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Barakol, a natural anxiolytic, inhibits striatal dopamine release but not uptake in vitro

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Abstract

The present study investigated the effects of barakol on the in vitro release of endogenous and radiolabelled dopamine from rat striatal slices in comparison with the dopamine receptor agonists, quinelorane dihydrochloride (1 μM) and pergolide methanesulfonate (100 μM), and the dopamine receptor antagonist, *S*(−)-eticlopride hydrochloride (10 μM) using a semi-superfusion method and high-performance liquid chromatography with electrochemical detector measurement of endogenous dopamine. Barakol (1, 10 and 100 μM) reduced K^+-stimulated endogenous dopamine release as did the dopamine D_2 receptor agonists but had no effect on [^{3}H]dopamine release. The inhibition of barakol (10 μM) on K^+-stimulated endogenous dopamine release was antagonised by a dopamine D_2 receptor antagonist, eticlopride. Barakol (0.1 nM–10 μM) had no effect on [^{3}H]dopamine uptake except at the highest concentration (100 μM) when inhibition was observed. The results indicate that barakol might act as a dopamine agonist to inhibit endogenous dopamine release without a change in dopamine uptake.

Keywords: Cassia siamea; Barakol; Striatum, rat; Dopamine release; Dopamine uptake, in vitro

1. Introduction

Barakol, a 3α,4-dihydro-3α,8-dihydroxy-2,5-dimethyl-1,4-dioxaphenalene ring structure (Thongsaard et al., 1996a), is a biologically active constituent of extracts of *Cassia siamea*, a plant widely cultivated in Southeast Asia and used locally to treat insomnia and various other medical conditions such as diabetes, hypertension, asthma, constipation and diuresis (Satyavati et al., 1979; Mokasmit, 1981; Kinghorn and Balandrin, 1992). In animal studies, barakol has been shown to lower blood pressure (Suwan et al., 1992), increase tension in smooth muscle (Arunlakshana, 1949) and produce a non-sedative anxiolytic effect (Thongsaard et al., 1995b, 1996a). It was first extracted in 1969 (Hassanali-Walji et al., 1969) and the structure was characterised in 1970 (Bycroft et al., 1970). It has been shown to have dopamine agonist-like effects in behavioural tests, to increase nociceptive threshold and to have possible serotonergic antagonist properties by decreasing head shake behaviour induced by 5-hydroxytryptophan (Tongroach et al., 1992). In our previous study, barakol was shown to exhibit an anxiolytic profile in the rat elevated plus-maze similar to diazepam, but it also had effects on exploratory and locomotor behaviour which were dissimilar to those produced by diazepam (Thongsaard et al., 1995b, 1996a).

The present work compares the effects of barakol with two dopamine agonists and an antagonist on the in vitro release of endogenous dopamine and [^{3}H]dopamine from striatal slices. The study also investigated the effects of barakol on dopamine uptake. Some of the results in this current study have been presented in a preliminary form to

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the British Pharmacological Society (Thongsaard et al., 1995a).

2. Materials and methods

2.1. Animals

Male hooded Lister rats weighing 200–250 g (Animal Unit, Medical School, Nottingham University, Nottingham, UK) were used. They were housed in groups of 5 in a temperature-controlled room with a 12 h light/12 h dark cycle (07.00–19.00 light on), with access to food and water ad libitum.

2.2. In vitro endogenous dopamine release

The method used to study in vitro dopamine release was adapted from the column method previously described to measure synaptosomal release (Ebstein et al., 1982). Small plastic columns (Econo-columns) obtained from Bio-Rad Laboratories were fixed onto a stand (12 cm height). Sephadex G-10 resin (Sigma, Poole, UK) was mixed thoroughly with double-distilled deionized water further purified in a ELGA purifier (1 g Sephadex G-10 in 7 ml water) and aliquots of the mixture (1 ml) were used to fill each column. The mixture was pre-washed with 10 ml of normal Krebs-Henseleit buffer (KHB) by gravity flow at room temperature and left to settle in the columns for at least 30 min before the experiments. KHB was freshly prepared on the day of the experiment and contained 123 mM NaCl, 1.3 mM KCl, 1.2 mM $MgSO_4 \cdot 7H_2O$, 1.185 mM KH_2PO_4, 25 mM $NaHCO_3$, 10 mM D-glucose, and 2 mM $CaCl_2 \cdot 2H_2O$. Nominally Ca^{2+}-free KHB was identical to normal KHB with the $CaCl_2.2H_2O$ omitted. For the K^+-stimulation experiments, KHB was prepared containing the same concentration of $MgSO_4 \cdot 7H_2O$, D-glucose and $CaCl_2 \cdot 2H_2O$ as normal KHB, but the concentrations of $NaHCO_3$, NaCl, KCl, and KH_2PO_4 were adjusted to give final concentrations of 15, 20, 30, and 50 mM [K^+] without changing the osmolarity of the buffer solution. KHB was prepared using double-distilled deionized water further purified in an ELGA purifier. The solutions were gassed with 95% O_2/5% CO_2 and kept in a water bath (37°C) throughout the experiments.

