Dopamine Uptake through the Norepinephrine Transporter in Brain Regions with Low Levels of the Dopamine Transporter: Evidence from Knock-Out Mouse Lines

José A. Morón, Alicia Brockington, Roy A. Wise, Beatriz A. Rocha, and Bruce T. Hope

Behavioral Neuroscience Branch, National Institute on Drug Abuse, National Institutes of Health, Baltimore, Maryland 21224

Selective blockers of the norepinephrine transporter (NET) inhibit dopamine uptake in the prefrontal cortex. This suggests that dopamine in this region is normally cleared by the somewhat promiscuous NET. We have tested this hypothesis by comparing the effects of inhibitors selective for the three monoamine transporters with those of a nonspecific inhibitor, cocaine, on uptake of ^{3}H-dopamine into synaptosomes from frontal cortex, caudate nucleus, and nucleus accumbens from wild-type, NET, and dopamine transporter (DAT) knock-out mice. Dopamine uptake was inhibited by cocaine and nisoxetine, but not by GBR12909, in frontal cortex synaptosomes from wild-type or DAT knock-out mice. At transporter-specific concentrations, nisoxetine and GBR12909 failed to block dopamine uptake into frontal cortex synaptosomes from NET knock-out mice. The efficacy of cocaine at the highest dose (1 mM) was normal in DAT knock-out mice but reduced by 70% in NET knock-out mice. Nisoxetine inhibited dopamine uptake by 20% in caudate and nucleus accumbens synaptosomes from wild-type and DAT knock-out mice but had no effect in those from NET knock-out mice. Cocaine failed to block dopamine uptake into caudate or nucleus accumbens synaptosomes from DAT knock-out mice. Cocaine and GBR12909 each inhibited dopamine uptake into caudate synaptosomes from NET knock-out mice, but cocaine effectiveness was reduced in the case of nucleus accumbens synaptosomes. Thus, whereas dopamine uptake in caudate and nucleus accumbens depends primarily on the DAT, dopamine uptake in frontal cortex depends primarily on the NET. These data underscore the fact that which transporter clears dopamine from a given region depends on both the affinities and the local densities of the transporters.

Key words: nucleus accumbens; caudate; frontal cortex; synaptosomes; nisoxetine; GBR 12909; cocaine

Monoamines have long been thought to play roles in depression. Even though dopamine (DA) is the monoamine most closely associated with reward and affect, the DA hypothesis of depression has received little recent attention because of the success of antidepressant medications that selectively target the norepinephrine transporter (NET) or the serotonin transporter (SERT) with little or no affinity for the dopamine transporter (DAT) (Eriksson, 2000; Gumnick and Nemeroff, 2000; Svensson, 2000). Although antidepressants can be very selective for the NET or the SERT, these transporters are not equally selective for their nominal substrates. The SERT has too weak an affinity to be likely to take up DA at physiological levels (Hoffman et al., 1991), but the NET can transport DA as well as norepinephrine (NE) (Horn, 1973; Raiteri, 1977) and, indeed, has greater affinity for DA than does the DAT itself (Giros et al., 1994; Gu et al., 1994; Eshleman et al., 1999). Indeed, the NET-selective antidepressant desipramine elevates levels of DA as well as NE in the frontal cortex (FCx) (Carboni et al., 1990; Di Chiara et al., 1992; Tanda et al., 1994; Yamamoto and Novotney, 1998), where the NET is more concentrated than the DAT (Moll et al., 2000).

The present study was designed to compare the effects of blockade of the NET and DAT on DA uptake into synaptosomes prepared from FCx and other DA terminal fields. This assay allows us to dissociate changes in extracellular DA that result from altered DA clearance from changes that result from altered DA release, which can be secondary to elevations in NE or 5-HT (Pozzi et al., 1994, 1999; Matsumoto et al., 1999; Sakaue et al., 2000). We compared the effects of inhibitors for each of the three monoamine transporters: nisoxetine (which selectively blocks the NET), GBR 12909 (which selectively blocks the DAT), and fluoxetine (which selectively blocks the SERT), with the effects of the nonspecific inhibitor cocaine. We contrasted basal DA uptake and drug-induced inhibition of DA uptake into synaptosomes from FCx, where DAT expression is minimal (Freed et al., 1995; Sesack et al., 1998), with uptake into synaptosomes from caudate nucleus, where DAT expression is maximal, and from nucleus accumbens where intermediate levels of the DAT are expressed. In each case, we compared uptake of [^{3}H]DA into synaptosomes from DAT knock-out, NET knock-out, and wild-type mice.

