

Transporter-mediated actions of *R*-(–)-1-(benzofuran-2-yl)-2-propylaminopentane

Seiichiro Shimazu^{a,*}, Hiroko Tsunekawa^a, Fumio Yoneda^a, Hiroshi Katsuki^b,
Akinori Akaike^b, Aaron Janowsky^c

^a*Institute of Research and Development, Fujimoto Pharmaceutical Corporation, 1-3-40 Nishiotsuka, Matsubara, Osaka 580-0011, Japan*

^b*Department of Pharmacology, Graduate School of Pharmaceutical Sciences, Kyoto University,
46-29 Yoshida-Shimoadachi-cho, Sakyo, Kyoto 606-8501, Japan*

^c*V.A. Medical Center, and Department of Psychiatry, Physiology and Pharmacology, and Behavioral Neuroscience,
Oregon Health and Science University, Portland, OR 97201, USA*

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Abstract

R-(–)-1-(Benzofuran-2-yl)-2-propylaminopentane [(–)-BPAP] is a catecholaminergic and serotonergic activity enhancer that increases impulse-evoked catecholamine and serotonin release from nerve terminals, and is a candidate for symptomatic treatment of early Parkinson's disease. We now report the catecholamine and serotonin transporter-mediated actions of (–)-BPAP. The effects of (–)-BPAP on inhibition of neurotransmitter uptake and radioligand binding were assessed using human embryonic kidney 293 cells (HEK 293 cells) expressing cDNA for the human dopamine transporter (hDAT), norepinephrine transporter (hNET), and serotonin transporter (hSERT). The IC₅₀ values for the effects of (–)-BPAP on [³H]dopamine, [³H]norepinephrine, and [³H]serotonin uptake were 42 ± 9, 52 ± 19, and 640 ± 120 nM, respectively. The IC₅₀ values for the effects of (–)-BPAP on [¹²⁵I]3β-(4-iodophenyl)tropane-2β-carboxylic acid methyl ester ([¹²⁵I]RTI-55) binding to hDAT, hNET, and hSERT were 16 ± 2, 211 ± 61, and 638 ± 63 nM, respectively. The effects of (–)-BPAP on spontaneous and tyramine-induced norepinephrine and dopamine release from rat brain synaptosomes using a superfusion system were also assessed. Tyramine but not (–)-BPAP potentiated norepinephrine release. Furthermore, (–)-BPAP inhibited tyramine-induced norepinephrine release. Thus, (–)-BPAP may block tyramine-induced adverse effects such as hypertensive crisis. The actions of (–)-BPAP on the spontaneous and tyramine-induced dopamine release resembled its effects on norepinephrine release. We conclude that (–)-BPAP is not only catecholaminergic and serotonergic activity enhancer, but also a norepinephrine and dopamine uptake inhibitor and a weak serotonin uptake inhibitor that does not possess a tyramine-like action on catecholamine release, and is an inhibitor of tyramine-induced release of norepinephrine.

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Keywords: *R*-(–)-1-(Benzofuran-2-yl)-2-propylaminopentane [(–)-BPAP]; Catecholaminergic and serotonergic activity enhancer; Human embryonic kidney 293 cell; Parkinson's disease; Transporter; Tyramine

1. Introduction

β-Phenylethylamine and tryptamine enhance the electrically stimulated catecholamine and serotonin release from isolated rat brain stem (Knoll et al., 1999), suggesting a novel regulatory mechanism underlying impulse-propagation-mediated release of catecholamines and serotonin, and

suggesting that these amines are endogenous substances for this regulation. The regulatory mechanism is designated as a “catecholaminergic and serotonergic enhancer (CAE/SAE)” mechanism (Knoll et al., 1999).

R-(–)-1-(Benzofuran-2-yl)-2-propylaminopentane [(–)-BPAP] was synthesized following the study of the structure–activity relationship between the structures of β-phenylethylamine, tryptamine, and analogs, and their actions at enhancing electrically stimulated dopamine, norepinephrine and serotonin release from the brain stem (Knoll et al., 1999; Yoneda et al., 2001). (–)-BPAP is a potent synthetic CAE/SAE (Knoll et al., 1999). CAE/SAEs

* Corresponding author. Tel.: +81-72-332-5151; fax: +81-72-332-8482.