2.2.1. Tissue preparation

Rats were killed by stunning and decapitation. The brains were removed quickly and the striatum dissected out. The striatum was then sliced using a manually-operated brain tissue slicer which produces slices (approx. 0.35 mm thick) suitable for the measurement of endogenous dopamine release (Bennett et al., 1983). The slices were placed in a flask containing 10 ml nominally Ca^{2+}-free KHB and pre-washed 3 times with 10 ml Ca^{2+}-free KHB. The flask was filled with 10 ml Ca^{2+}-free KHB, gassed, capped and placed in a shaking water bath for 60 min at 37°C. The Ca^{2+}-free KHB was changed 3 times (every 20 min) during the pre-incubation. At the end of the pre-incubation the incubation medium was discarded and the suspension of packed striatal slices was dispensed using a Micro-Doser pipette (Oxford Laboratory International Corporation, Ireland) onto the top of the Sephadex G-10 columns (50 μl packed slices/column). Initially, normal KHB (3 ml) was washed through the striatal slices on the columns by gravity flow leaving the washed striatal slices on the top of the column. The columns were capped and placed in a water bath (37°C) ready for the experimental incubation.

2.2.2. Calcium-dependent [K^+]-induced endogenous dopamine release

The effect of elevated K^+ concentrations (15, 20, 30 and 50 mM) on dopamine release was studied using 4 individual columns for each concentration. The striatal slices were incubated for 10 min after addition of 300 μl normal KHB (basal release). The columns were removed from the water bath, 1 ml of normal KHB was added to the columns and the total 1300 μl eluate collected into Eppendorf tubes containing 130 μl of an antioxidant solution (0.3% sodium metabisulfite and 0.1 M perchloric acid). After collection of the basal samples the columns were replaced in the waterbath and a further 300 μl KHB added but this time containing varying [K^+] (15, 20, 30 or 50 mM). The columns were incubated for 10 min again and the eluate collected as described above using 1 ml of normal KHB to wash out the columns. The samples were snap-frozen in liquid nitrogen and kept at −70°C until analysed. A concentration of K^+ (20 mM) that produced a consistent but submaximal increase in the release of dopamine in the K^+-stimulation experiment was used in this study. The experiment was performed in a similar manner to the first experiment except that the Ca^{2+}-free samples were incubated in nominally Ca^{2+}-free normal KHB for basal release and nominally Ca^{2+}-free, high [K^+]KHB for K^+-stimulated release.

2.2.3. Effects of dopamine D_2 receptor agonists, antagonist and barakol on endogenous dopamine release

This experiment was divided into 2 parts. The first experiments were performed using the same method previously described (i.e., using Sephadex G-10 columns and striatal slices produced using the manually-operated brain slicer). All drugs (dopamine receptor agonists, antagonist and barakol) were added only to the high [K^+]KHB (20 mM) to determine the effect of the drugs on K^+-stimulated dopamine release; prior to this the tissue was incubated in normal KHB to allow measurement of basal release free of drugs. This method was used to measure the effects of the selective dopamine D_2 receptor agonist, quinelorane dihydrochloride (1 μM), and the selective dopamine D_2 receptor antagonist, *S*(−)-eticlopride hydrochloride (1 μM), on

dopamine release in the presence of high [K^+]KHB compared with basal release in the presence and absence of the drugs. The effects of barakol (0.1 nM, 1 nM, 10 nM, 0.1 μM, 1 μM, 10 μM and 100 μM) were studied under the same conditions.

In the second experiments, striatal slices were prepared using a McIlwan tissue chopper (0.35 × 0.35 mm) and Sephadex G-10 resin was omitted from the columns as it was found that improved release was obtained without Sephadex G-10 (Thongsaard et al., 1996b). The non-selective dopamine D_1/D_2 receptor agonist, pergolide methanesulfonate (100 μM), and barakol (1 pM, 0.1 nM and 10 μM) were added to both the normal KHB and high [K^+]KHB (20 mM), so that the drugs were present throughout the study. The basal and K^+-stimulated release were carried out in the same manner as in the first experiments.

