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Short communication

Cocaine inhibits the release of MPP^+ but not dopamine through the rat dopamine transporter

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Abstract

Transporter-mediated release of dopamine and the parkinsonism-inducing neurotoxin 1-methyl-4-phenylpyridinium (MPP^+) was examined in COS cells, a cell line derived from monkey kidney, expressing the rat dopamine transporter. The release of preloaded [^{3}H]MPP^+ but not [^{3}H]dopamine was dose-dependently inhibited by cocaine and other compounds known as dopamine uptake inhibitor, 1-[2-(diphenylmethoxy)ethyl]-4-(3-phenylpropyl)-piperazine (GBR12935) and nomifensine, although the uptake of both [^{3}H]dopamine and [^{3}H]MPP^+ was sensitive to these compounds. The different sensitivity of the outward transport of dopamine and MPP^+ to the uptake inhibitors might provide a clue to the identity of the specific site(s) for cocaine on the transporter.

Keywords: Dopamine; Transporter; Dopamine efflux

1. Introduction

The dopamine transporter acts to terminate dopaminergic neurotransmission by reaccumulating released dopamine (Horn, 1990). Evidence has been accumulated that the process is reversible, supporting the existence of a transporter-mediated release of dopamine, although its physiological relevance is as yet unclear (Levi and Raiteri, 1993). Recent cloning of cDNAs for neurotransmitter transporters including the dopamine transporter makes it possible to investigate directly the structure-function relationship of the transporter molecules (Uhl, 1992). Functional expression of the wild-type and mutant dopamine transporter in COS cells, a cell line derived from monkey kidney, reveals the relevance of different amino acid residues to the transporter functions and selective drug interactions, such as with cocaine (Kitayama et al., 1992, 1993). Furthermore, the expressed dopamine transporter was shown to be under inhibitory modulation by protein kinase C (Kitayama et al., 1994). To obtain further insight into the molecular aspects of the transporter function, we examined the effects of cocaine and several other compounds known as dopamine uptake inhibitors on the reverse process of transport for different substrates, i.e. dopamine and the parkinsonism-inducing neurotoxin 1-methyl-4-phenylpyridinium (MPP^+) through the dopamine transporter expressed in COS cells.

2. Materials and methods

Materials and methods for the uptake of [^{3}H]dopamine and [^{3}H]MPP^+ in the transfected COS cells were as described previously (Kitayama et al., 1993). For the efflux measurement, cells were preloaded with [^{3}H]dopamine (10 nM, 20.3 Ci/mmol; NEN Dupont) or [^{3}H]MPP^+ (5 nM, 77.2 Ci/mmol; NEN Dupont) by incubation in Krebs-Ringer HEPES-buffered solution (KRH; 125 mM NaCl, 4.8 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 5.6 mM glucose and 25 mM HEPES, pH 7.35) containing 0.1 mM ascorbic acid and 50 μM pargyline (for dopamine) for 60 min at 37°C. The loaded cells were washed three times with ice-cold KRH, and then incubated in KRH with or without the compounds under investigation at 37°C for 2 min. After that, the incubation solution was immediately removed and counted for radioactivity. Radioactivity in the cells retained after incubation was extracted with NaOH and after neutralization with HCl measured by liquid scintillation counting. The net release of [^{3}H]dopamine and [^{3}H]MPP^+ was calculated as % of initial radioactivity loaded in the cells and the effects

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of drugs on the release were expressed as % of control release in the absence of drugs. Statistical analyses were performed using analysis of variance (ANOVA) with pairwise comparison by Bonferroni method (Wallenstein et al., 1980).

3. Results

COS cells transfected with the rat dopamine transporter cDNA have the ability to take up both [^{3}H]dopamine and [^{3}H]MPP$^+$ robustly. Cocaine and 1-[2-(diphenylmethoxy)ethyl]-4-(3-phenylpropyl)-piperazine (GBR12935), known as a specific dopamine uptake inhibitor, both inhibited the uptake of [^{3}H]dopamine and [^{3}H]MPP$^+$ in a concentration-dependent fashion (Shimada et al., 1991; Kitayama et al., 1992). The release of preloaded [^{3}H]dopamine and [^{3}H]MPP$^+$ through the transporter was observed in COS cells expressing the dopamine transporter with a time-course as shown in Fig. 1A. A rapid release of [^{3}H]dopamine within the initial 2 min occurred followed by a slight increase in efflux: initial release within 2 min was 15% of total preloaded content and after 10 min only 30% of total content was released. The release seems to be mediated by the expressed transporter, since it was reduced by lowering the incubation temperature and increased by omission of Na^+ from the incubation solution (data not shown). Preloaded [^{3}H]MPP$^+$ was released persistently and more than 60% of the total content was released within 10 min, although the rate of release in the initial phase (first 2 min) was faster than that of the late phase.