E-mail address: soyaku@fujimoto-pharm.co.jp (S. Shimazu).

may serve as therapeutic agents for neurologic diseases (e.g., Alzheimer's disease, Parkinson's disease, and age-related depression) in which reduction of catecholamines and serotonin is observed. Based on the dopamine deficiency theory (Barbeau, 1962), activation of the dopaminergic system by (–)-BPAP may be useful for the improvement of motor deficits in patients with early Parkinson's disease. (–)-BPAP is also expected to improve the non-motor functional deficits such as depression that is observed in Parkinson's disease, is unresponsive to anti-Parkinsonian drugs (Fibiger, 1984; Mayberg and Solomon, 1995), and may be related to noradrenergic and serotonergic deficits in addition to a dopaminergic deficit (Agid et al., 1984; Gerlach et al., 1994). In fact, (–)-BPAP reverses the hypolocomotion in reserpine-pretreated rats (Shimazu et al., 2001), and also is able to enhance the action of 3,4-dihydroxyphenylalanine (L-DOPA) in reserpine-pretreated rats and reduce the required dose of L-DOPA, i.e., L-DOPA-sparing effects (Shimazu et al., 2003). Furthermore, (–)-BPAP reverses reduced active avoidance performance in the tetrabenazine-induced depression model in rats, and this effect may be due to its antidepressant action (Knoll et al., 1999, 2002).

However, the details of (–)-BPAP's neurochemical actions are not yet clear. In the present study, the affinities and actions of (–)-BPAP on dopamine, norepinephrine and serotonin uptake sites were studied to further clarify the pharmacological profile of (–)-BPAP related to the regulation of catecholaminergic and serotonergic tone in the CNS. In addition, the effects of (–)-BPAP on the spontaneous and tyramine-induced release of norepinephrine and dopamine from rat brain synaptosomes were examined using a superfusion system, and results suggest that (–)-BPAP does not have tyramine-like releasing effects on catecholamines and has inhibitory actions on tyramine-induced release of catecholamines.

2. Materials and methods

2.1. Filtration assay for measuring inhibition of [¹²⁵I]RTI-55 binding to hDAT, hSERT or hNET in HEK293 cells

The inhibitory actions of test compounds at neurotransmitter transporters was assessed using [¹²⁵I]3β-(4-iodophenyl)tropane-2β-carboxylic acid methyl ester ([¹²⁵I]RTI-55) binding according to the methods of Eshleman et al. (1999). The human embryonic kidney 293 cells transfected with human dopamine transporter cDNA (HEK-hDAT cells) and human serotonin transporter cDNA (HEK-hSERT cells) were transfected and selected as previously described (Eshleman et al., 1994, 1995, 1999). The hSERT cDNA and the HEK 293 cells transfected with human norepinephrine transporter cDNA (HEK-hNET cells) were generously supplied by Dr. Randy Blakely (Vanderbilt University, Nashville, TN, USA; Ramamoorthy et al., 1993; Galli et al., 1995).

2.1.1. Cell preparation

The HEK-hDAT cells, HEK-hNET cells or HEK-hSERT cells were grown to 80% confluence on 150 mm diameter tissue culture dishes. To prepare cell membranes, medium was poured off the plate, the plate was washed with 10 ml of calcium- and magnesium-free phosphate-buffered saline (PBS), and lysis buffer [10 ml; 2 mM 4-(2-hydroxyethyl)-1-piperazineethansulfonic acid (HEPES) with 1 mM EDTA] was added. After 10 min, cells were scraped from plates, poured into centrifuge tubes, and centrifuged at 30,000 × g for 20 min. The supernatant fluid was removed and the pellet is resuspended in 12–32 ml of 0.32 M sucrose using a Polytron. The resuspension volume depended on the density of binding sites within a cell line and was chosen to reflect binding of 10% or less of the total radioactivity.

2.1.2. RTI-55 binding assay conditions

Each assay tube contained 50 μl membrane preparation (about 10–25 μg of protein), 25 μl (–)-BPAP solution, compound used to define non-specific binding, or buffer (Krebs-HEPES, pH 7.4; 122 mM NaCl, 2.5 mM CaCl₂, 1.2 mM MgSO₄, 10 μM pargyline, 100 μM tropolone, 0.2% glucose and 0.02% ascorbic acid buffered with 25 mM HEPES), 25 μl [¹²⁵I]RTI-55 (40–80 pM, final concentration) and additional buffer sufficient to bring the final volume up to 250 μl. Membranes were preincubated with (–)-BPAP for 10 min prior to the addition of the [¹²⁵I]RTI-55, and assay tubes were incubated at 25 °C for 90 min. Binding was terminated by filtration over Whatman GF/C filters using a Tomtec 96-well cell harvester. Filters were washed for 6 s with ice-cold saline. Scintillation cocktail was added to each spot and radioactivity remaining on the filter was determined using a Wallac micro- or beta-plate reader. Specific binding was defined as the difference in binding observed in the presence and absence of 5 μM mazindol (HEK-hDAT and HEK-hNET) or 5 μM imipramine (HEK-hSERT). At least three independent competition experiments were conducted each with duplicate determinations.

