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SINGLE ADMINISTRATION OF 1-BENZYL-1,2,3,4-TETRAHYDROISOQUINOLINE INCREASES THE EXTRACELLULAR CONCENTRATION OF DOPAMINE IN RAT STRIATUM

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Abstract—We performed a combined neurochemical and behavioral study to determine the effects of 1-benzyl-1,2,3,4-tetrahydroisoquinoline (1-BnTIQ) on the extracellular dopamine concentrations in the striatum. Single dose administration of 1-BnTIQ (20, 40, and 80 mg/kg i.p.) increased striatal dopamine extracellular levels in a dose-dependent manner when an *in vivo* microdialysis technique was used to assess dopamine levels in the striatum of rats. Enhancement of striatal dopamine levels by systemic administration of a single dose of 1-BnTIQ was suppressed by perfusion of tetrodotoxin and a calcium ion-free solution into the striatum. This 1-BnTIQ-induced increase in extracellular dopamine concentration was also inhibited by pre-treatment with a dopamine uptake inhibitor, GBR12909 (1-(2-[bis(4-Fluorophenyl)-4-(3-phenylpropyl)piperazine dihydrochloride). Local application of 1-BnTIQ into the striatum via a dialysis probe failed to enhance the extracellular concentration of dopamine. However, microinjection of 1-BnTIQ into the substantia nigra pars compacta increased the extracellular dopamine levels in the striatum. Locomotor activity was increased by systemic administration of a single dose of 1-BnTIQ in a dose-dependent manner. This 1-BnTIQ-induced locomotor activity was attenuated by pre-treatment with SCH23390 (R(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochlodride) and raclopride, D_1 and D_2 dopaminergic receptor antagonists, respectively. Moreover, 1-BnTIQ induced ipsilateral rotational behavior in 6-hydroxydopamine–lesioned rats. These results suggest that systemic administration of a single dose of 1-BnTIQ increases striatal extracellular dopamine concentration through activation of dopaminergic nigra striatal neurons via the dopamine transporter.

Key words: Parkinson's disease, 1-benzyl-1,2,3,4-tetrahydroisoquinoline, dopamine, striatum, substantia nigra pars compacta, *in vivo* microdialysis.

The discovery of the exogenous Parkinson's disease–inducing compound 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) suggests that endogenous toxins that accumulate in the CNS may cause Parkinson's disease. This has inspired research into the possibility of MPTP-like endogenous agents. Hao et al. (1995a) have reported that a low molecular weight fraction (i.e. less than 10,000 kDa) collected from the cerebrospinal fluid of subjects with parkinsonian features is cytotoxic to mesencephalon cells *in vitro*. Moreover, Hao et al. (1995b) have reported reduced cytotoxicity following the application of L-deprenyl, a therapeutic drug in Parkinson's disease. These results suggest that substances causing Parkinson's disease might exist within the cerebrospinal fluid of subjects with Parkinson's disease. Several 1,2,3,4-tetrahydroisoquinoline (TIQ) derivatives have been identified as possible MPTP-like endogenous or environmental neurotoxins that may cause Parkinson's disease (Abe et al., 2005).

One TIQ derivative, 1-benzyl-1,2,3,4-tetrahydroisoquinoline (1-BnTIQ), which occurs naturally in animal brain tissue, has been observed to induce parkinsonism in monkeys and mice (Kotake et al., 1995, 1996). This compound has been observed to pass through the blood–brain barrier in rats following systemic administration (Song et al., 2006). L-Deprenyl, a drug used in the treatment of Parkinson's disease, has been reported to decrease endogenous 1-BnTIQ content in the mouse brain (Kotake et al., 1998). In addition, 1-BnTIQ has been identified as a potential neurotoxin capable of causing Parkinson's disease in humans, given that it is present in the cerebrospinal fluid of patients with parkinsonian features at approximately three times the concentrations normally observed in healthy individuals (Kotake et al., 1995). Systemic administration of 1-BnTIQ has been reported to decrease dopamine levels and increase levels of homovanillic acid within the rat striatum (Antkiewicz-Michaluk et al., 2001). In fact, 1-BnTIQ has been observed to decrease dopamine content

