfactory tract 4 h after administration was significantly higher than at other time points and than in the other brain tissue samples within the same group (Fig. 2 b).

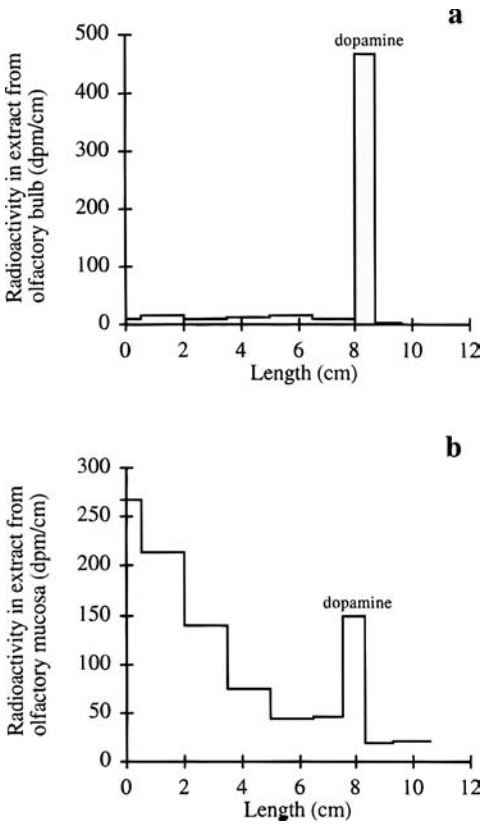

Fig. 1. The profile of radioactivity (dpm/cm) in different regions of the TLC plates 2 h after unilateral nasal (right side) administration of [^{3}H]-dopamine (5 μCi; 0.08 μg) to a mouse. The concentration of radioactivity in the extracts of homogenate from (a) the right olfactory bulb and (b) the olfactory mucosa from the right nasal cavity are demonstrated.

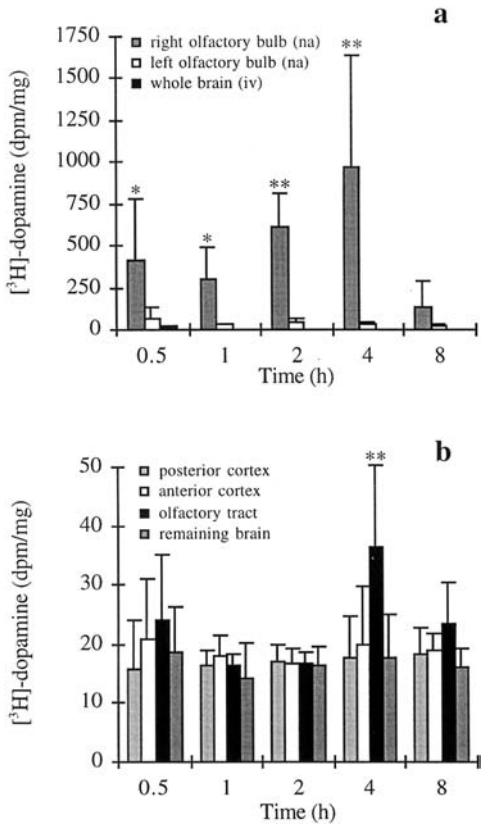

Fig. 2. The amount of radioactivity in selected brain tissue samples after unilateral nasal administration (right side) of [^{3}H]-dopamine (5 μCi; 0.08 μg) to mice (n = 5–7). (a) Mean radioactivity in the right and left olfactory bulbs after nasal administration (n = 5–7; * $p < 0.05$, ** $p < 0.01$ by Student's paired t-test for right vs left olfactory bulbs) and in whole brain tissue samples (including bulbs) 30 min after intravenous administration (n = 3) of [^{3}H]-dopamine (5 μCi; 0.08 μg). (b) Mean radioactivity in the olfactory tract, posterior and anterior portions of cortex and the remaining parts of the brain after nasal administration (** $p < 0.01$ by the Fisher PLSD for the olfactory tract at 4 h vs other time points and other brain tissue samples. Data are expressed as means ± S.D. Note that different scales are used on the y-axes.

In three mice which received intravenous [^{3}H]-dopamine, the mean radioactivity in the brain tissue samples (including the olfactory bulbs) at 30 min post-dose was 22 ± 5 dpm/mg (Fig. 2 a).

The right to left ratio of the radioactivity content of the olfactory bulbs reached 6 as early as 30 min after administration. The ratio had increased to 27 after 4 h; i.e. the amount of radioactivity in the right bulb (the side of administration) was 27 times higher than in the left bulb.