The concentration range of (–)-BPAP in binding assays was 2.16 nM–10 μM (for hDAT) or 21.6 nM–10 μM (for hSERT and hNET). (–)-BPAP was first weighed and dissolved in dimethylsulfoxide (DMSO) to make a 10 mM stock solution, and subsequent dilutions were made with assay buffer supplemented with DMSO to maintain a final concentration 0.1% DMSO.

2.2. Filtration assay for inhibition of [³H]neurotransmitter uptake in HEK293 cells expressing recombinant biogenic amine transporters

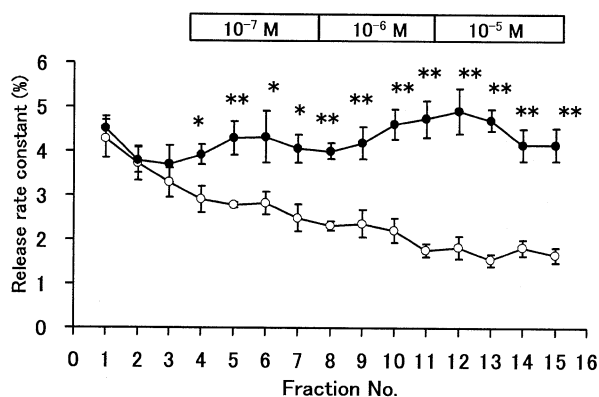
The inhibitory effects of test compounds on [³H]neurotransmitter uptake into HEK 293 cells expressing biogenic amine transporters were assessed according to the methods of Eshleman et al. (1999).

2.2.1. Cell preparation

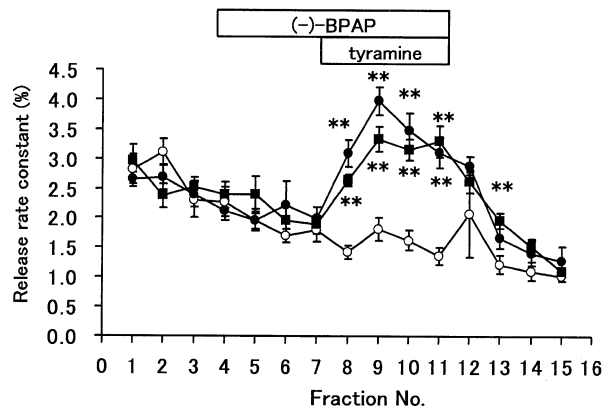
Cells were grown to confluence as described above. The medium was removed, and cells were washed twice with PBS at room temperature. Following the addition of

3 ml Krebs–HEPES buffer, the plates were warmed in a 25 °C water bath for 5 min. The cells were gently scraped and triturated, and cells from multiple plates were combined.

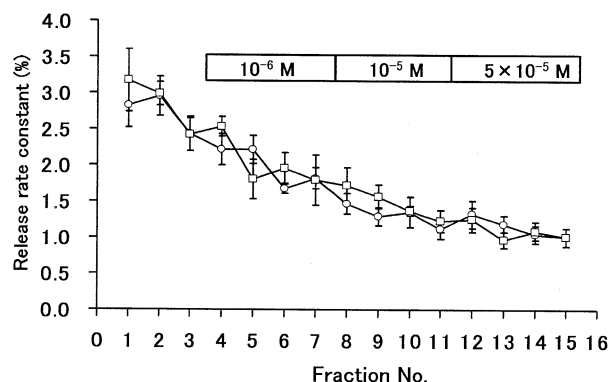
A-1: Tyramine



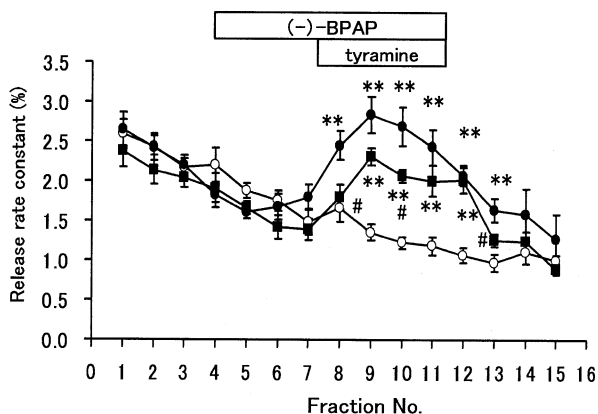
B-1: 10^{-7} M (–)–BPAP + 10^{-6} M Tyramine



