Noradrenaline transporter blockers raise extracellular dopamine in medial prefrontal but not parietal and occipital cortex: differences with mianserin and clozapine

V. Valentini, R. Frau and G. Di Chiara

Department of Toxicology and Centre of Excellence ‘Neurobiology of Addiction’, University of Cagliari, Cagliari, Italy

Abstract
This study compared the interaction between noradrenaline (NA) and dopamine (DA) mechanisms in the prefrontal (PFCX) and in the parietal (ParCX) and occipital (OccCX) cortex. The effect of reboxetine and desipramine, two NA transporter blockers, of mianserin, an antagonist of \( \alpha_2 \) and 5-HT2 receptors, and of clozapine, an atypical antipsychotic, on dialysate DA in the medial PFCX, ParCX and OccCX was studied. We also assessed the influence of a prior 6-hydroxydopamine (6-OHDA) lesion of the dorsal noradrenergic bundle (DNAB) on the effect of reboxetine and clozapine on dialysate DA in the PFCX and ParCX. Systemic administration of reboxetine and desipramine dose-dependently increased dialysate DA in the PFCX but not in the ParCX and OccCX. In contrast, mianserin and clozapine raised dialysate DA in the ParCX and OccCX to an even larger extent than in the PFCX. 6-OHDA lesions of DNAB abolished the increase of dialysate DA elicited by reboxetine in the PFCX and by clozapine both in the PFCX and in the ParCX. It is concluded that, although PFCX and ParCX/OccCX share the presence of a strong control of DA transmission by NA through \( \alpha_2 \) receptors, they differ in the extent to which DA is cleared from the extracellular compartment by uptake through the NA transporter. This process, although extensive in the PFCX, appears insignificant in the ParCX and OccCX, probably as a result of the higher ratio of NA to DA resulting in exclusion of DA from NA transporter.

Keywords: antidepressants, clozapine, cortex, microdialysis, monoamines.

located in the ventral tegmentum or in the PFCX itself. Thus, α-adrenergic receptor antagonists increase the efflux of both NA and DA in the PFCX (Tanda et al. 1996; Gobert et al. 1997; Hertel et al. 1999). Finally, it has been recently proposed that DA acts as a cotransmitter of NA at cortical NA terminals (De Vito et al. 2001).

Although existing studies on the interaction between cortical DA and NA have been focused on the PFCX, other cortical areas such as the occipital (OccCX) and parietal cortex (ParCX) show a coexistence of DA and NA (Berger 1992). We thought that, given the lower ratio of DA to NA innervations in the OccCX and ParCX as compared to the PFCX (Berger 1977; Berger et al. 1991), it would be interesting to study in these areas the effect of various drugs known to affect DA transmission in the PFCX.

We therefore compared the effect of reboxetine and desipramine, two selective NA reuptake inhibitors (Sacchetti et al. 1999; Invernizzi et al. 2001; Linner et al. 2001), GBR 12909, a selective DA reuptake inhibitor (Nissbrandt et al. 1991), mianserin, an antagonist of 5-HT2 receptors (Conn and Sanders-Bush 1987; De Boer et al. 1994; Tanda et al. 1996), and clozapine, an atypical antipsychotic (Chen et al. 1992; Moghaddam 1994; Brunello et al. 1995; Westerink et al. 1998), on dialysate DA in the medial PFCX, ParCX and OccCX. Moreover, we further investigated the relationship between DA and NA transmission by assessing the influence of a prior 6-hydroxodopamine (6-OHDA) lesion of the dorsal noradrenergic bundle (DNAB) (Berger et al. 1974) upon basal extracellular levels of DA and NA and the sequelae of these lesions on the effect of reboxetine and clozapine in the PFCX and in the ParCX.

Materials and methods

Animals

Male Sprague-Dawley rats (275–300 g) were obtained from Charles River (Calco, Italy) and kept six per cage with standard food and water ad libitum under constant temperature (23°C) and humidity (60%) and in a 12-h light–dark cycle (lights from 8.00 a.m. to 8.00 p.m.).