2.2.4. Sample analysis

Samples were removed from storage at −70°C, thawed, and filtered through 0.45 μm filters (Gelman Sciences) prior to injection onto a high performance liquid chromatography column with an electrochemical detector (HPLC-ECD) (ANTEC). The mobile phase prepared using HPLC grade water (Fisher Scientific International Company, UK) contained 156 mM $NaH_2PO_4 \cdot 2H_2O$, 1 mM diaminoethanetetra-acetic acid (EDTA), 1 mM sodium octyl sulfate and 10% methanol with the pH adjusted to 3 with phosphoric acid. Before use it was filtered through a 0.45 μm filter (Millipore) and degassed by sonication for 30 min. The mobile phase was pumped through the column (Hypersil ODS 3 μ, Phenomenex) at a flow rate of 0.3 ml/min using an HPLC Technology pump (RR1066L). Samples were filtered through 0.45 μm filters (Gelman Sciences) and injected onto the column via a Rheodyne injection unit (7125) fitted with a 20 μl injection loop. The dual glassy carbon working electrode was held at a potential of +0.65 V against a Ag/AgCl reference electrode and currents produced by the oxidation of dopamine and metabolites measured. The dopamine level in each sample was compared to standard dopamine (2 pmol/20 μl, Sigma) and the data presented as dopamine release (pmol/μl). The limit of detection for dopamine was approximately 10 fmol.

2.3. In vitro [^{3}H]dopamine release

2.3.1. Preparation of striatal slices

Rat were killed by stunning and decapitation. The brains were removed quickly, the striatum dissected out and sliced using a McIlwan tissue chopper as described above. The slices were pre-washed 3 times with 10 ml nominally Ca^{2+}-free KHB similar to that used in the previous experiment. The slices were pre-incubated in a shaking waterbath for 60 min at 37°C with the incubation medium changed 3 times (every 20 min) during the pre-incubation. The striatal slices were further pre-incubated with 2.5 ml normal KHB containing 10 μCi [^{3}H]dopamine for another 30 min. The incubation medium was then discarded. The slices were washed 3 times with 10 ml normal KHB and the suspension of packed slices was dispensed using a Micro-Doser pipette (Oxford Laboratories International Corporation, Ireland) onto the columns (25 μl packed slices/column) which were divided into 2 sets, with and without Sephadex G-10 resin. An initial 3 ml normal KHB was washed through the slices on the columns by gravity flow and the eluate discarded. All KHB used in this experiment contained 0.05 mM pargyline, a monoamine oxidase inhibitor. The striatal slices were then incubated in 1 ml normal KHB for 5 min and the eluate collected into scintillation vials. The process was repeated 4 times. The third and fourth collections were taken as basal release. Dopamine release was stimulated by incubating slices in 1 ml of 30 mM [K^+]KHB (fifth collection) followed by further incubation with normal KHB (sixth collection). The fifth and sixth collections were considered as K^+-stimulated release. Finally, 2 ml of 1 M HCl was added to permeabilize the tissue and the radioactivity counted in both perfusates and the tissue by liquid scintillation spectroscopy using 8 ml scintillation cocktail (Packard Instrument, Groningen, The Netherlands). Data are presented as % of total [^{3}H]dopamine release.

2.3.2. Effects of dopamine D_2 receptor agonist, antagonist and barakol on [^{3}H]dopamine release

The effects of the dopamine D_1/D_2 receptor agonist, antagonist and barakol on [^{3}H]dopamine release in the presence of high [K^+] (10 mM) were studied using the method already described. The slices were incubated in medium containing either single drug alone or a combination of drugs as stated in Section 3. The antagonist was always added during the second incubation period and before either agonist or barakol which were added during the third incubation period. The agonist, pergolide methanesulfonate (100 μM), antagonist, *S*(−)-eticlopride hydrochloride (10 μM), and barakol (10 and 100 μM) were prepared in appropriate KHB prior to use.