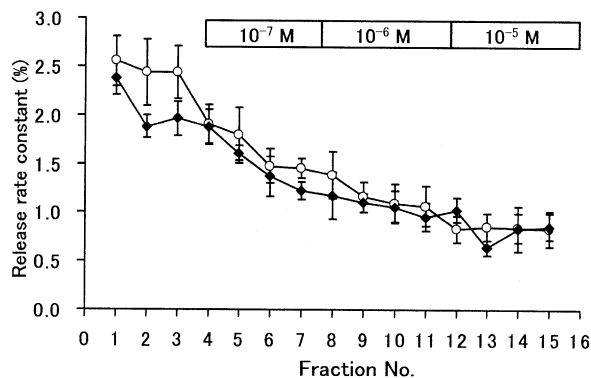
A-2: (–)–BPAP



B-2: 10^{-6} M (–)–BPAP + 10^{-6} M Tyramine



A-3: Nomifensine



B-3: 10^{-5} M (–)–BPAP + 10^{-6} M Tyramine

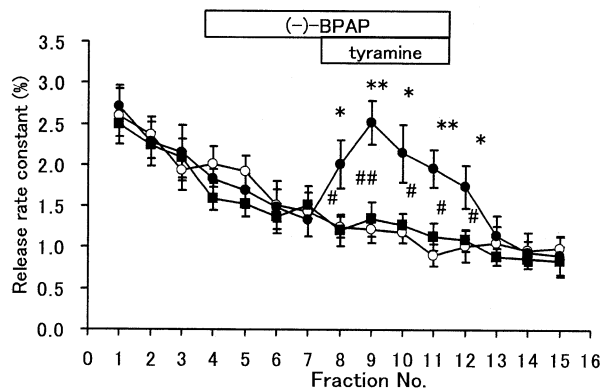


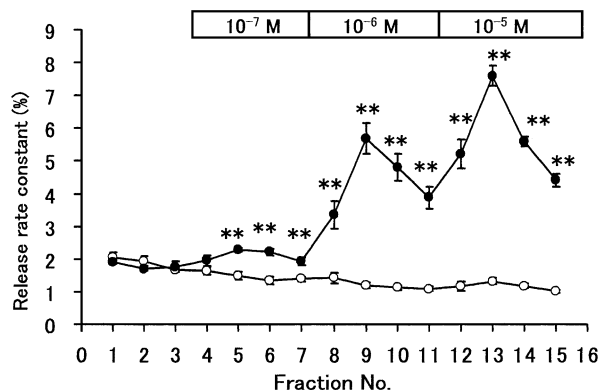
Fig. 1. The effects of (–)–BPAP on tyramine-evoked release of [3 H] from synaptosomes incubated with [3 H]norepinephrine. Results were shown as a release rate constant derived from the recovered radioactivity in each fraction and expressed as a percentage of the total radioactivity taken up into the synaptosomes. Values are mean \pm S.E.M. of six independent experiments. (A) The effects of tyramine alone (A-1), (–)–BPAP alone (A-2), and a catecholamine uptake inhibitor (nomifensine) alone (A-3) on [3 H] release from synaptosomes incubated with [3 H]norepinephrine. Symbols; superfusion buffer (○), tyramine (●), (–)–BPAP (□), and nomifensine (◆). (B) The effects of (–)–BPAP on tyramine-evoked release of [3 H] from synaptosomes. Symbols; superfusion buffer (○), tyramine (●), and 10^{-6} M tyramine + (–)–BPAP (■). Concentrations of (–)–BPAP were 10^{-7} (B-1), 10^{-6} (B-2) and 10^{-5} M (B-3). * P < 0.05 and ** P < 0.01, vs. the control group with superfusion buffer. # P < 0.05 and ## P < 0.01, vs. the control group with tyramine.