All animal experimentation have been conducted in accordance with the statement revised and approved by the Society for Neuroscience in January 1995 and with the Guidelines for Care and Use of Experimental Animals of the European Economic Community (86/609; D.L. 27.01.1992, No. 116).

Drugs

Reboxetine methansulphonate was a gift of Pharmacia & Upjohn (Milan, Italy); clozapine was kindly donated by Polfa (Starogard, Poland); desipramine, GBR 12909 and mianserin HCl were obtained from Sigma (Milan, Italy). All drugs were dissolved in saline and administered intraperitoneally (i.p., injection volume: 0.3 mL/100 g); only mianserin was administrated subcutaneously (injection volume: 0.1 mL/100 g). 6-Hydroxydopamine HCl was obtained from Sigma (Milan, Italy).

Probe preparation

Concentric dialysis probes (dialysing portion 3.0 mm) were prepared with AN 69 (sodium methallyl sulphate copolymer) dialysis fibre (310 mm o.d. 220 mm i.d., Hospal, Dasco, Italy), according to the method of Di Chiara et al. (1993) as modified by Tanda and Di Chiara (1998).

Lesions

Bilateral lesions of the DNAB were performed by stereotaxically injecting 6-hydroxydopamine HCl solution (1.5 μL, 6 mg/mL vehicle; vehicle, 0.4 mg/mL ascorbic acid in 0.9% saline) at the following coordinates: posterior −5.2 mm; lateral ±1.1 mm from bregma and vertical −5.3 mm from dura, according to the atlas of Paxinos and Watson (1998). Sham lesions were performed by injecting vehicle at the same coordinates.

Rats were implanted with dialysis probes 2 weeks after DNAB lesioning. Lesions were effective when rats showed at least a 90% reduction of basal dialysate NA. This was obtained in 95% of the rats infused with 6-OHDA in the DNAB.

Surgery and experiments

Rats were anaesthetized with 100 mg/kg of ketamine, administered i.p. (Ketalar; Parke Davis, Milan, Italy) and placed in a stereotaxic apparatus. The skull was exposed, and a small hole was drilled on one side. The probe was implanted vertically in the PFCX (anterior 3.5; lateral 0.8 from bregma; vertical −5.0 from dura), in the ParCX (30° inclin., anterior 1.2; lateral 2.3 from bregma; vertical −5.0 from dura), and in the OccCX (posterior −6.8, lateral −5.8 from bregma; vertical −5.5 from dura), according to the atlas of Paxinos and Watson (1998), and then fixed on the skull with dental cement. Rats were housed in transparent plastic (Plexiglas) hemispheric bowls with food and water available.

Experiments were performed on freely moving rats 24 h after probe implant. A Ringer’s solution (147 mM NaCl; 2.2 mM CaCl2; 4 mM, KCl) was pumped through the dialysis probe at a constant rate of 1 μL/min. In some experiments a calcium-free Ringer’s solution, with Mg2+ in place of Ca2+ ions, was utilized (147 mM NaCl; 2.2 mM MgCl2; 4 mM KCl). Samples from PFCX, ParCX and OccCX were taken every 20 min and analysed.

Analytical procedure

Dialysate samples were injected without purification into HPLC apparatus equipped with reverse-phase column (C8 3.5 μm, Waters, Milford, MA, USA) and a coulometric detector (ESA Coulochem II, Bedford, MA, USA) to quantify DA and in some experiments also NA. The first electrode was set at +125 mV and the second electrode at −175 mV. The following solution was utilized as mobile phase: 0.1 M sodium acetate, 1.8 mM octanesulphonic acid, 0.3 mM Na2EDTA and 120 mM/L methanol. In a first series of experiments this solution was adjusted with acetic acid to pH 4.1. This pH, while allowing the reliable estimation of DA in dialysates, did not allow that of NA, due to the presence of an interfering peak. Therefore, since the experiments involving lesions of the DNAB bundle the pH of the solution was adjusted to pH 5.5 (with acetic acid), which allowed the reliable estimation of NA in addition to DA in dialysates from the various cortical areas. In the present study, whenever NA values are reported, they have been obtained using a mobile phase at pH 5.5. The sensitivity of the assay for DA and NA
was 2 fmol per sample. The mobile phase was pumped with a Jasco pump at a flow rate of 0.6 mL/min.