Effects of dopamine uptake inhibitors on [^{3}H]dopamine and [^{3}H]MPP$^+$ efflux were examined during 2-min incubations. Cocaine, nomifensine and GBR12935 failed to affect [^{3}H]dopamine release. These inhibitors significantly reduced [^{3}H]MPP$^+$ release in a concentration-dependent fashion (Fig. 1B). Rank order of potency for inhibiting MPP$^+$ efflux was similar to that observed for dopamine uptake (Shimada et al., 1991; Kitayama et al., 1992), although IC_{50} values for efflux inhibition were a hundred-fold larger than those for uptake inhibition. One possible explanation for this result is that these antagonists may enter the cell and act at binding sites on the transporter.

It is known that the extracellular presence of analogues transported by the same carrier affects transporter-mediated neurotransmitter release, i.e. homo- and heteroexchange (Levi and Raiteri, 1993). We examined the effects of the presence of extracellular substrates such as dopamine and MPP$^+$ on the release of preloaded [^{3}H]dopamine and [^{3}H]MPP$^+$ and modification of their release by cocaine. Addition of dopamine to the incubation medium caused a slight but significant increase in [^{3}H]dopamine efflux (Table 1). Efflux of [^{3}H]MPP$^+$ was also facilitated by extracellular dopamine and MPP$^+$. The effects of dopamine and

Table 1
Effects of substrates and cocaine on the efflux of [^{3}H]dopamine and [^{3}H]MPP$^+$ in COS cells expressing the rat dopamine transporter

Treatment	[^{3}H]dopamine efflux (% of initial content)	[^{3}H]MPP$^+$ efflux (% of initial content)
None (control)	9.51 ± 0.52	20.44 ± 0.75
Cocaine	8.50 ± 0.16	7.00 ± 1.31 [a]
Dopamine	11.49 ± 0.53 [a]	33.78 ± 1.42 [a]
Cocaine + dopamine	8.49 ± 0.16	15.64 ± 2.13
MPP$^+$	10.82 ± 0.14	26.94 ± 0.98 [a]
Cocaine + MPP$^+$	9.45 ± 0.16	10.66 ± 0.39 [a]

Cells preloaded with 10 nM [^{3}H]dopamine or 2.5 nM [^{3}H]MPP$^+$ were washed with ice-cold KRH and then incubated for 2 min at 37°C with or without cocaine (100 μM), dopamine (100 μM) and/or MPP$^+$ (100 μM). [a] $P < 0.05$ vs. control.