MATERIALS AND METHODS

Animals. The original breeding pairs from DAT and NET knock-out mice were obtained from the laboratory of Dr. Marc Caron (Duke University Medical Center, Durham, NC). They contained the DNA constructs previously shown to produce genetic deletions of the DAT and NET, respectively (Giros et al., 1996; Rocha et al., 1998; Wang et al., 1999; Xu et al., 2000). These mice were produced from 20 or more generations of backcrossing on to a 129/SvJ background inbred strain. We used female homozygous mice and their wild-type littermates derived from the crossing of heterozygous breeding pairs. The animals were

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Correspondence should be addressed to Dr. Bruce T. Hope, Behavioral Neuroscience Branch, National Institute on Drug Abuse, National Institutes of Health, 5500 Nathan Shock Drive, Baltimore, MD 21224. E-mail: bhope@intra.nida.nih.gov.

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housed (four or five per cage) on a 12 hr light/dark cycle with *ad libitum* access to water and food. All animal procedures were in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Measurement of [^{3}H]DA uptake into synaptosomes. DA uptake was measured using synaptosomal fractions of tissue pooled from five or six mice for each brain region. Each experiment was repeated three or four times on different days using freshly pooled tissue from five or six mice each time. The mice were killed by decapitation, and their brains were dissected on an ice-cold dish. The FCx samples were cut from the frontal tip of the brain with a razor blade. The caudate and accumbens (Acb) samples were dissected from a 1 mm coronal slice. The Acb sample included both the core and shell regions.

The pooled tissues from FCx, caudate, and Acb were placed in ice-cold Krebs'–Ringer's solution buffer (in mM: NaCl 125, KCl 1.2, $MgSO_4$ 1.2, $CaCl_2$ 1.2, $NaHCO_3$ 22, NaH_2PO_4 1, and glucose 10, adjusted to pH 7.4) containing 0.32 M sucrose and homogenized using a glass homogenizing tube and a Teflon pestle. The samples were centrifuged for 10 min at 1000 × *g*, the pellet was discarded, and the remaining supernatant was centrifuged for an additional 15 min at 16,000 × *g*. The resulting P2 pellet containing the synaptosomes remained on ice until it was resuspended for the uptake assay.

The synaptosomal uptake assay used in our experiments has previously been described by Morón et al. (1998). The assay was performed in Krebs'–Ringer's buffer containing 0.64 mM ascorbic acid, 0.8 mM pargyline, and 0.1 μM [^{3}H]DA (50 Ci/mmol). This concentration of DA (0.1 μM) is the approximate K_m value for DA uptake in brain synaptosomes (Izenwasser et al., 1990, 1994; Elsworth et al., 1993; Copeland et al., 1996). The uptake assay was initiated by the addition of aliquots (FCx: 100–130 μg; Acb: 70–100 μg; caudate: 50–100 μg) of the synaptosomal fraction followed by incubation for 4 min at 37°C. Nonspecific uptake and adsorption of [^{3}H]DA was determined by incubation of a parallel set of samples at 4°C (the specific monoamine transporters are inactive at this temperature). The assay was terminated by placing the samples on ice and adding 5 ml of ice-cold Krebs'–Ringer's buffer. The synaptosomes were then separated from the assay solution by filtration through Whatman glass microfiber filters (GF/C), that had been presoaked in 0.1% polyethylenimine to reduce nonspecific binding, using a Brandel cell-harvester filtration apparatus. The synaptosomes, trapped on the filters, were washed twice with 5 ml of ice-cold Krebs'-Ringer's buffer. The filters were placed in scintillation vials, and 3 ml of Bio-Safe II scintillation fluid (Research Products, Mount Prospect, IL) were added to each vial, and the radioactivity was determined by liquid scintillation spectrometry. Under these experimental conditions, total [^{3}H]DA uptake increased linearly with both protein concentration and time over the 4 min incubation period in samples from each of the three brain regions (data not shown).

Protein was measured using the Bio-Rad assay (Hercules, CA). The uptake inhibition curves were obtained by the addition of varying concentrations of the monoamine uptake blockers to the reaction mix. IC_{50} values were determined using nonlinear curve fitting (Prism 2.0; GraphPad Software, San Diego, CA).