**Histology**

At the end of the experiment, rats were transcardially perfused with 100 mL of saline (0.9% NaCl) and 100 mL of formaldehyde (10%). The probes were removed, and brains were cut on a vibratome in serial coronal slices orientated according to Paxinos and Watson (1998) to locate the position of fibre.

**Statistics**

Analysis of variance (ANOVA) for repeated measures was applied to the data obtained from the serial assays of DA after each treatment. Results from treatments showing significant overall changes were subjected to post-hoc Tukey test with significance for means ± SEM of four subjects expressed as fmoles/sample). Basal values in OccCX: DA 7.4 ± 1.1, NA 32.7 ± 4.3; 1st sample: DA 4.7 ± 1.4 (−36%), NA 21.5 ± 1.7 (−41%); 2nd sample: DA 2.9 ± 0.4 (−70%), NA 5.5 ± 0.5 (−74%); 3rd sample: DA, undetectable; NA, undetectable (means ± SEM of four subjects expressed as fmoles/sample). Basal values in OccCX (10 mg/kg) (Fig. 3). Three-way ANOVA comparing the effects of reboxetine (5 and 10 mg/kg i.p.) on dialysate DA in the different brain areas showed a significant effect of area (F2, 12 = 51.7, p < 0.0001), dose (F2, 60 = 14.3, p < 0.0001) and time (F6, 360 = 15.2, p < 0.0001) and a significant area × dose × time interaction (F24, 360 = 6.3, p < 0.0001). Post-hoc test revealed that reboxetine significantly increased basal dialysate DA only in the PFCX.

**Reboxetine**

As shown in Fig. 2, reboxetine increased extracellular DA in a dose-dependent manner in the PFCX (two-way ANOVA [F(4,33)= 22.3, p < 0.0001]), but failed to modify DA output from the ParCX and from the OccCX. In the PFCX a low dose of reboxetine, 1.25 mg/kg, increased extracellular DA maximally by +56%, whereas doses of 5 and 10 mg/kg increased it by +84% and +122%, respectively. Doses of 20 mg/kg of reboxetine did not further increase the concentration of DA in the PFCX (data not shown). Two hours after administration of these doses of reboxetine the extracellular levels of DA were still high. Saline administration did not modify basal DA output in dialysates from the three areas. Three-way ANOVA, comparing the effects of reboxetine (5 and 10 mg/kg i.p.) on dialysate DA in the different brain areas, showed a main effect of area (F2, 60 = 51.7, p < 0.0001), dose (F2, 60 = 14.3, p < 0.0001) and time (F6, 360 = 15.2, p < 0.0001) and a significant area × dose × time interaction (F24, 360 = 6.3, p < 0.0001). Post-hoc test revealed that reboxetine significantly increased basal dialysate DA only in the PFCX.

**Desipramine**

A significant increase of dialysate DA was elicited by desipramine (5 and 10 mg/kg i.p.) in the PFCX (+93% and +175%, respectively) [two-way ANOVA (F2, 12 = 49.47, p < 0.0001)] but not in the ParCX (10 mg/kg) and in the OccCX (10 mg/kg) (Fig. 3). Three-way ANOVA comparing the effects of desipramine (10 mg/kg) on dialysate DA in the different brain areas showed a significant effect of area (F2, 18 = 42.0, p < 0.0001), dose (F1, 18 = 64.6, p < 0.0001) and time (F6, 108 = 10.4, p < 0.0001), and a significant area × dose × time interaction (F12, 108 = 7.5, p < 0.0001).
Post-hoc test revealed that desipramine increased basal dialysate DA in a dose-dependent manner in the PFCX, but failed to increase extracellular DA in the ParCX and OccCX.