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Abbreviations: AUC, area under the curve; DAT, dopamine transporter; DOPAC, 3,4-dehydroxyphenylacetic acid; HPLC, high performance liquid chromatography; HVA, 4-hydroxy-3-methoxyphenylacetic acid; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; SNc, substantia nigra pars compacta; TIQ, 1,2,3,4-tetrahydroisoquinoline; TTX, tetrodotoxin; 1-BnTIQ, 1-benzyl-1,2,3,4-tetrahydroisoquinoline; 3-MT, 3-methoxytyramine; 6-OHDA, 6-hydroxydopamine.

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within cultured organotypic slices of mesencephalon and striatum in rats (Kotake et al., 2003). Therefore, it seems likely that 1-BnTIQ is a neurotoxin capable of producing Parkinson's disease in humans. However, we have previously demonstrated that systemic administration of 1-BnTIQ does not induce a loss of tyrosine hydroxylase (the rate-determining enzyme in dopamine synthesis)–positive cells in the substantia nigra, despite its bradykinesia-inducing effect. Interestingly, we have also demonstrated significantly increased striatal dopamine levels as a result of 1-BnTIQ administration in mice (Abe et al., 2001). Thus, it is unclear whether a single systemic administration of 1-BnTIQ increases extracellular dopamine concentration in the striatum of conscious rats.

In the present study, using an *in vivo* microdialysis technique, we investigated the effect of systemically or locally applied single doses of 1-BnTIQ on the extracellular concentration of striatal dopamine.

EXPERIMENTAL PROCEDURE

In vivo microdialysis

Male Wistar rats (Charles River Laboratories, Kanagawa, Japan) weighing 250–320 g were used. All rats were housed individually under automatically controlled environmental conditions, using a 12-h light/dark cycle (light on from 08:00 to 20:00 h) with free access to food and water. All animals were quarantined in centralized animal facilities for at least 7 days upon arrival. Each animal was used only once. Experiments were carried out according to the guidelines for animal care and use published by the National Institutes of Health and the committee of Showa Pharmaceutical University. All efforts were made to minimize the number of animals used and their suffering.

After an acclimation period, the animals were anesthetized with ketamine (100 mg/kg i.p.) and xylazine (10 mg/kg i.p.) and placed in a stereotaxic apparatus. A midline incision on each scalp was made and a burr hole was drilled through the skull. The coordinates of the striatum were determined according to the atlas of Paxinos and Watson (1986) as follows: bregma 0.3 mm, lateral 3.0 mm, and depth −6.0 mm. Each guide cannula was placed firmly using dental acrylic and anchored to the skull using stainless steel screws, after which the animals were observed for 1 week after surgery to confirm that no complications resulted from the procedure.

Microdialysis probes (dialysis membrane length, 3.0 mm; diameter, 0.3 mm; MW cutoff, 20,000 kDa; Eicom Co., Kyoto, Japan) were inserted into the striatum through each guide cannula. Each animal was placed in a Plexiglas box (30 cm×30 cm×38 cm) and connected to a syringe pump and collection vials via polyethylene inflow and outflow tubes (CMA 100; Carnegie Medicine, Stockholm, Sweden). Perfusion solution (125 mM NaCl, 3 mM KCl, 1.3 mM $CaCl_2$, 1 mM $MgCl_2$, and 23 mM $NaHCO_3$) in aqueous potassium phosphate buffer (1 mM, pH 7.4) was perfused into each dialysis probe at a rate of 2 μl/min. The perfusate samples were collected at 15-min intervals. Concentrations of dopamine and its metabolites [3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxytyramine (3-MT), and 4-hydroxy-3-methoxyphenylacetic acid (HVA)], as well as concentrations of serotonin (5-HT), were analyzed within the perfusate. Samples were analyzed by reverse-phase high performance liquid chromatography (HPLC) coupled with electrochemical detection. The HPLC system and conditions were as follows: an Eicom HTEC-500 system was used with an Eicompak SC-5ODS column (4.6 mm i.d.×150 mm) and pre-column using a mobile phase of 83% 0.1 M citric acid/0.1 M sodium acetate buffer, 17% methanol, and 0.023% sodium 1-octanesulfonate containing 5 mg/l disodium-EDTA. A flow rate of 500 μl/min was used with an Eicom WE-3G graphite electrode and Eicom RE-100 Ag–AgCl reference electrode, with an applied voltage of 700–750 mV vs. Ag/AgCl. The column temperature was maintained at 25 °C.