Chemicals. Chemicals and reagents were obtained from the following sources: 7,8-[^{3}H]DA (50 Ci/mmol) from Amersham (Arlington Heights, IL); pargyline hydrochloride and ascorbic acid from Sigma (St. Louis, MO); cocaine hydrochloride from the National Institute on Drug Abuse (Bethesda, MD); and GBR12909 and nisoxetine hydrochloride from Research Biochemicals (Natick, MA).

RESULTS

DA uptake into synaptosomes from DAT and NET knock-out mice differed as a function of brain structure (Fig. 1). The rate of DA uptake was 0.45 pmol · min^{-1} · mg^{-1} into FCx synaptosomes, 3.44 pmol · min^{-1} · mg^{-1} into caudate synaptosomes, and 2.70 pmol · min^{-1} · mg^{-1} into Acb synaptosomes. Uptake in FCx synaptosomes was normal in DAT knock-out mice but severely attenuated in NET knock-out mice (Fig. 1). DA uptake was 40% less in Acb and caudate synaptosomes from NET knock-out mice and was >70% less in Acb and caudate synaptosomes from DAT knock-out mice. An overview of the effects of cocaine, nisoxetine, and GBR 12909 is shown in Table 1.

Cocaine inhibited DA uptake into synaptosomes differentially as a function of brain structure and mouse genotype (Fig. 2). The highest concentration of cocaine used (1 mM) inhibited 50% of DA uptake into FCx synaptosomes, 90% of DA uptake into caudate synaptosomes, and 70% of DA uptake into Acb synaptosomes from wild-type mice. The apparent IC_{50} values for cocaine-dependent inhibition of DA uptake were ~1 mM in FCx, 1 μM in caudate, and 10 μM in Acb synaptosomes. Cocaine blocked DA uptake equally into FCx synaptosomes from both wild-type and DAT knock-out mice but failed to block DA uptake into caudate or Acb synaptosomes from DAT knock-out mice. Cocaine blocked DA uptake equally into caudate synaptosomes

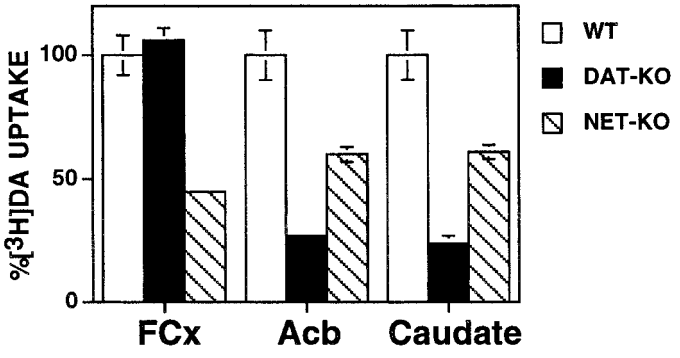

Figure 1. Total [^{3}H]DA uptake in synaptosomes obtained from FCx, Acb, and caudate from DAT knock-out (*DAT-KO*), NET knock-out (*NET-KO*), and wild-type (*WT*) mice. Absolute values for total [^{3}H]DA uptake rates in each genotype of mice are expressed as a percentage of that observed in wild-type mice. Values represent mean ± SEM obtained from three or four independent experiments using fresh tissue pooled from five or six mice each time.

Table 1. Summary of inhibition of DA uptake by cocaine, nisoxetine, and GBR 12909 in wild-type, DAT, and NET knock-out mice

Inhibition of DA uptake by cocaine, nisoxetine, and GBR 12909

FCx	Wild-type	NET KO	DAT KO
Cocaine	↓↓	0	↓↓
Nisoxetine High	↓	0	↓
Nisoxetine Low	↓	0	↓
GBR 12909	0	0	N.D.

Caudate	Wild-type	NET KO	DAT KO
Cocaine	↓↓↓	↓↓↓	0
Nisoxetine High	↓	0	↓
Nisoxetine Low	↓↓	↓↓↓	0
GBR 12909	↓↓↓	↓↓↓	N.D.

Acb	Wild-type	NET KO	DAT KO
Cocaine	↓↓↓	↓↓	0
Nisoxetine High	↓	0	↓
Nisoxetine Low	↓↓	↓↓	↓↓
GBR 12909	↓↓↓	↓↓↓	N.D.

The arrows "↓, ↓↓, ↓↓↓" qualitatively indicate increasing levels of inhibition in each brain region. "0" indicates DA uptake was insensitive to inhibitor. ND, Not determined. There were both "high"- and "low"-sensitive components of nisoxetine-dependent inhibition of DA uptake.