GBR 12909

The selective dopamine reuptake inhibitor GBR 12909 increased dialysate DA in the PFCX at doses of 5 and 10 mg/kg i.p., whereas doses of 2.5 mg/kg did not modify it significantly (Fig. 4). In the ParCX, GBR 12909 increased DA only at the highest dose (10 mg/kg) and in the OccCX was ineffective at both doses (Fig. 4). Three-way ANOVA of the results obtained showed a significant effect of area ($F_{2,29} = 20.45$, $p < 0.0001$), dose ($F_{2,29} = 25.20$, $p < 0.0001$) and time ($F_{6,174} = 10.74$, $p < 0.0001$) and a significant area $\times$ dose $\times$ time interaction ($F_{24,174} = 1.73$, $p = 0.023$). Post-hoc analysis showed that GBR 12909, at the dose of 5 mg/kg, increased significantly basal dialysate DA only in the PFCX; at the dose of 10 mg/kg GBR 12909 increased basal dialysate DA in the PFCX more effectively than in the ParCX.

Mianserin

Figure 5 shows the effect of mianserin (0.5 and 1 mg/kg s.c.) on DA output from the three cortical areas. Mianserin increased extracellular DA equally in the PFCX, ParCX and OccCX.
Three-way ANOVA of the results obtained showed a significant effect of area ($F_{2, 36} = 5.34, \ p < 0.09$), dose ($F_{2, 36} = 36.02, \ p < 0.0001$), and time ($F_{6, 216} = 43.85, \ p < 0.0001$) and a significant area $\times$ dose $\times$ time interaction ($F_{24, 216} = 3.26, \ p < 0.0001$). Post-hoc test revealed that mianserin (0.5 and 1 mg/kg) significantly increased dialysate DA output in the three areas. Furthermore, the increase elicited by the lower dose of mianserin (0.5 mg/kg) was significantly higher in the OccCX than in the PFCX.

Clozapine
The effect of clozapine (2.5 and 5.0 mg/kg i.p.) on dialysate DA in the PFCX, ParCX and OccCX is shown in Fig. 6. Three-way ANOVA showed a significant effect of area ($F_{2, 36} = 15.3, \ p = 0.0001$), dose ($F_{2, 36} = 100.10, \ p < 0.0001$) and time ($F_{6, 216} = 50.92, \ p < 0.0001$) and a significant area $\times$ dose $\times$ time interaction ($F_{24, 216} = 2.73, \ p = 0.0006$). Post-hoc test after three-way ANOVA showed that clozapine increased dialysate DA in the PFCX only at the highest dose. At this dose the effect of clozapine was higher in the ParCX and in the OccCX than in the PFCX. Post-hoc test after two-way ANOVA with dose and time as factors showed a significant increase of DA in the PFCX also after 2.5 mg/kg [main effect of dose = ($F_{2, 14} = 26.84, \ p < 0.001$), of time = ($F_{6, 84} = 22.83, \ p < 0.0001$) and dose $\times$ time interaction = ($F_{12, 84} = 6.15, \ p < 0.0001$)].
Effect of dorsal bundle lesions on reboxetine and clozapine

Figure 7 shows the effect of 6-OHDA lesion of the DNAB on the increase of dialysate DA by reboxetine in the PFCX (Fig. 7a), and of clozapine on dialysate in the PFCX (Fig. 7b) and in the ParCX (Fig. 7c). DNAB lesions did not affect basal dialysate values of DA (fmoles/sample) in the PFCX and in the ParCX. Although one-way ANOVA of basal dialysate DA in the PFCX and in the ParCX did not show significant differences between sham and DNAB-lesioned groups in both cortical areas (PFCX: \( p = 0.13 \), and ParCX: \( p = 0.07 \)), in the ParCX the difference tended to approach the \( p < 0.05 \) level. In sham-lesioned rats, dialysate NA values were superimposable in the PFCX and in the ParCX [28 ± 2.3 \(( n = 11)\) and 24 ± 2.4 \(( n = 3)\) fmoles/sample, respectively]. In DNAB-lesioned rats, NA values in dialysates were down to values below the sensitivity of the technique (< 2 fmoles).