2.2.2. Uptake inhibition assay conditions

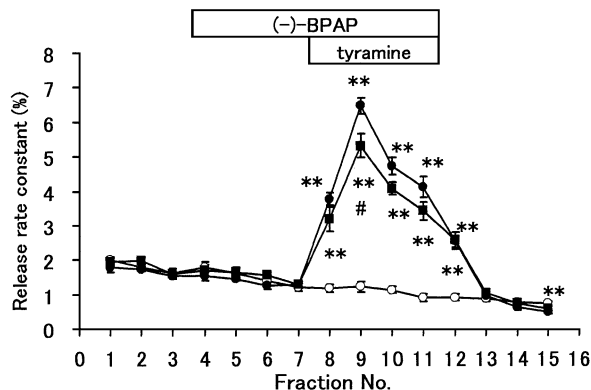
Assays were conducted in 96-well 1-ml vials. Krebs-HEPES (350 μ l), 50 μ l (-)-BPAP, compounds used to define non-specific uptake, or buffer were added to vials and placed in a 25 °C water bath. Specific uptake was defined as the difference in uptake observed in the pres-

ence and absence of 5 μ M mazindol (HEK-hDAT and HEK-hNET) or 5 μ M imipramine (HEK-hSERT). Cells (50 μ l) were added and preincubated with compounds for 10 min and the assay was initiated by the addition of [3 H]dopamine, [3 H]serotonin or [3 H]norepinephrine (50 μ l, 20 nM final concentration). Filtration through Whatman

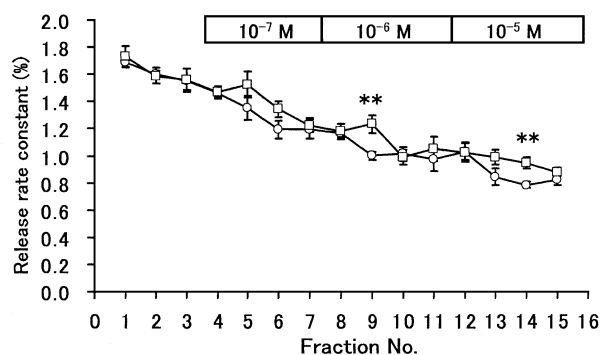
A-1: Tyramine



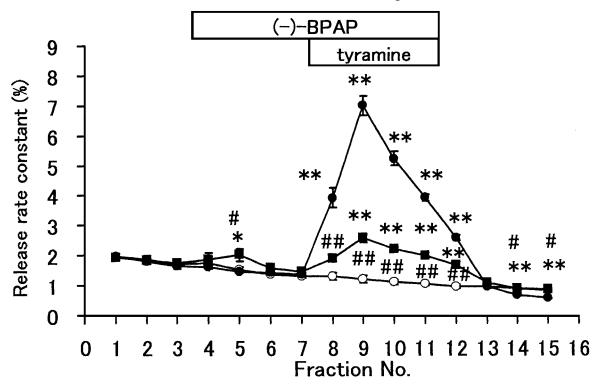
B-1: 10⁻⁷ M (-)-BPAP + 10⁻⁶ M Tyramine



A-2: (-)-BPAP



B-2: 10⁻⁶ M (-)-BPAP + 10⁻⁶ M Tyramine



B-3: 10⁻⁵ M (-)-BPAP + 10⁻⁶ M Tyramine

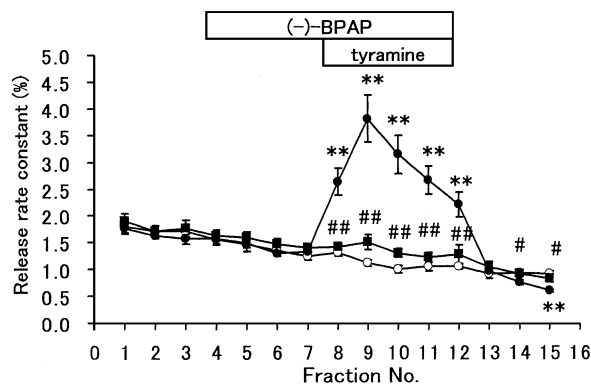


Fig. 2. The effects of (-)-BPAP on tyramine-evoked release of [3 H] from synaptosomes incubated with [3 H]dopamine. Results are expressed as a release rate constant, as explained in the legend to Fig. 1. Values are mean \pm S.E.M. of six independent experiments. (A) The effects of tyramine alone (A-1) and (-)-BPAP alone (A-2) on [3 H] release from synaptosomes incubated with [3 H]dopamine. Symbols; superfusion buffer (○), tyramine (●), and (-)-BPAP (□). (B) The effects of (-)-BPAP on tyramine-evoked release of [3 H] from synaptosomes. Symbols; superfusion buffer (○), tyramine (●), and 10⁻⁶ M tyramine+(-)-BPAP (■). Concentrations of (-)-BPAP were 10⁻⁷ (B-1), 10⁻⁶ (B-2) and 10⁻⁵ M (B-3). * P <0.05 and ** P <0.01, vs. the control group with superfusion buffer. # P <0.05 and ## P <0.01, vs. the control group with tyramine.