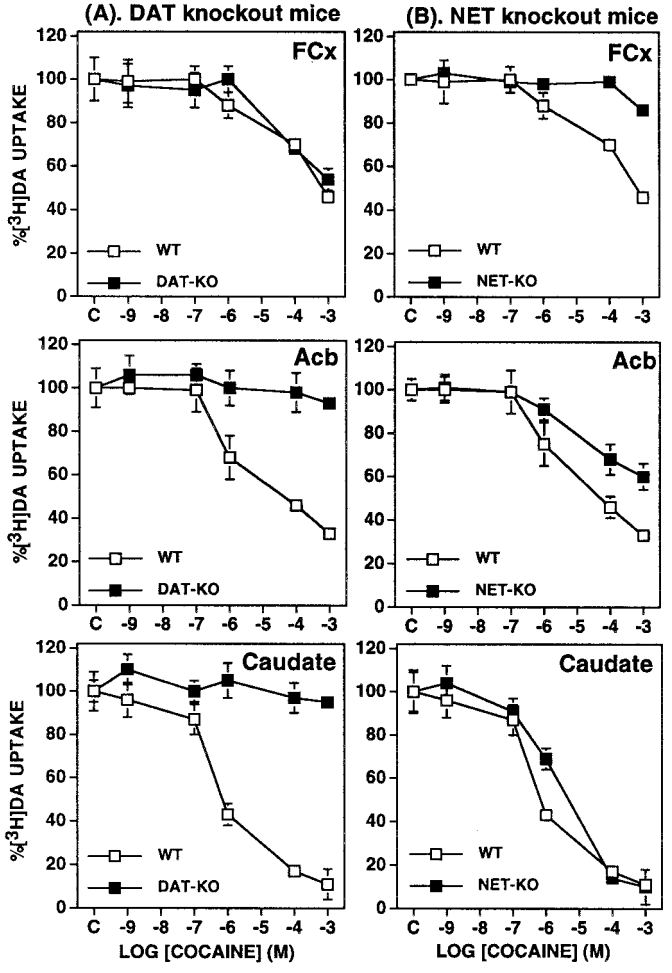

Figure 2. Effects of cocaine on [^{3}H]DA uptake in synaptosomes obtained from FCx, Acb, and caudate from DAT knock-out (*DAT-KO*) (*A*), NET knock-out (*NET-KO*) (*B*), and wild-type (*WT*) mice. The rate of [^{3}H]DA uptake at each concentration of cocaine is expressed as a percentage of that observed for each genotype with only the vehicle present in the assay. Values represent mean ± SEM obtained from three or four independent experiments using fresh tissue pooled from five or six mice each time.