In order to investigate if noradrenergic terminals contribute to the effect of reboxetine on dialysate DA in the PFCX, the effect of reboxetine (10 mg/kg, i.p.) was examined. As shown in Fig. 7, DNAB lesions completely prevented the increase of PFCX DA output induced by reboxetine (Fig. 7a). Two-way ANOVA of the results obtained in sham-
lesioned and DNAB-lesioned rats showed a significant effect of group \( (F_{1, 11} = 17.62, \ p < 0.0001) \) and lesion \( (F_{1, 11} = 96.32, \ p < 0.0001) \) and a significant area \times \) time interaction \( (F_{6, 66} = 13.75, \ p < 0.0001) \). Post-hoc test revealed that reboxetine increases dialysate DA in the PFCX of sham-lesioned rats but not of DNAB-lesioned rats.

Figures 7(b) and (c) show the effect of clozapine (5 mg/kg i.p.) on dialysate DA of the PFCX and ParCX in sham and DNAB-lesioned rats. Three-way ANOVA of the results obtained showed a significant effect of area \( (F_{1, 11} = 17.62, \ p = 0.001) \) and lesion \( (F_{1, 11} = 96.32, \ p < 0.0001) \) and time \( (F_{6, 66} = 29.90, \ p < 0.0001) \) and a significant area \times \) lesion \times \) time interaction \( (F_{6, 66} = 10.03, \ p < 0.0001) \). Post-hoc test revealed that clozapine increases dialysate DA in the PFCX and in the ParCX of sham-lesioned rats but not of DNAB-lesioned rats and this increase were higher in the ParCX than in the PFCX.

Reboxetine attenuates the increase of DA by clozapine and mianserin in the ParCX

Figure 8 shows that the pre-treatment with reboxetine (5 mg/kg i.p.) reduces the effect of clozapine and mianserin on DA output from the ParCX. Two-way ANOVA of the results obtained showed after clozapine a significant effect of group \( (F_{1, 10} = 18.82, \ p < 0.001) \) and time \( (F_{11, 110} = 39.14, \ p < 0.0001) \) and a significant group \times \) time interaction \( (F_{11, 110} = 11.90, \ p < 0.0001) \) and after mianserin a significant effect of time \( (F_{11, 77} = 15.41, \ p < 0.0001) \) and a significant group \times \) time interaction \( (F_{11, 77} = 2.45, \ p = 0.01). Post-hoc test revealed that reboxetine attenuates the increase of dialysate DA in the ParCX induced by either drugs and this effect is more emphasized with clozapine.