Tape Section Autoradiography

Autoradiograms of mice killed 1, 2 and 4 h after nasal administration of [^{3}H]-dopamine (Fig. 3; 2 h) showed that there was a high level of radioactivity in the olfactory mucosa of the right nasal cavity (site of administration). Low or negligible levels of radioactivity occurred in the mucosa of the left nasal

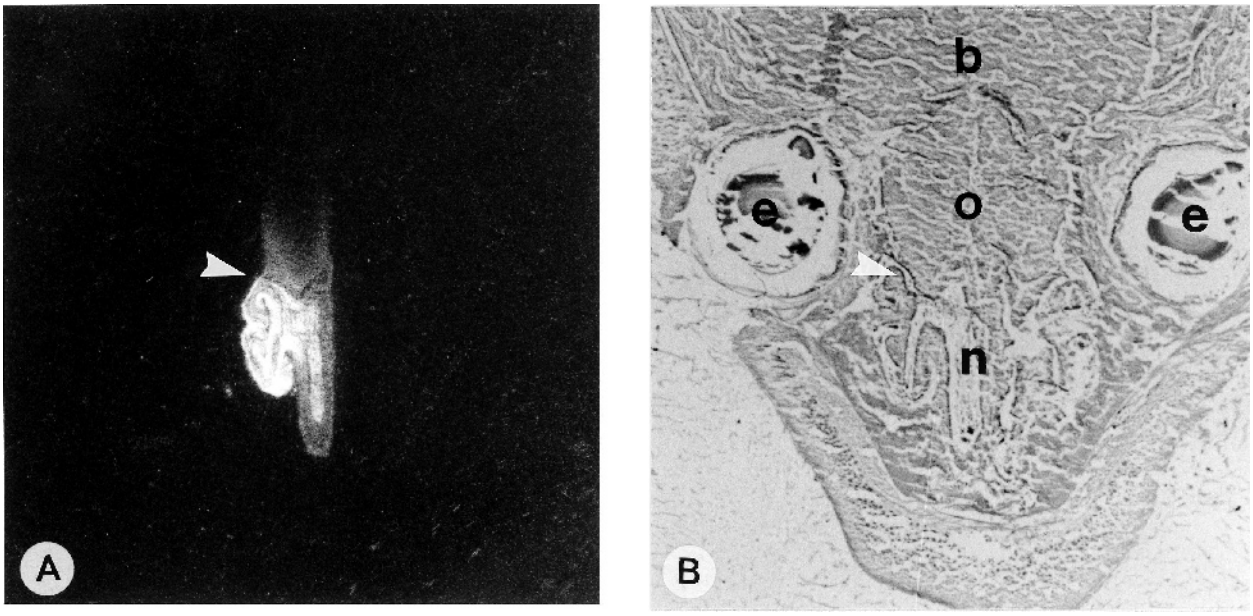

Fig. 3. (A) Autoradiogram of a mouse skull (horizontal section) 2 h after unilateral nasal administration (right side) of [^{3}H]-dopamine (5 μCi; 0.08 μg). (B) Corresponding hematoxylin-eosin stained tissue section at same order of magnification. There is a high level of radioactivity in the right olfactory mucosa, the right axonal nerve layer and the glomerular layer of the olfactory bulb. Radioactivity is present only at low levels in the other brain regions. The arrowheads in both figures point at the border between the olfactory mucosa in the nasal cavity and the olfactory bulb. The different regions in B are nasal cavity (n), olfactory bulbs (o), brain (b), and eye (e).

cavity. One to 4 h after administration, a distinct localisation of radioactivity was observed in the outer layer of the right olfactory bulb. Selective uptake of radioactivity was seen in the right axonal nerve layer and the glomerular layer of the right olfactory bulb. There was a decreasing level of radioactivity towards the centre of olfactory bulb. Twenty-four hours after administration, no radioactivity was observed in the right olfactory bulb.

High levels of radioactivity were observed in the mucosa of the nasopharynx and oesophagus 1 to 4 h after the nasal administration of [^{3}H]-dopamine. High levels of radioactivity were also observed in the urine at 1 and 2 h after administration. A low level of radioactivity was noted in the contents of the gastrointestinal tract 4 h after nasal administration of [3H]-dopamine.