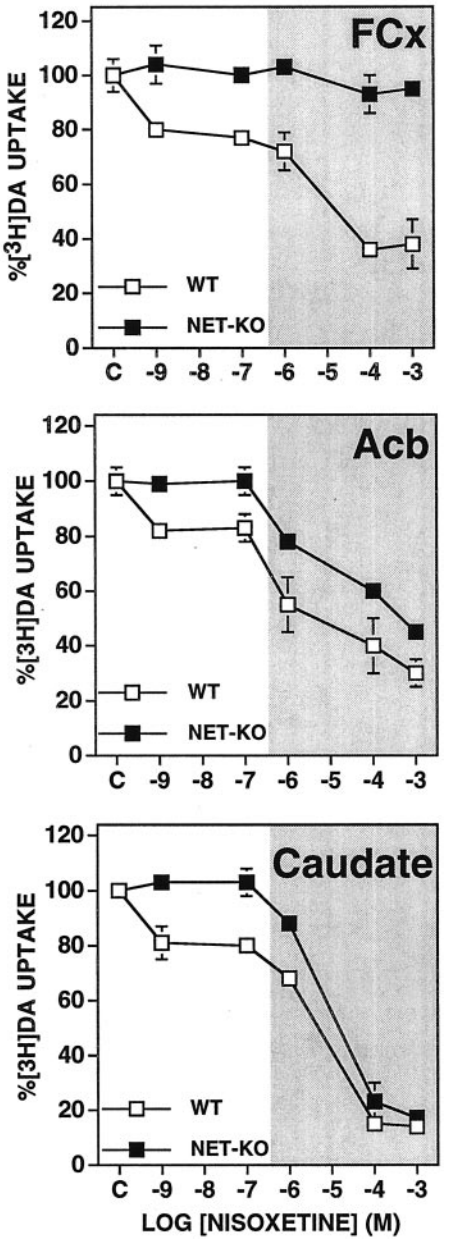

Figure 3. Effects of nisoxetine on [^{3}H]DA uptake in synaptosomes obtained from FCx, Acb, and caudate from NET knock-out (*NET-KO*) and wild-type (*WT*) mice. The rate of [^{3}H]DA uptake at each concentration of nisoxetine is expressed as a percentage of that observed for each genotype with only the vehicle present in the assay. Values represent mean ± SEM obtained from three or four independent experiments using fresh tissue pooled from five or six mice each time. The *shaded area* indicates the effects of higher nonselective concentrations (>100 nM) of nisoxetine.

from wild-type and NET knock-out mice. Cocaine also blocked DA uptake into Acb synaptosomes from NET knock-outs, although there was a decrease in cocaine potency—across the range of doses tested—for inhibition of DA uptake into Acb synaptosomes from NET knock-out mice. Cocaine blocked DA uptake into FCx synaptosomes from wild-type but not from NET knock-out mice.

Nisoxetine attenuated DA uptake into wild-type but not NET-knock-out synaptosomes from all three regions (Fig. 3). At low concentrations (10^{-9} to 10^{-7} M), nisoxetine attenuated by 20% the DA uptake into synaptosomes from each of the three brain regions of wild-type mice. At nonselective concentrations of 10^{-6} to 10^{-3} M, nisoxetine attenuated DA uptake into synaptosomes from both caudate and Acb of both wild-type and NET knock-out mice. At these higher concentrations, nisoxetine blocked 60% of the DA uptake into FCx synaptosomes from wild-type mice. Nisoxetine, even at high concentrations, failed to block DA uptake into FCx synaptosomes from NET-knock-out mice.

In the case of DAT-knock-out synaptosomes, nisoxetine blocked DA uptake at both low and high concentrations into synaptosomes from both wild-type and knock-out mice (Fig. 4). At low (10^{-9} M) concentration, nisoxetine blocked ~20% of DA uptake into synaptosomes from each of the three brain regions of both wild-type and DAT knock-out mice. At high concentrations (10^{-6} to 10^{-3} M), nisoxetine blocked DA uptake by ~60% in FCx and Acb synaptosomes from both wild-type and DAT-knock-out mice. At the highest concentrations used (10^{-4} and 10^{-3} M), nisoxetine blocked uptake into caudate synaptosomes from wild-type, but not from DAT-knock-out mice, by >80%.

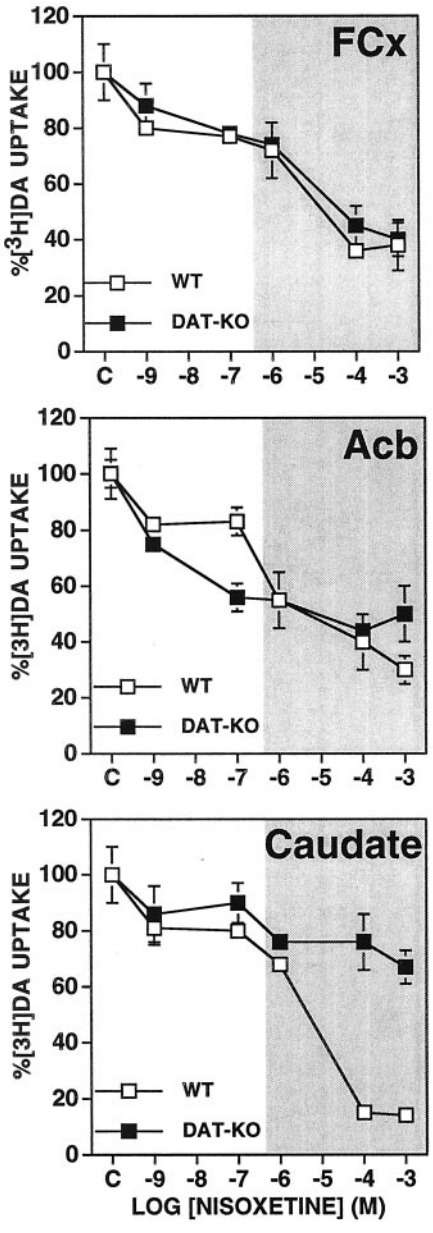

Figure 4. Effects of nisoxetine on [^{3}H]DA uptake in synaptosomes obtained from FCx, Acb, and caudate from DAT knock-out (*DAT-KO*) and wild-type (*WT*) mice. The rate of [^{3}H]DA uptake at each concentration of nisoxetine is expressed as a percentage of that observed for each genotype with only the vehicle present in the assay. Values represent mean ± SEM obtained from three or four independent experiments using fresh tissue pooled from five or six mice each time. The *shaded area* indicates the effects of higher nonselective concentrations (>100 nM) of nisoxetine.