Discussion

This study reports three major findings. First, reboxetine and desipramine, two relatively selective inhibitors of the NA reuptake carrier, differentially affect dialysate DA in the PFCX as compared to the ParCX and OccCX. Although reboxetine and desipramine dose dependently increased dialysate DA in the PFCX, they did not affect dialysate DA in the ParCX and OccCX. Second, mianserin and clozapine, in contrast to the two NA reuptake inhibitors, raised dialysate DA in the ParCX and OccCX to an even larger extent than in the PFCX. Third, 6-OHDA lesions of the DNAB, although reducing to unmeasurable levels dialysate NA in the PFCX and in the ParCX, did not modify basal dialysate DA in both cortical areas but abolished the increase of dialysate DA elicited by reboxetine in the PFCX and by clozapine both in the PFCX and in the ParCX. The inability of the two NA uptake inhibitors to raise dialysate DA in the ParCX and in the OccCX at doses that are fully active in raising dialysate DA in the PFCX suggests that in these areas the NA uptake carrier is differentially involved in the clearance of DA from the extracellular compartment. Assuming that the removal of DA from the extracellular compartment through the NA carrier is expressed by the increase of dialysate DA induced by its pharmacological blockade, the differential effect of NA carrier blockers on dialysate DA of the ParCX/OccCX as compared to the PFCX indicates that the contribution of the NA carrier to the clearance of extracellular DA, while remarkable in the PFCX, is insignificant in the ParCX and OccCX. This difference might be related to the lower ratio of DA to NA concentrations in the ParCX and OccCX compared to the PFCX. In fact, since extracellular DA and NA compete for binding to the NA transporter, the efficiency of DA clearance will depend on the ratio between its extracellular concentrations and those of NA. This ratio, as deduced directly from the relative concentrations of dialysate DA and NA reported in the present study, is lower in the ParCX and OccCX as compared to the PFCX (0.43 and 0.18 vs. 0.66). One might argue that this difference, particularly that between the ParCX and the PFCX, is rather small to fully justify the different response of dialysate DA to the NA reuptake inhibitors in the two areas. However, these observations are consistent at least qualitatively with the notion that the density of the DA innervation is higher in the PFCX as compared to the ParCX and OccCX (Berger et al. 1976, 1978; Lindvall et al. 1977, 1978; Berger 1992). It is possible that the basal differences in dialysate DA and NA in these cortices do not quantitatively reflect the differences between the different cortical areas in the extracellular concentrations of NA and DA.

If indeed in the ParCX and OccCx the NA transporter does not contribute to a significant extent to the clearance of DA from the extracellular space, one would expect that in these...
areas DA be cleared largely by other mechanisms, such as the DA transporter. This, however, does not appear to be the case, at least as judged from the failure of the specific inhibitor of the DA transporter, GBR 12909, to raise dialysate DA in the OccCX and by the lesser action in the ParCX as compared to the PFCX. In the PFCX, the maximal increase of extracellular induced by GBR 12909 was only 50% of basal, i.e. less than one fourth compared to the increase induced by NA reuptake blockers in the same area. This observation, while confirming the role of the NA carrier for the removal of DA from the extracellular space in the PFCX (Carboni et al. 1990; Pozzi et al. 1994; Mundorf et al. 2001; Moron et al. 2002), leaves open the issue of the mechanism by which DA clearance takes place in the ParCX/OccCX under basal conditions.

Devoto et al. (2001), using transcerebral probes, failed to observe differences in dialysate DA between the PFCX and the OccCX. Visual inspection of the diagrams in the study by Devoto et al. (2001) shows that their probes, whose dialysing surface extended for 8 mm, assayed a dorsally and laterally placed area of the frontal cortex. In this area the DA innervation becomes progressively sparser going medio-laterally (Berger et al. 1976; Lindvall et al. 1978). In our previous studies (Carboni et al. 1990; Tanda et al. 1994) this inadequacy of the transcerebral microdialysis technique was in part overcome by covering the whole dialysis membrane with epoxy glue except for a median 3 mm length corresponding to the medially most aspect of the dorsal prefrontal cortex, where the density of DA terminals is reportedly high. Therefore, the differences between our observations and those of Devoto et al. (2001) can be accounted for by the lower overall density of the DA innervation of the laterally placed frontal cortical areas assayed by their microdialysis probes. Indeed, more recently, the same group (Devoto et al. 2003), utilizing concentric probes in the PFCX and transcerebral probes in the OccCX, has reported a small difference (29%) in basal DA in dialysates from the two areas. However, direct comparison of these observations with the present ones is made difficult by the fact that whereas in the present study the same concentric probes with a 3 mm dialysing surface were utilized, in the study by Devoto et al. (2003) the two probes were different not only in geometry but also in dialysing surface (8 mm for the transcerebral OccCX probes and 4 mm for the concentric PFCX probes). As a result of this, while in our study dialysate values of DA and NA in the different cortical areas are directly comparable, in the study of Devoto et al. (2003) comparison of dialysate DA and NA in the PFCX and OccCX required normalization to unitary membrane length. This approach, however, fails to take into account differences in the recovery of DA from the extracellular compartment of the PFCX and OccCX due to the different probe geometry and non-linear behaviour of transmitter recovery by microdialytic membranes (Bungay et al. 2001).