2.4. In vitro [^{3}H]dopamine uptake

Striatal slices were prepared as for [^{3}H]dopamine release. After 60 min pre-incubation, the packed slices were carefully placed into flat-bottomed plastic tubes (25 μl packed slices/tube) which had been pre-warmed in a water bath (37°C) for 15 min. The slices were then incubated in 450 μl of KHB containing either one of the drugs or no drug (see below) for 10 min ($n = 6$); 1.25 μCi [^{3}H]dopamine was added to each tube and incubation continued for a further 30 min. Uptake was stopped by vacuum filtration onto Whatman GFB filters (2.5 mm)

which were then washed with 10 ml of cold KHB (2°C). The filters containing the slices were placed into scintillation vials and 2 ml of 1 M HCl added to permeabilize the tissue. The radioactivity was counted by liquid scintillation spectroscopy after adding scintillation cocktail (Packard Instrument). Non-specific dopamine uptake was measured in the same way as the controls except that the incubation was performed on ice at 0°C. Dopamine uptake was measured in the presence of various concentrations of barakol (0.1 nM, 1 nM, 10 nM, 0.1 μM, 1 μM, 10 μM and 100 μM), cocaine hydrochloride (1 and 50 μM), or GBR-12909 (0.1 and 1 μM), the dopamine uptake inhibitor, prepared in normal KHB.

2.5. Plant material and drugs

Barakol was extracted from fresh young leaves of *Cassia siamea* which were cut into small pieces and boiled twice with 2% acetic acid for 1 h (Chaichantipyuth, 1979). All fractions of water extract were filtered, combined and alkalinised with concentrated ammonia solution. The mixture was further extracted with chloroform and the chloroform extract was washed with water. The solution was concentrated and shaken with 5% aqueous acetic acid until the extract became colourless. The acidified chloroform extract was neutralised carefully with concentrated ammonia solution and cooled. The crude barakol was crystallised as greenish yellow needles. The purity of the barakol was established by nuclear magnetic resonance (Thongsaard et al., 1996a) and chromatographic techniques using HPLC-ECD and no contaminants were identified. Pargyline (Sigma), quinelorane dihydrochloride (LY-163,502; (5α *R-trans*)-5,5α,6,7,8,9α,10-octahydro-6-propyl-pyridol-[2,3-*g*]quinazolin-2-amine dihydrochloride, Research Biochemicals, Natick, MA, USA), pergolide methanesulfonate (8-[(methylthio)methyl]-6-propylergoline methanesulfonate, Research Biochemicals), *S*(−)-eticlopride hydrochloride (*S*(−)-3-chloro-5-ethyl-*N*-[(1-ethyl-2-pyrolidinyl]-6-hydroxy-2-methoxy-benzamide hydrochloride, Research Biochemical), cocaine hydrochloride (Sigma) and GBR-12909 (1-[2-[*bis*(4-fluorophenyl)methoxy]ethyl]-4-(3-phenylpropyl)piperazine dihydrochloride, Research Biochemicals) were dissolved first in water to a concentration of 10 mM before further dilution to tested concentrations in appropriate KHB prior to use. [7,8-^{3}H]Dopamine was obtained from Amersham Life Sciences (Amersham, UK).

2.6. Statistics

All data are presented as mean ± standard error of mean (S.E.M). The data were analysed using a non-parametric test (Mann-Whitney U-test) with $P < 0.05$ considered significant. The standard errors are included so as to give an indication of the range of the data but are independent of the statistical analysis.

3. Results

3.1. In vitro endogenous dopamine release

3.1.1. Effects of elevated [K^+] Krebs and Ca^{2+} on striatal dopamine release

Elevated K^+ (15, 20, 30 and 50 mM) induced a significant increase in endogenous dopamine release when compared to basal release (Fig. 1A). KHB containing 20 mM [K^+] was used in the later studies as this concentration produced a consistent but submaximal increase in dopamine release. When Ca^{2+} was left out of the KHB, 20 mM [K^+] failed to increase endogenous dopamine release when compared to basal release. There was no significant difference in basal dopamine release in the presence and nominal absence of Ca^{2+} (Fig. 1B).

3.1.2. Effects of dopamine D_2 receptor agonists, antagonist and barakol on endogenous dopamine release

Barakol reduced the dopamine release from striatal slices stimulated with [K^+] (20 mM) at all concentrations tested (0.1 nM, 1 nM, 10 nM, 0.1 μM, 1 μM, 10 μM and 100 μM) (Table 1). The dopamine D_2 receptor agonist, quinelorane dihydrochloride (1 μM), significantly ($P < 0.05$) reduced the K^+-stimulated endogenous dopamine release by 35% when compared with K^+-stimulated release in control (Fig. 2) while *S*(−)-eticlopride hydrochloride (1 μM), a dopamine D_2 receptor antagonist, significantly potentiated the K^+-stimulated dopamine release by 29% ($P < 0.05$). When *S*(−)-eticlopride hydrochloride was added together with quinelorane dihydrochloride (1 μM) or barakol (10 μM), the reduction in dopamine release seen with barakol or quinelorane dihydrochloride alone was not observed (Fig. 2). There were no significant differences in the basal release of dopamine with any of the drugs.