GBR 12909 blocked DA uptake equally in wild-type and NET-knock-out synaptosomes regardless of brain region (Fig. 5). GBR 12909 blocked DA uptake into FCx synaptosomes only at the highest concentration (10^{-3} M). At DAT-selective concentrations (10^{-7} and 10^{-6} M), GBR 12909 blocked 70–80% of DA uptake into Acb and caudate synaptosomes. Because of the limited productivity of our breeding pairs, the effects of GBR 12909 were not tested on synaptosomes from DAT knock-out mice.

Fluoxetine, a SERT-specific blocker, had no effect on DA uptake into FCx or caudate synaptosomes from wild-type mice (data not shown).

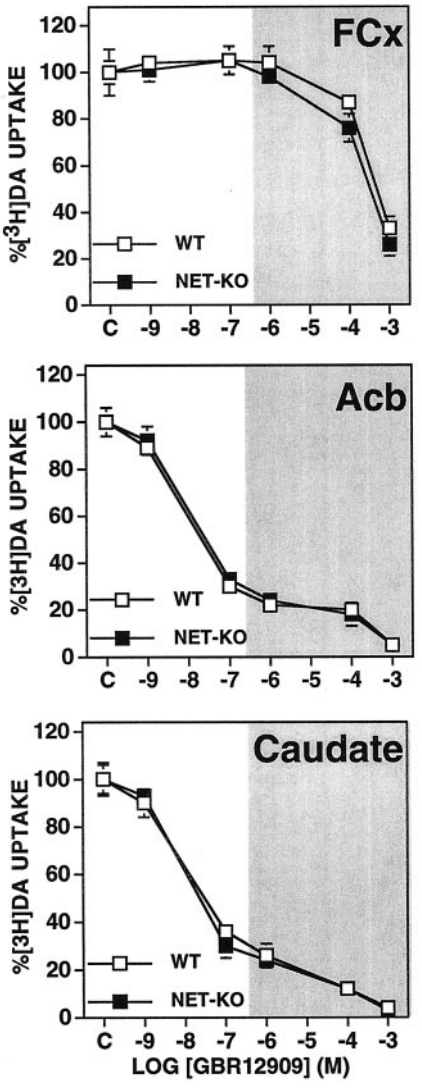

Figure 5. Effects of GBR 12909 on [^{3}H]DA uptake in synaptosomes obtained from FCx, Acb, and caudate from NET knock-out (*NET-KO*) and wild-type (*WT*) mice. The rate of [^{3}H]DA uptake at each concentration of GBR 12909 is expressed as a percentage of that observed for each genotype with only the vehicle present in the assay. Values represent mean ± SEM obtained from three or four independent experiments using fresh tissue pooled from five or six mice each time. The *shaded area* indicates the effects of nonselective concentrations (>100 nM) of GBR12909.

DISCUSSION

DA uptake in FCx

The present study confirms that extracellular DA is cleared from FCx primarily by the NET. This possibility was first suggested on the basis of microdialysis studies (Carboni et al., 1990; Di Chiara et al., 1992; Tanda et al., 1994; Yamamoto and Novotney, 1998) that did not reveal whether the observed elevations in DA levels were because of clearance of DA by the NET or rather were secondary to a presynaptic action such as that of elevated NE on DA release (Pozzi et al., 1994). In the present assay, exogenous NE was not present and thus could not affect the DA nerve terminals. Moreover, DA of intracellular origin, which was unlabeled, could not be confused with the exogenous, labeled, DA of extracellular origin that was taken up by synaptosomes. Furthermore, use of the DAT and NET knock-out mice allowed an

examination of DA uptake mechanisms under conditions where all contributions of the DAT or NET could be ruled out.