Drug administration during *in vivo* microdialysis

(1) Perfusate was collected continuously over a minimum of 2 h prior to drug administration to obtain a stable baseline. Following this, 1-BnTIQ (20, 40, and 80 mg/kg) was administered as a single i.p. dose. (2) The effect of a sodium channel blocker, tetrodotoxin (TTX, 1 μM), as well as the effect of a perfusate devoid of calcium ions, on 1-BnTIQ-induced dopaminergic changes in the striatum was examined. 1-BnTIQ (40 mg/kg i.p.) was administered 2–3 h after commencement of the TTX or calcium ion-free perfusion. (3) The effect of pre-treatment with a dopamine transporter (DAT) inhibitor, GBR12909 (10 mg/kg i.p.), 30 min prior to 1-BnTIQ (40 and 80 mg/kg i.p.) administration was investigated. (4) 1-BnTIQ was administered locally into the striatum via a dialysis probe at a final concentration of 10, 100, and 1000 μM for 60 min (5) A guide cannula enabled direct injection of 1-BnTIQ into the substantia nigra pars compacta (SNc). A microinjection needle (30 gauge stainless steel tubing) terminating 2 mm below the tip of the guide cannula was inserted into the SNc at the following coordinates: bregma −5.3 mm, lateral 1.9∼2.0 mm, and depth −7.6∼−7.7 mm, according to the atlas of Paxinos and Watson (1986). After 1 week, a 2 μl volume of 1-BnTIQ (50, 100, and 200 μg/site) was administered via the microinjection needle and dialysate samples in the striatum were collected. After the administration of 1-BnTIQ or saline, the injection cannula was carefully withdrawn.

Location of the microdialysis probe and injection site

Following each experiment, the positions of the probe and injection cannula within the striatum or SNc were verified. Correct placement of each microinjection was verified in 1-BnTIQ-injected animals by injecting Methylene Blue into the striatum and SNc. Animals were killed by i.v. injection of pentobarbital–Na and perfusion through the heart of phosphate-buffered saline (PBS) followed by 4% formalin dissolved in isotonic saline. Brains were further fixed in 4% paraformaldehyde overnight. Following this, the brain tissue was frozen in an embedding compound (Sakura Finetechnical, Tokyo, Japan) and stored at −80 °C until the time of use. Several days later, coronal slices of brain tissue were stained with Cresyl Violet and probe placement in the striatum and SNc was confirmed by light microscopy.

Spontaneous locomotor activity

The effect of systemic administration of a single dose of 1-BnTIQ on spontaneous locomotor activity was measured using a MK-Animex Activity meter (Model SE, Muromachi Co., Tokyo, Japan) in animals observed through a Plexiglas box (30 cm×30×38 cm). The injections were made between 10:00 and 13:00 h, and behavioral testing was performed between 10:00 and 16:00 h. Animal movement induced signals due to variations in the inductance capacity of the resonance circuit of the apparatus. The animals were acclimated to observation through a Plexiglas box for at least 60 min, after which 1-BnTIQ was administered intraperitoneally. Their behavior was analyzed over consecutive 10 min periods for 90 min from the time of injection. Some animals in the 1-BnTIQ treatment groups, including those treated with SCH23390 (dissolved in saline, 0.05 mg/kg) or raclopride (dissolved in saline, 0.1 mg/kg), were pre-treated by s.c. injection 20 min prior to systemic administration of a single dose of 1-BnTIQ.