In contrast to the effects of the NA uptake blockers, mianserin and clozapine were more effective in raising dialysate DA in the ParCX and in the OccCX compared to the PFCX. Thus, the lower dose of mianserin increased dialysate DA to a larger extent in the OccCX than in the PFCX. Moreover, both doses of clozapine increased dialysate DA to a larger extent in the OccCX than in the PFCX. The same also applied to the higher dose of clozapine in the ParCX. An increase of dialysate DA in the OccCX has been recently reported by Devoto et al. (2003). Given the potent a2 receptor blocking properties of clozapine and mianserin, the increase of DA induced by these drugs in the ParCX and OccCX can be accounted for by relief of the release of DA from an inhibitory tone exerted by endogenous NA through a2 receptors. The same mechanism has been proposed to explain the increase in dialysate DA induced by the same drugs in the PFCX (Chen et al. 1992; Moghaddam 1994; Tanda et al. 1996; Westerink et al. 1998; Millan et al. 2000a). Therefore, parietal and occipital areas share with prefrontal areas an a2-mediated control of DA transmission. Although clozapine has pronounced a1 receptor blocking properties (Brunello et al. 1995), a role of these properties in the increase of DA in cortical areas is unlikely, given the evidence that relatively selective blockade of a1 receptors by prazosin does not increase dialysate DA in the PFCX (Gobert et al. 1998). Indeed, stimulation of DA release in the cortex might eventually result from stimulation rather than blockade of a1 receptors in the ventral tegmental area (VTA), through facilitation of burst firing of DA neurones (Grenhoff et al. 1993, 1995). However, Hertel et al. (1999) and Linner et al. (2001) failed to increase extracellular DA in the medial PFCX after intra-VTA infusion of a2 antagonists or reboxetine. Therefore, an a1 mechanism located in the VTA might eventually amplify the increase of dialysate DA secondary to blockade of the NA carrier or of cortical presynaptic a2 receptors (Grenhoff et al. 1995) but is unlikely to play a primary role in the systemic effect of these drugs on extracellular DA in the PFCX. The above observations (Hertel et al. 1999) also tend to exclude an intra-VTA origin of the stimulatory effects of a2 blockade on DA release in the cortex.

The ParCX and OccCX do not seem to differ, at least qualitatively, from the PFCX as far as regards the control exerted by NA over the activity of DA transmission. This conclusion is strengthened by the finding that reboxetine, while not increasing per se dialysate DA in the ParCX, actually reduced the increase of DA induced by mianserin and clozapine. This interaction can be explained as the result of the increased inhibitory tone exerted by the rise of endogenous NA concentrations following reboxetine-induced blockade of the NA carrier. The observation that reboxetine reduced the increase of dialysate DA induced by mianserin and clozapine also indicates that endogenous NA increased by reboxetine efficiently competes with clozapine.
and mianserin for $\alpha_2$ receptors. If this interpretation is correct, one might hypothesize that the increase of extracellular DA in the PFCX by reboxetine and desipramine is the differential result of the interaction between two opposite influences of NA carrier blockade: inhibition of DA uptake by NA terminals, which tends to increase extracellular DA, increase of extracellular NA and stimulation of receptors, which tends to reduce the accumulation of extracellular DA by NA carrier blockade.