DA uptake into FCx synaptosomes from NET knock-out mice was 55% lower than those from wild-type mice. This was not a consequence of developmental compensations in the knock-out mice, because nisoxetine, an uptake inhibitor selective for the NET, caused similar inhibition of DA uptake into FCx synaptosomes from wild-type mice. The residual DA uptake into FCx synaptosomes from NET knock-out mice was not because of uptake via the DAT because DA uptake into FCx synaptosomes from NET knock-out mice was not inhibited by selective concentrations of GBR 12909, an uptake inhibitor selective for the DAT. This is consistent with the observation of similar levels of DA uptake into FCx synaptosomes from DAT knock-out mice and wild-type mice. The DAT appears to be ineffective in clearing DA from FCx (Carboni et al., 1990; Di Chiara et al., 1992; Tanda et al., 1994; Yamamoto and Novotney, 1998) because of its sparse concentration (Sesack et al., 1998) relative to the dense concentration of the NET (Schroeter et al., 2000), which has a stronger affinity for DA than does the DAT (Giros et al., 1994; Gu et al., 1994; Eshleman et al., 1999). The rate of DA uptake is so low around the sites of DA release and in the surrounding regions, that DA is able to diffuse to a much larger volume (Stamford et al., 1988; Garris and Wightman, 1994; Cass and Gerhardt, 1995; Jones et al., 1996) where the NET is the predominant transporter capable of transporting DA (Schroeter et al., 2000).

The remaining of DA uptake into FCx synaptosomes from NET knock-out mice is likely attributable to a cocaine-insensitive transporter similar to that observed in rat brain (Izenwasser et al., 1990; Elsworth et al., 1993). Thus, the NET is the only transporter in FCx likely to have mediated the observed cocaine-dependent inhibition of DA uptake into FCx synaptosomes from wild-type and DAT-knock-out mice; cocaine had no effect on DA uptake in FCx synaptosomes from NET knock-out mice. This is consistent with the finding that reverse dialysis of the NET blocker desipramine blocks the increase in DA levels in rat prefrontal cortex after intraperitoneal cocaine administration (Tanda et al., 1997).

DA uptake in caudate

DA uptake into caudate synaptosomes from DAT knock-out mice was depressed 76%. This was not a consequence of developmental compensations in the knock-out mice, because DAT-selective concentrations of GBR 12909 produced a similar level of inhibition of DA uptake into caudate synaptosomes from wild-type mice. The remaining amount of DA uptake into caudate synaptosomes from DAT knock-out mice was reduced only 20% by nisoxetine, similar to that in wild-type mice. These results suggest that DA uptake in caudate is mediated primarily by the DAT with only a minor contribution from the NET. This is consistent with the fact that the DAT is abundant and the NET is sparse in caudate (Schroeter et al., 2000). Despite the fact that the NET contributes 20% of total DA uptake into caudate synaptosomes from DAT knock-out mice, DA uptake via the NET does not appear to play an important role in regulation of DA levels in the intact caudate. Systemic administration or reverse dialysis of desmethyimipramine (DMI) into rat caudate does not significantly increase DA levels in this brain region (Carboni et al., 1990; Di Chiara et al., 1992; Yamamoto and Novotney, 1998). One possibility is that DA is normally intercepted by the DAT, which is located perisynaptically (Nirenberg et al., 1997), before it can reach the higher-affinity but more distant and sparse NET in this brain structure. Even in DAT knock-out mice, the NET does not transport a significant amount of DA in the intact caudate; cyclic voltammetry studies demonstrated that the clearance rate for DA in caudate slices from DAT knock-out mice is similar to the calculated rate for diffusion-mediated clearance and unaffected by the addition of DMI (Jones et al., 1998).

Cocaine-dependent inhibition of DA uptake in caudate seems almost entirely attributable to inhibition of the DAT because cocaine-dependent inhibition of DA uptake into caudate synaptosomes was similar in wild-type and NET knock-out mice but completely absent in DAT knock-out mice. Surprisingly, the NET in caudate synaptosomes from DAT knock-out mice was insensitive to cocaine, whereas it remained somewhat sensitive to nisoxetine.