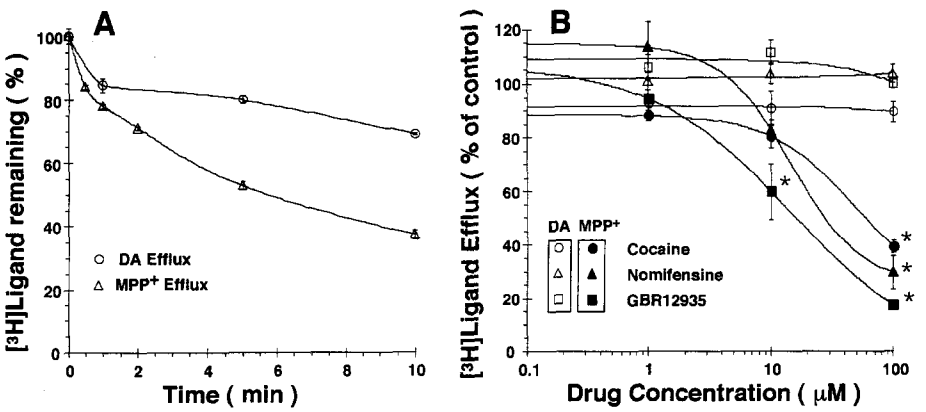

Fig. 1. (A) Time-course of the release of [^{3}H]dopamine and [^{3}H]MPP$^+$ in COS cells expressing the rat dopamine transporter. Cells preloaded with 10 nM [^{3}H]dopamine (circle) or 5 nM [^{3}H]MPP$^+$ (triangle) were incubated in the ligand-free KRH for various times and radioactivity remaining in cells after incubation and washing was determined. (B) Effect of cocaine, nomifensine and GBR12935 on the release of [^{3}H]dopamine and [^{3}H]MPP$^+$ through the transporter in COS cells expressing the rat dopamine transporter. Cells preloaded with 10 nM [^{3}H]dopamine (open symbols) or 2.5 nM [^{3}H]MPP$^+$ (closed symbols) were incubated for 2 min with or without various concentrations of cocaine (circle), nomifensine (triangle) or GBR12935 (square). Values represent the mean ± S.E.M. of three determinations. Control efflux of [^{3}H]dopamine and [^{3}H]MPP$^+$ in the absence of inhibitors was 9.14 ± 0.28% and 22.05 ± 1.96% of the initial content loaded in the cells (4553 ± 211 and 1808 ± 94 dpm/well), respectively. * $P < 0.05$ vs. control.

MPP^+ in the extracellular medium were dose-dependent between 1 and 100 μM (data not shown). The enhanced efflux of MPP^+ and dopamine elicited by the substrates was blocked by cocaine (Table 1), in contrast to the specific inhibition of spontaneous MPP^+ efflux by cocaine without the spontaneous dopamine efflux being affected.

4. Discussion

The present results demonstrate that the ability of cocaine and other uptake inhibitors to block the reversed transport through the rat dopamine transporter is selective for a substrate, probably without affecting transport processes common to other substrates, such as translocation or reorientation.

It is assumed that facilitation of the inward transport of substrates increases the probability that the transporter faces the intracellular side, which makes it possible to cause inverse transport of substrates present in cytosol. Therefore, it is likely that cocaine and other uptake inhibitors prevent homo- and heteroexchange by inhibition of the uptake of substrates added to the incubation medium, as observed in a variety of preparations reported previously, e.g. synaptosomes in vitro (Raiteri et al., 1979) and in vivo rat brain microdialysis (Butcher et al., 1988). The present results suggest the possibility that the substrate specificity of cocaine inhibition of spontaneous efflux through the rat dopamine transporter is mediated by a mechanism different from that for inhibition of uptake.

Our present results show some differences to those recently reported by Eshleman et al. (1994) and Wall et al. (1995). The former showed that cocaine failed to affect [^{3}H]dopamine efflux in COS cells transiently expressing the human dopamine transporter, while GBR12935 and mazindol caused a significant decrease of [^{3}H]dopamine release which was antagonized by cocaine. In contrast, Wall et al. (1995) observed that both cocaine and mazindol were without an effect on MPP^+ efflux through the rat dopamine transporter. Wall et al. (1995) used a stably transfected cell line and measured the efflux of [^{3}H]MPP^+ instead of [^{3}H]dopamine because of considerable degradation of dopamine in LLC-PK cells, a cell line derived from pig kidney. Although Wall et al. (1995) explained the dissimilarity in results as being due to cell line used and difference of transient or stable expression of the transporter, the present results suggest the possibility that uptake inhibitors show substrate specificity for the reversed transport.

Another important aspect to explain these differences is species difference in the transporter. It is interesting to compare the amino acid sequence of the human and rat dopamine transporter. In transmembrane regions, only few amino acids present in the 3rd, 8th, 9th and 11th putative transmembrane regions are different from each other. Among those, tyrosine is present in the 11th transmembrane region of the rat dopamine transporter while phenylalanine is present at corresponding positions in the human dopamine transporter. We have demonstrated that substitution of polar amino acids in the 11th transmembrane region including tyrosine to alanine in the rat dopamine transporter by in vitro site-directed mutagenesis increased affinity for MPP^+ while it had no or little effect on dopamine uptake or cocaine analogue binding (Kitayama et al., 1993). Therefore, this region may be important for a specific interaction with MPP^+ for its recognition with respect to reversed transport and for the interaction with cocaine and other compounds known as dopamine uptake inhibitors.

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