GF/C filters presoaked in 0.05% polyethylenimine was used to terminate uptake after 10 min. At least three independent determinations, each conducted in triplicate were conducted.

The concentrations range of (–)-BPAP that was tested in uptake inhibition assays was 3.16 nM–10 μM (for hDAT), or 31.6 nM–10 μM (for hSERT and hNET). (–)-BPAP solutions for each assay were prepared as described above.

2.3. Superfusion study

2.3.1. Synaptosome preparation

Striatum (for dopamine release) or forebrain (for norepinephrine release) tissue isolated from rats (Male Wistar strain, 8–13 weeks old, Nihon SLC, Shizuoka, Japan) was gently homogenized in 10 volumes (tissue wet weight) of 0.32 M sucrose (pH 7.4) using a glass-Teflon homogenizer. The homogenate was centrifuged at $900 \times g$ for 10 min, and the supernatant was recentrifuged at $11,500 \times g$ for 20 min. The final pellet containing synaptosomes was resuspended in ice-cold superfusion buffer [NaCl 115 mM, K_2HPO_4 1.5 mM, $MgCl_2 \cdot 7H_2O$ 1.5 mM, D-glucose 10 mM, $NaHCO_3$ 25 mM, $CaCl_2 \cdot 2H_2O$ 2 mM, L-(+)-ascorbate 0.1 mM, EGTA 0.5 mM, pargyline hydrochloride 10 μM].

2.3.2. Labeling of synaptosomes with [3H]catecholamines

The methods used for labeling the synaptosomes with [3H]dopamine or [3H]norepinephrine were identical to those of Bowyer et al. (1984). The protein concentration of the synaptosomal suspension was determined by the method of Lowry et al. (1951), and was adjusted to a concentration of 0.6 mg/ml. An aliquot (5 ml) of the total synaptosomal suspension was equilibrated at 35 °C during continuous aeration with 95% O_2 –5% CO_2 for 5 min. [3H]Dopamine (34 Ci/mmol) or [3H]norepinephrine (12 Ci/mmol) was then added to a final concentration in the buffer of 25 nM, and the synaptosomes were incubated for an additional 5 min.

2.3.3. Superfusion studies

Methods used for the superfusion studies were identical to those of Bowyer et al. (1984), with some modifications. The apparatus (Superfusion system, SF-06, Neuroscience, Tokyo, Japan) used for superfusion and collection of superfusate consisted of a fraction collector, a variable-speed multi-channel peristaltic pump, six polypropylene holders as superfusion chambers, and 35 °C water bath. All tubing used in the apparatus, other than silicon pump tubing, was made of Teflon. The suspended, labeled synaptosomes were pipetted into six superfusion chambers (0.25 ml/chamber of synaptosomal suspension) to measure release. The synaptosomes were embedded in 6.35-mm Whatman GF/C filters in superfusion chambers, and the superfusion chambers, filled with superfusion buffer equilibrated with 95% O_2 –5% CO_2 , and maintained at 35 °C before delivery to the superfusion chambers.

The synaptosomes were superfused at a rate of 0.5 ml/min with buffer for 30 min before determining the spontaneous release of tritium. This period was necessary to obtain a stable rate of spontaneous release. Subsequently, the superfusates from each chamber were collected continuously at 2-min intervals. The first two or three collected fractions were used to determine spontaneous release. Test compounds were then added to the superfusion buffer. The collected superfusates were added to 3.5 ml scintillation cocktail for liquid scintillation counting (LS-3500, Aroka, Tokyo, Japan). The total radioactivity of [3H]dopamine or [3H]norepinephrine taken up into the synaptosomes in 1 ml suspension was determined by solubilizing the synaptosomes, which were trapped on Whatman GF/C filters presoaked in 0.05% BSA and washed with ice-cold saline, in 7 ml of scintillation cocktail.

Results are shown as a release rate constant derived from the recovered radioactivity in each fraction and expressed as a percentage of the total radioactivity taken up into the synaptosomes.

2.4. Chemicals

(–)-BPAP hydrochloride was synthesized by Fujimoto Pharmaceutical (Osaka, Japan). Nomifensine maleate was purchased from Research Biochemicals International (Natick, MA, USA), pargyline hydrochloride from Sigma (St. Louis, MO, USA), and tyramine hydrochloride from Nacalai Tesque (Kyoto, Japan). Radiolabeled compounds were purchased from Du Pont-New England Nuclear (Boston, MA, USA). Scintillation cocktail (Picoflow™ 40) was purchased from Packard Instrument (Meriden, CT, USA). All other reagents and chemicals were of the highest purity that is commercially available.