DISCUSSION

The results of this study indicate that dopamine is transferred along the olfactory pathway into the olfactory bulb in the brain following nasal administration in mice. Following intravenous administration, the uptake of [^{3}H]-dopamine in the brain was low (Fig. 2) due to the limited ability of dopamine to pass the BBB. These results are in agreement with a study by Anand Kumar *et al.* (27) on the nasal administration of [^{3}H]-dopamine in monkeys. Radioactivity was detected in the CSF 15 min after nasal but not after intravenous administration of [^{3}H]-dopamine; however, the source of the radioactivity was not further examined in that study (27). Data from our study of the metabolic fate of [^{3}H]-dopamine using TLC indicated that although extensive metabolism of dopamine takes place in the olfactory mucosa, most of the radioactivity transferred into the olfactory bulb was unchanged [^{3}H]-dopamine.

It is important to take samples or measurements at several time points when investigating whether a substance has been transferred into the CNS after nasal administration. In this study, 5 brain tissue samples were obtained over the 8 hours following administration and a concentration-time profile was plotted. If samples had been collected less often, e.g., only at 30 min or 1 h after administration, the peak at 4 h would have been overlooked. In the previously mentioned [^{3}H]-dopamine study in monkeys (27), the last CSF sample was collected 1 h after administration and the maximum level of radioactivity in the CSF may have been overlooked. In another study in which zidovudine was administered to rats (28), the concentration of drug in the CSF after nasal administration was no higher after 15 min than after intravenous administration, i.e. direct transport appeared not to have taken place. Samples of CSF taken at several different time points might have led to another conclusion.

Autoradiography showed a distinct localisation of radioactivity in the peripheral layers of the olfactory bulb (Fig. 4) following intranasal administration of [^{3}H]-dopamine. Dopamine did not seem to be transferred further into the brain, since selective uptake of radioactivity was not seen in other regions of the brain. However, the liquid scintillation data showed that the olfactory tract had a significantly higher radioactivity content 4 h after nasal administration than the other selected brain tissue samples (not olfactory bulbs) (Fig. 2 b). In the present study a trace dose of [3H]-dopamine was administered nasally and totally 0.12% was detected in the brain after 4 h. It may be that higher doses of dopamine would be required in order for the drug to be transferred further into the brain. Increased extracellular concentration of dopamine in the ipsilateral neostriatum was observed by de Souza Silva *et al.* (29)

after unilateral nasal administration of 50 mg/kg levodopa methylesther to rats.

The autoradiography study also demonstrated that the administration of [^{3}H]-dopamine into the right nasal cavity was successful, since a high level of radioactivity occurred in the olfactory mucosa at the site of administration and only a negligible amount was transferred to the opposite nasal cavity. However, liquid scintillation data showed that a few mice inhaled or swallowed large amounts of the administered dose. This probably caused the wide standard deviation ranges seen in the level of radioactivity of the brain tissue samples. The mice that had less radioactivity in the right olfactory bulb had higher activity in the oesophagus and trachea (data not shown).

In this study, the peak concentration of dopamine in the right olfactory bulb appeared later than in previous studies of [^{3}H]-dopamine in the CSF (27). In the study by Anand Kumar *et al.*, the level of radioactivity in the CSF reached a plateau 15 min after nasal administration of [^{3}H]-dopamine and the plateau was maintained throughout the experiment for 60 min. The difference in time to peak levels between our results and the previous observations with [^{3}H]-dopamine, may be explainable by the two alternative pathways for transfer of substances from the olfactory mucosa into the CNS (axonal and epithelial) as mentioned in the introduction. The different methods used in the studies should, however, also be taken into consideration. The dopamine may have reached the olfactory bulb by rapidly (30 min in our study) traversing the perineural space that is continuous with the subarachnoid space in the epithelial pathway (30). The appearance of the peak after 4 h may have been the result of additional transport of dopamine within the axons.

Taken together, the results of this study and earlier observations from other research groups suggest that the olfactory pathway may be a potential route of administration for CNS-active drugs. Further experiments with nasally administered dopamine at higher doses are required in order to reveal whether the nose-brain pathway could be an alternative to the current oral levodopa therapy for Parkinson's disease. In addition, studies of the nasal morphology after nasal administration of clinically relevant doses of levodopa and dopamine are required. Levodopa contains a carboxylic moiety and previous studies have shown morphological changes of the olfactory mucosa after nasal administration of benzoic acid (9). Thus dopamine given by the nasal route may prove to be preferable to oral levodopa for clinical use.

In conclusion, this study shows that unchanged dopamine is transferred into the ipsilateral olfactory bulb following unilateral nasal administration of [^{3}H]-dopamine to mice.

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