The interaction between reboxetine and mianserin/clozapine provides some insight on the contrasting influence exerted on extracellular DA by the blockade of the NA uptake carrier. In fact, the increase of extracellular NA following blockade of the NA carrier tends to reduce any increase of extracellular DA induced by blockade of DA uptake by the NA carrier. As a result of this, the final effect of an inhibitor of the NA carrier on extracellular DA in cortical areas will depend not only on the extent of the DA uptake through the NA carrier but also on the degree of saturation of the inhibitory $\alpha_2$-mediated mechanism. In view of this, the differential responsiveness of medial PFCX DA and ParCX/OccCX DA to NA uptake inhibitors might be at least in part related to strengthening of the $\alpha_2$-mediated inhibition of DA release by the increase of extracellular NA brought about by NA carrier blockade. This given, one might predict that down-regulation of inhibitory $\alpha_2$ control of DA transmission will result in the appearance of a stimulatory response of DA transmission in areas, such as the ParCX and the OccCX, that would be otherwise unresponsive. Indeed such a change might take place after chronic antidepressant administration (Invernizzi et al. 2001). Studies are currently in progress to investigate this possibility (Valentini et al. in preparation).

Throughout this discussion we have left open the issue of the precise intracortical origin of extracellular DA in cortical areas. Indeed the present results are, in principle, equally compatible with an origin of extracellular DA from DA as well as from NA neurones, as suggested by Devoto et al. (2001). Thus, the observation of the present study that DNAB lesions, which delete cortical NA transmission, also abolish the effects on dialysate DA of all the drugs we tested would not be incompatible with the possibility that DA is coreleased with NA and that its extracellular concentrations are controlled by the same sites that control extracellular NA. However, consistent with an origin of extracellular DA from cortical DA projections separate from NA projections (Carboni et al. 1990; Pozzi et al. 1994), 6-OHDA lesions did not significantly reduce extracellular DA in the PFCX as well as in the ParCX. It is notable, however, that in the ParCX we observed at least a tendency ($p < 0.07$) towards a decrease of basal extracellular DA after DNAB lesions. It is possible, therefore, that in the ParCX at least a portion of the extracellular DA pool is contributed as a cotransmitter by NA terminals. Along this vein one might also interpret the observation that GBR 12909, the selective inhibitor of the DA transporter, increased extracellular DA in the PFCX but not in the ParCX and OccCX, as deduced from post-hoc comparison with saline controls (see Results). The relative importance of the contribution of DA released from NA terminals to dialysate DA in the cortex will depend on the size of the DA innervation in a given cortical area. In the PFCX, where the density of the DA innervation is higher, the contribution of DA released from NA terminals would be relatively insignificant, whereas it might be more robust in the ParCX and in other isocortical areas. Clearly, a definitive answer to the above issues must await the results of selective lesioning of cortical DA innervation.

The observation of clear-cut topographic differences between the medial PFCX and isocortical areas in the neurochemical effects of NA-selective uptake inhibitors and $\alpha_2$ receptor antagonists raises the problem of their clinical significance. Thus, one might ask if differences in the ability to raise extracellular DA in isocortical areas, i.e. outside the medial PFCX, underlie differences in the therapeutic spectrum of these drugs. In relation to this it might be worth noting that in the isocortical areas, where relatively low basal levels of DA are present, one can show even more clearly than in the medial PFCX the consequences of blockade of $\alpha_2$ receptors for the regulation of DA transmission. Obviously, an answer to these issues requires a preliminary knowledge of the function of DA in isocortical areas that, at the moment, is largely unknown.

Acknowledgements
This study was performed with funds from Ministero dell’Università e della Ricerca, Centro di Eccellenza per le Dipendenze e Progetti di Interesse Nazionale. A preliminary account of these studies was presented at the 3rd FENS Forum, Paris, July 2002.

References
Brunello N., Masotto C., Steardo L., Markstein R. and Racagni G. (1995) New insights into the biology of schizophrenia through the...