2.5. Analyses

The IC_{50} values were calculated using GraphPAD Prism software from curves made up of six drugs concentration. For binding assays, IC_{50} values were converted to K_i values using the Cheng–Prusoff equation (Cheng and Prusoff, 1973).

Statistical analyses were performed using Student's *t*-test when comparing between two groups (Figs. 1-A and 2-A: SAS preclinical package version 5.0, SAS Institute, Cary, NC, USA). When comparing more than three groups, Dunnett's test was used (Figs. 1-B and 2-B). $P < 0.05$ was considered significant.

3. Results

3.1. The affinity and effects of (–)-BPAP at biogenic amine uptake site

In HEK-hDAT cells, the affinity of (–)-BPAP for the [^{125}I]RTI-55 binding site was higher than that of cocaine.

The K_i value for the displacement of [125 I]RTI-55 binding by (–)-BPAP was 16.3 nM, and the K_i value for cocaine was 469 nM. In the uptake assays, (–)-BPAP was more potent at blocking the uptake of [3 H]dopamine, with an IC_{50} value of 42.3 nM, as compared to the potency of cocaine (IC_{50} = 524 nM, see Table 1).

In HEK-hNET cells, the affinity of (–)-BPAP for the binding site was higher than the affinity of cocaine for the same site. The K_i value for the displacement of [125 I]RTI-55 binding from hNET cells by (–)-BPAP was 211 nM, and the K_i value for cocaine was 2420 nM. In the uptake assays, (–)-BPAP was more potent at blocking the uptake of [3 H]norepinephrine, with an IC_{50} value of 52 nM, as compared to the potency of cocaine (IC_{50} = 370 nM, see Table 1).

In HEK-hSERT cells, the affinity of (–)-BPAP for the binding site was lower than the affinity of cocaine. The K_i value for displacement of [125 I]RTI-55 binding by (–)-BPAP was 638 nM, and the K_i value for cocaine was 353 nM. In the uptake assays (–)-BPAP was less potent at blocking the uptake of [3 H]serotonin, with an IC_{50} value of 640 nM, as compared to the potency of cocaine (IC_{50} = 419 nM, see Table 1).

3.2. Effects of (–)-BPAP on spontaneous and tyramine-induced norepinephrine release from synaptosomes

Tyramine significantly potentiated spontaneous norepinephrine release from synaptosomes at concentrations of 10^{-5} , 10^{-6} and 10^{-7} M ($P < 0.01$ and 0.05 , Fig. 1-A-1), while (–)-BPAP had no effect (Fig. 1-A-2). A catecholamine uptake inhibitor, nomifensine, also failed to influence norepinephrine release (Fig. 1-A-3).

Next, the effects of (–)-BPAP on 10^{-6} M tyramine-induced norepinephrine release were examined. (–)-BPAP significantly inhibited tyramine-induced norepinephrine re-

lease at concentrations of 10^{-7} , 10^{-6} and 10^{-5} M, with complete inhibition at 10^{-5} M (Fig. 1-B).

3.3. Effects of (–)-BPAP on spontaneous and tyramine-induced dopamine release from synaptosomes

Tyramine significantly potentiated dopamine release from synaptosomes at the concentrations of 10^{-5} and 10^{-6} M ($P < 0.01$ and 0.05 , Fig. 2-A-1), while (–)-BPAP exerted little effect (Fig. 2-A-2).

(–)-BPAP significantly inhibited tyramine-induced dopamine release at concentrations of 10^{-6} and 10^{-5} M, with complete inhibition at 10^{-5} M (Fig. 2-B).

4. Discussion

Competition binding assays and uptake assays, using hDAT-, hNET- and hSERT-transfected HEK293 cells indicate that (–)-BPAP has an affinity for hDAT, hNET and hSERT, and acts as a dopamine and norepinephrine uptake inhibitor and a weak serotonin uptake inhibitor (Table 1). The effects of (–)-BPAP on dopamine and norepinephrine uptake were more potent than those of cocaine, while its potency at inhibiting serotonin uptake was weaker than that of cocaine (Table 1).

CAE/SAEs enhance release of catecholamines and serotonin from the isolated rat brain stem, but not without electrical stimulation (Knoll et al., 1996, 1999). Thus, CAE/SAEs behave as a kind of enhancer in amine release depending on the firing activity of neurons, and can be distinguished from the catecholamine releasing effects of tyramine-like compounds and effects of monoamine uptake inhibitors (Knoll et al., 1996, 1999; Yoneda et al., 2001). However, as a catecholamine uptake inhibitor is known to potentiate motor activity (Hemby et al., 1997; Stanford et al., 2002), the uptake inhibitory effect of (–)-BPAP may be involved in motor stimulant effects in addition to its CAE/SAE effect.