In the second set of experiments performed using the McIlwan chopped slices, no Sephadex G-10 and drugs present in both normal and high [K^+]KHB, there was a similar pattern of results to the first set of experiments except that the amounts of dopamine released were greater in the absence of Sephadex G-10 (compare values in Figs. 2 and 3). Pergolide methanesulfonate (100 μM), a non-selective dopamine receptor agonist, and barakol (10 μM) significantly reduced K^+-stimulated endogenous dopamine release by 40% ($P < 0.05$) and 57% ($P < 0.01$), respectively (Fig. 3), while the drugs had no significant effect on basal dopamine release.

3.2. Effect of dopamine D_2 receptor agonist, antagonist and barakol on [^{3}H]dopamine release

[K^+] 10 mM was used in the study of [^{3}H]dopamine release as this concentration produced a submaximal in-

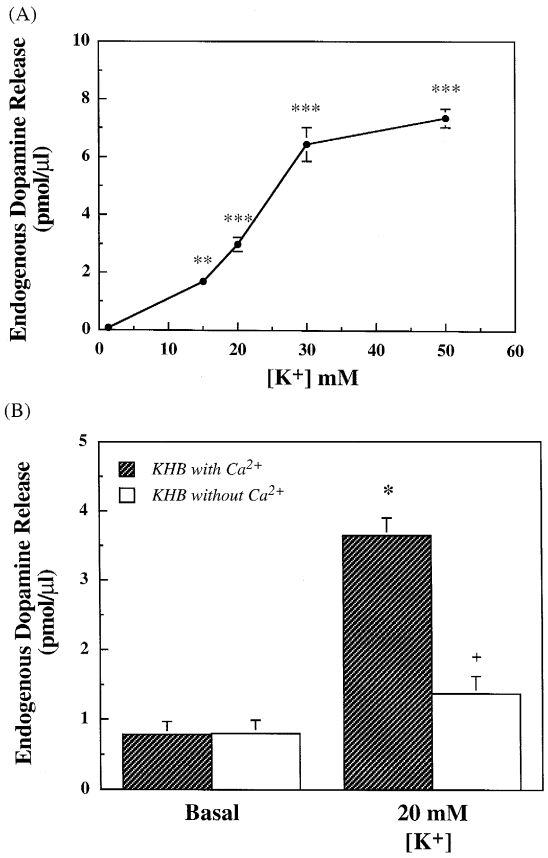

Fig. 1. K^+-stimulated endogenous dopamine release in response to increasing $[K^+]$ (A) and in the presence and nominal absence of Ca^{2+} in KHB (B) using Sephadex G-10 columns. Data are presented as mean ± S.E.M. * $P < 0.05$ and *** $P < 0.01$ compared to basal release using normal KHB, $^{+}$ $P < 0.05$ compared to K^+-stimulation using normal KHB without Ca^{2+}. Closed columns represent the release using KHB containing Ca^{2+} and the open columns represent the release using KHB without Ca^{2+}. $n = 4$ in all groups.

crease in radiolabelled dopamine release (Thongsaard et al., 1996b). The non-selective dopamine D_1/D_2 receptor agonist, pergolide methanesulfonate (100 μM) signifi-

Table 1
Effect of various concentrations of barakol on in vitro endogenous dopamine release from rat striatal slices

Groups	No.	Dopamine release (pmol/μl)	
		Basal	$[K^+]$ 20 mM
Control	40	1.37 ± 0.16	3.00 ± 0.26
Barakol			
0.1 nM	18	1.62 ± 0.29	2.29 ± 0.32
1.0 nM	10	1.09 ± 0.25	2.04 ± 0.39
10 nM	10	1.12 ± 0.16	2.01 ± 0.27
0.1 μM	9	1.22 ± 0.17	2.00 ± 0.31
1.0 μM	15	1.30 ± 0.31	1.60 ± 0.33[a]
10 μM	9	1.05 ± 0.25	1.30 ± 0.25[a]
100 μM	11	1.88 ± 0.33	0.62 ± 0.21[b]

Data are presented as mean ± S.E.M. [a] $P < 0.01$ and [b] $P < 0.001$ compared to control.