DA uptake in the nucleus accumbens

Transporter-specific inhibition of DA uptake into Acb synaptosomes was similar to that into caudate synaptosomes. This result suggests that total DA uptake in our preparations of both the shell and core subregions of Acb combined is mediated mostly by the DAT with a smaller contribution from the NET. However, Acb may not be homogenous in this regard. Reverse dialysis of DMI into the shell subregion of the intact rat Acb increased DA levels (Yamamoto and Novotney, 1998). As with FCx, it remains to be determined whether this DMI-dependent increase in DA was attributable to a decrease in transport through the NET or to noradrenergic interactions with DA terminals. The rightward shift in the dose–response curve for cocaine inhibition of DA uptake into our Acb synaptosomal samples from NET-knock-out mice suggests that the NET can transport a small but significant amount of DA in Acb from wild-type mice. This is consistent with the hypothesis that DMI in the microdialysis studies increased DA levels by directly inhibiting NET-mediated DA uptake, similar to that in FCx. The heterogeneous distribution of the DAT and NET in Acb suggests that the mechanisms for DA transport may vary dramatically depending on the microregion of Acb. The DAT is more densely concentrated in the core subregion than in the shell subregion (Ciliax et al., 1995; Freed et al., 1995; Hersch et al., 1997). The shell subregion itself is divided into patches of densely distributed DAT surrounded by areas with sparse DAT. The NET is present in the shell subregion and distributed along the rostrocaudal axis from low to medium density (Schroeter et al., 2000). Thus, in areas of the shell subregion with sparse DAT and higher levels of the NET, it is possible that DA uptake is locally dependent on the NET.

Cocaine-dependent inhibition of DA uptake into Acb synaptosomes from DAT-knock-out mice was not evident, whereas the dose dependence curve for cocaine-dependent inhibition of DA uptake into Acb synaptosomes from NET knock-out mice was shifted strongly to the right. This suggests that whereas cocaine-dependent inhibition of DA uptake in Acb is mostly attributable to inhibition of the DAT, inhibition of the NET plays some role, at least in wild-type mice. Indeed, the 20–25% difference between NET knock-out and wild-type mice for inhibition of DA uptake by 10^{-3} M cocaine was comparable with the 20% inhibition of DA uptake into Acb synaptosomes from wild-type mice by nanomolar concentrations of nisoxetine.

Differential cocaine-sensitivity between brain regions

In brain regions where the DAT mediates the majority of DA uptake, such as caudate, DA uptake is highly sensitive to cocaine. In brain regions where the NET mediates the majority of DA uptake, such as FCx, DA uptake has much lower sensitivity to

cocaine. The NET has been shown to have lower sensitivity to cocaine than the DAT in cell culture (Gu et al., 1994) and in rat brain (Ritz et al., 1990). Thus, the apparent cocaine sensitivity of total DA uptake may decrease in brain regions where the NET mediates the greatest portion of total DA uptake.

Not all DA uptake in the frontal cortex and accumbens was blocked by cocaine. Cocaine-insensitive DA uptake (not inhibited by 10^{-3} M cocaine) may be mediated by the recently cloned and characterized polyspecific cation–monoamine transporters Oct2 and Oct3/EMT, which are found in rat brain (Russ et al., 1996; Busch et al., 1998; Grundemann et al., 1998; Wu et al., 1998). The levels of cocaine-insensitive DA uptake are low. In brain regions where the DAT is abundant and total DA uptake rates are high, such as in caudate, cocaine-insensitive DA uptake does not contribute significantly toward total DA uptake. In brain regions where the DAT and NET are less abundant and total DA uptake rates are low, such as in FCx, cocaine-insensitive DA uptake contributes significantly toward total DA uptake. In our study, only millimolar levels of GBR 12909 could inhibit this cocaine-insensitive transporter.

Potential implications

The present data underscore the fact that transporter-selective uptake inhibitors are not necessarily transmitter-selective uptake inhibitors. This may explain the fact that cocaine self-administration, which is well known to be dopamine-dependent (de Wit and Wise, 1977; Roberts et al., 1977) is not lost in DAT knock-out mice (Rocha et al., 1998). For example, cocaine blockade of NET and consequent accumulation of DA in a critical subregion of Acb—presumably some portion of Acb shell (Carlezon et al., 1995)—could account for the rewarding effects of cocaine in these animals (Rocha et al., 1998; Sora et al., 2001). Transporter promiscuity might also explain why NET-selective uptake inhibitors are each effective in treatment of depression (Eriksson, 2000; Gumnick and Nemeroff, 2000; Svensson, 2000); it may be the transmitter, not the transporter, that is critical. Thus, it appears critical to determine transmitter selectivity for a given transporter blocker before assuming that the blocker's effectiveness is mediated by the transporter for which the blocker is most selective.

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