On the other hand, tyramine significantly potentiated the spontaneous release of norepinephrine (Fig. 1-A-1). In our superfusion system, the flow rate of 0.5 ml/min was used to reduce the influences of an uptake inhibitory effect, according to the method of Bowyer et al. (1984, 1987). Actually, a catecholamine uptake inhibitor, nomifensine, did not influence the spontaneous norepinephrine release (Fig. 1-A-3). Therefore, tyramine-stimulated norepinephrine release is mediated through reverse transport actions of norepinephrine by the transporter (Pifl et al., 1999; Sitte et al., 1998; Wayment et al., 1998). (–)-BPAP did not show any effects on spontaneous norepinephrine release (Fig. 1-A-2). Thus, (–)-BPAP did not exert tyramine-like norepinephrine releasing action. The heterocyclic compounds with a propylaminopentane-structure are unlikely to have this tyramine-like norepinephrine releasing action, even if the ring-structure is substituted (Yoneda et al., 2001).

Table 1

Inhibition of [125 I]RTI-55 binding and [3 H]biogenic amine uptake by (–)-BPAP and cocaine in HEK-hDAT, HEK-hNET and HEK-hSERT cells

	(–)-BPAP	Cocaine
<i>HEK-hDAT cells</i>		
[125 I]RTI-55 binding: K_i (nM)	16.3 ± 2.4	469 ± 37
Hill coefficient	0.85 ± 0.10	1.19 ± 0.12
[3 H]Dopamine uptake: IC_{50} (nM)	42.3 ± 9.3	524 ± 55
<i>HEK-hNET cells</i>		
[125 I]RTI-55 binding: K_i (nM)	211 ± 61	2420 ± 120
Hill coefficient	0.93 ± 0.16	0.87 ± 0.04
[3 H]Norepinephrine uptake: IC_{50} (nM)	52 ± 19	370 ± 56
<i>HEK-hSERT cells</i>		
[125 I]RTI-55 binding: K_i (nM)	638 ± 63	353 ± 30
Hill coefficient	1.11 ± 0.07	1.07 ± 0.03
[3 H]Serotonin uptake: IC_{50} (nM)	640 ± 120	419 ± 37

Values are mean ± S.E.M. from at least three independent experiments, each conducted with duplicate (for binding assays) or triplicate (for uptake assays) determinations.

Furthermore, we demonstrated that (–)-BPAP inhibited tyramine-induced norepinephrine release (Fig. 1-B). Several laboratories have previously demonstrated that (–)-deprenyl reduces tyramine-induced contractions in various isolated contractile systems (Knoll, 1978; Finberg et al., 1981) and tyramine-induced norepinephrine release from brain slice preparation (Glover et al., 1983). Glover et al. (1983) used brain slice preparations as a model system that reflects the peripheral system. The reported effects imply a blockade of (–)-deprenyl against the ‘cheese effect’ induced by tyramine, and the tyramine uptake inhibitory effect of (–)-deprenyl is proposed as a possible mechanism (Knoll, 1978). Conversely, clorgyline and (+)-deprenyl enhance tyramine-induced norepinephrine release from brain slices, and the actions are considered to be adverse effects of these compounds related to hypertensive crisis (Glover et al., 1983). However, (–)-BPAP, as demonstrated here, has a similar action to (–)-deprenyl concerning potential to participate in hypertensive crisis. As (–)-BPAP has an affinity to catecholamine transporters which are also carriers for tyramine, the inhibitory action of (–)-BPAP on tyramine-induced norepinephrine release may be due to its tyramine uptake inhibitory action. In the present study, (–)-BPAP was also demonstrated not to influence spontaneous dopamine release, and to reduce tyramine-induced dopamine release, as was the case for norepinephrine. Thus, (–)-BPAP inhibits the effect of tyramine.

In conclusion, (–)-BPAP is a dopamine and norepinephrine uptake inhibitor and a weak serotonin uptake inhibitor, and both the catecholamine uptake inhibition and CAE/SAE effect may contribute to motor stimulant and L-DOPA-sparing effects of (–)-BPAP. (–)-BPAP does not exert the tyramine-like releasing action of catecholamines, and also acts as an inhibitor of tyramine-induced catecholamine release.

Acknowledgements

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