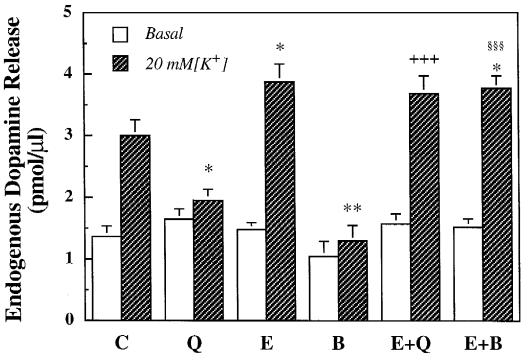

Fig. 2. Effects of dopamine D_2 receptor agonist, quinelorane dihydrochloride (1 μM), dopamine D_2 receptor antagonist, *S*(−)-eticlopride hydrochloride (1 μM) and barakol (10 μM) and combinations of *S*(−)-eticlopride hydrochloride and quinelorane dihydrochloride or barakol on in vitro endogenous dopamine release using columns containing Sephadex G-10. Data are presented as mean ± S.E.M. * $P < 0.05$, ** $P < 0.01$ compared to K^+-stimulated dopamine release in control. $^{+++}$ $P < 0.001$ compared to K^+-stimulated release in quinelorane dihydrochloride alone. §§§ $P < 0.001$ compared to K^+-stimulated release in barakol alone. C, control ($n = 40$); Q, quinelorane dihydrochloride ($n = 12$);, E, *S*(−)-eticlopride hydrochloride ($n = 18$); B, barakol ($n = 9$); E + Q, *S*(−)-eticlopride hydrochloride plus quinelorane dihydrochloride ($n = 11$); E + B, *S*(−)-eticlopride hydrochloride plus barakol ($n = 18$).

cantly reduced the effect of 10 mM $[K^+]$ stimulation on $[^3H]$dopamine release ($P < 0.01$) while *S*(−)-eticlopride hydrochloride (10 μM), the dopamine D_2 receptor antagonist, significantly potentiated K^+-stimulated dopamine release ($P < 0.05$) (Fig. 4). The presence of *S*(−)-eticlopride hydrochloride (10 μM) in normal KHB caused a significant increase in basal $[^3H]$dopamine release when compared to control (control = 5.42 ± 0.41, *S*(−)-eticlopride hydrochloride = 17.88 ± 1.49 pmol/μl) ($P < 0.01$). Unlike its effect on endogenous dopamine release, barakol (10 and 100 μM) failed to inhibit $[^3H]$dopamine release induced by 10 mM $[K^+]$. There were no significant differ-

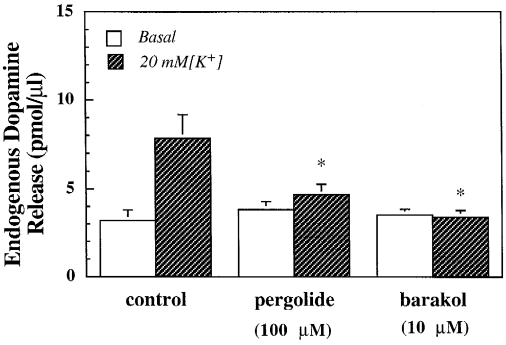

Fig. 3. Effects of dopamine D_2 receptor agonist, pergolide methanesulfonate (100 μM), and barakol (10 μM) on K^+-stimulated endogenous dopamine release using a McIlwan tissue chopper in an absence of Sephadex G-10 resin. Drugs were presence throughout the experiment. Data are presented as mean ± S.E.M. * $P < 0.05$ compared to K^+-stimulated release in control. $n = 6$ in all groups.

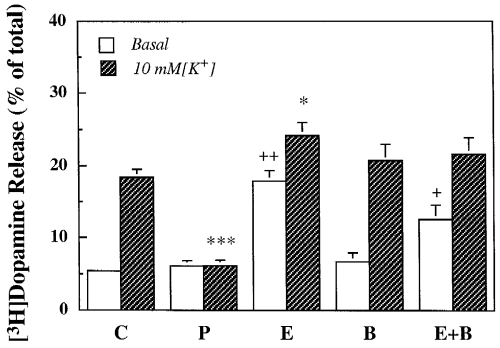

Fig. 4. Effects of a non-selective dopamine D_1/D_2 receptor agonist, pergolide methanesulfonate (100 μM), dopamine D_2 receptor antagonist, *S*(−)-eticlopride hydrochloride (10 μM) and barakol (10 μM) and the combinations of antagonist and barakol on K^+-stimulated [^{3}H]dopamine release using columns with no Sephadex G-10. Data are presented as mean ± S.E.M. $^{+}$ $P < 0.05$ and $^{++}$ $P < 0.01$ compared to basal release in control and * $P < 0.05$ and *** $P < 0.001$ compared to K^+-stimulated release in control. C, control; P, pergolide methanesulfonate; E, *S*(-)-eticlopride hydrochloride; B, barakol; E + B, *S*(−)-eticlopride hydrochloride plus barakol. $n = 6$ in all groups.

ences in K^+-stimulated [^{3}H]dopamine release when *S*(−)-eticlopride hydrochloride was added together with 10 and 100 μM barakol compared to control.

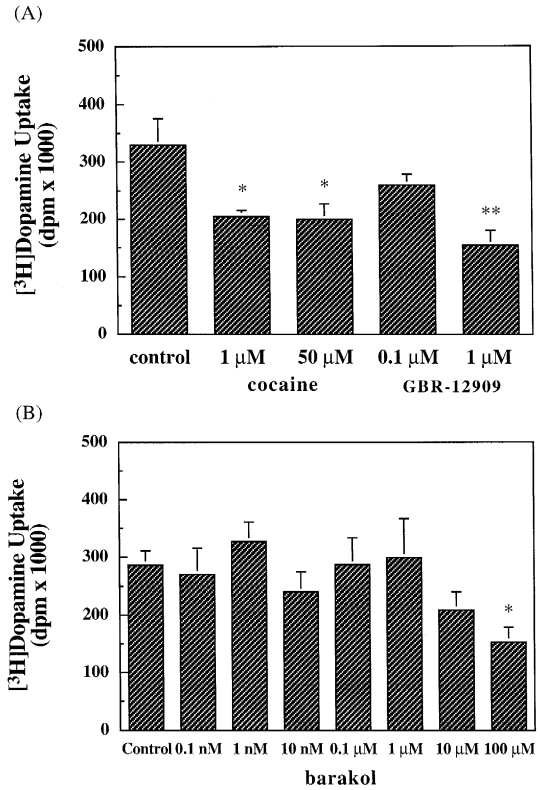

Fig. 5. Effects of dopamine uptake blockers, cocaine hydrochloride (1 and 50 μM) and GBR-12909 (0.1 and 1 μM) in (A) and various concentrations of barakol (0.1 nM, 1 nM, 10 nM, 0.1 μM, 1 μM, 10 μM and 100 μM) in (B) on [^{3}H]dopamine uptake in striatal slices. Data are presented as mean ± S.E.M. * $P < 0.05$ and ** $P < 0.01$ compared to control. $n = 6$ in all groups.

3.3. Effects of dopamine uptake blockers and barakol on uptake of [^{3}H]dopamine

Cocaine hydrochloride (1 and 50 μM) and GBR-12909 (1 μM) significantly decreased the uptake of [^{3}H]dopamine into rat striatal tissue slices incubated at 37°C (Fig. 5A). The uptake were reduced by 38 and 40% by 1 and 50 μM cocaine hydrochloride ($P < 0.05$) and 53% by 1 μM GBR-12909 ($P < 0.01$), respectively, compared with the control value. The presence of barakol (0.1 nM–100 μM) in the incubation medium had no effect on the dopamine uptake except at the highest concentration (100 μM) when a significant reduction was observed (Fig. 5B).

4. Discussion

The results demonstrated that barakol extracted from *Cassia siamea* is a potent inhibitor of K^+ stimulated endogenous dopamine release from striatal slices but that this inhibition is not observed when the release of radiolabelled [^{3}H]dopamine is measured under identical conditions to those used for assessment of endogenous release. The inhibition of endogenous release was prevented by the dopamine D_2 receptor antagonist, *S*(−)-eticlopride hydrochloride, indicating involvement of the dopamine D_2 presynaptic autoreceptor (Hall et al., 1985; Cooper et al., 1991; Tyler and Galloway, 1992, Rowlett et al., 1995) in the barakol-induced inhibition. Previous behavioural studies (Tongroach et al., 1992) have shown that barakol (75–150 mg/kg) increased rotation in rats with unilateral 6-hydroxydopamine lesions in a dose-dependent pattern—an effect also seen with the selective dopamine D_2 receptor agonist quinelorane dihydrochloride (0.01–1.0 mg/kg) using the same experimental protocol (Foreman et al., 1989). These findings further support the view that barakol may have dopamine D_2 receptor agonist properties. These data might suggest that quinelorane dihydrochloride is a more potent dopamine D_2 receptor agonist than barakol, though the present in vitro data indicate that they are about equipotent. The difference between the in vivo and in vitro data remain to be explained. In the present experiments, however, the potency of quinelorane dihydrochloride observed, using endogenous release measurement, was consistently lower than the value reported by Foreman et al. (1989) using [^{3}H]dopamine release, suggesting a difference in response when measuring endogenous rather than radiolabelled dopamine release.

It is possible that barakol could act by a mechanism independent of the dopamine D_2 receptor or another dopamine receptor, to reduce dopamine release. Such mechanisms could include effects on either K^+ or Ca^{2+} channels. In contrast to cocaine hydrochloride (Galloway, 1988; Kiyatkin, 1995) and GBR-12909 (Heikkila and Manzino, 1984; Baldo and Kelley, 1991; Nakachi et al., 1995) barakol had no effect on striatal dopamine uptake

except at a very high concentration (100 μM). Barakol inhibited endogenous dopamine release at concentration as low as 1 μM, indicating a clear dissociation between the effects of barakol on release and reuptake. In a further study, an inhibitory effect of barakol on striatal dopamine release was also found in the freely moving rat using the microdialysis technique (Thongsaard et al., 1996c) when barakol (10 and 100 mg/kg, i.p.) reduced striatal dopamine release by a maximum of 30 and 60%, respectively. An unexpected finding in the present release experiments was the failure of barakol to inhibit release of [^{3}H]dopamine. Other studies have shown that measurement of endogenous and [^{3}H]dopamine release may not produce identical results (Herdon et al., 1985), suggesting that radiolabelled amine release may not always provide an accurate marker of endogenous release. A major difference between the two experimental approaches could be that endogenous release essentially reflects release from a newly synthesised store of dopamine which cannot be monitored by [^{3}H]dopamine release. If this is correct, it might imply that barakol has selective effects on the release of newly synthesised dopamine possibly through a dopamine-D_2-mediated mechanism.

Barakol (10–50 mg/kg) produces anti-anxiety effects (Thongsaard et al., 1995b, 1996a) using the conventional elevated plus-maze test (Handley and Mithani, 1984; Pellow et al., 1985; File, 1987). The role of dopaminergic function in this test has not been widely studied and it remains to be determined whether the inhibition of dopamine release by barakol is of importance in the behavioural effects of this compound in this model of anxiety. However, in the previous studies barakol at the lowest doses (10 mg/kg, i.p.) increased locomotor and exploratory behaviour (Thongsaard et al., 1995b, 1996a). Both quinelorane dihydrochloride (0.3–1.0 mg/g.) and pergolide methanesulfonate (1 mg/kg) also increased locomotor activity and stereotypic behaviour probably by activation of post-synaptic dopamine D_2 receptors (Foreman et al., 1989, 1995). The pharmacological effects of barakol have some common features with the dopamine D_2 receptor agonists, quinelorane dihydrochloride and pergolide methanesulfonate, the later being used to reduce the severity of dyskinesias in Parkinson patients (Langtry and Clissold, 1990; Pahwa and Koller, 1995). More studies, however, are needed to explain the precise mechanism of action of barakol. Barakol can suppress 5-hydroxytryptophan head shakes in mice (Tongroach et al., 1992) which is considered a 5-hydroxytryptamine$_2$ (5-HT$_2$)-mediated response, indicating that other mechanisms (e.g., 5-HT$_2$ receptor antagonism) apart from altered dopamine function may be involved in the behavioural effects of barakol. The 5-HT$_2$-receptor antagonist, ritanserin, has been reported to have anxiolytic effects (Colpaert et al., 1985); therefore, the effects of barakol on plus-maze behaviour observed in our previous study (Thongsaard et al., 1995b, 1996a) could also involve 5-HT mechanisms.

In summary, the present results demonstrate an effect of barakol on endogenous K^+-stimulated dopamine release from striatal slices which may involve the dopamine D_2 receptor. Alternatively, barakol may act by a mechanism independent of dopamine D_2 receptor. It remains to be determined whether an action on dopaminergic function is important in the behavioural effects of barakol associated with the use of *Cassia siamea* as a medicinal plant in Southeast Asia.

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