Alpha₂-adrenoceptor mediated co-release of dopamine and noradrenaline from noradrenergic neurons in the cerebral cortex

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Abstract
Previous results suggest that extracellular dopamine (DA) in the rat cerebral cortex originates from dopaminergic and noradrenergic terminals. To further clarify this issue, dialysate DA, dihydroxyphenylacetic acid (DOPAC) and noradrenaline (NA) were measured both in the medial prefrontal cortex (mPFC) and in the occipital cortex (OCC), with dense and scarce dopaminergic projections, respectively. Moreover, the effect of the α₂-receptor antagonist RS 79948 and the D₂-receptor antagonist haloperidol on extracellular DA, DOPAC and NA was investigated. Extracellular DA and DOPAC concentrations in the OCC were 43% and 9%, respectively, those in the mPFC. Haloperidol (0.1 mg/kg i.p.) increased DA and DOPAC (by 35% and 150%, respectively) in the mPFC, but was ineffective in the OCC. In contrast, RS 79948 (1.5 mg/kg i.p.) increased NA, DA and DOPAC, both in the mPFC (by approximately 50%, 60% and 130%, respectively) and the OCC (by approximately 50%, 80% and 200%, respectively). Locally perfused, the DA transporter blocker GBR 12909 (10 μM) was ineffective in either cortex, whereas desipramine (DMI, 100 μM) markedly increased extracellular NA and DA in both cortices. The weak haloperidol effect on DA efflux was not enhanced after DA- and NA-transporter blockade, whereas after DMI, RS 79948 markedly increased extracellular NA, and especially DA and DOPAC in both cortices. The results support the hypothesis that most extracellular DA in the cortex is co-released with NA from noradrenergic terminals, such co-release being primarily controlled by α₂-adrenoceptors.

Keywords: desipramine, dihydroxyphenylacetic acid, GBR 12909, microdialysis, occipital cortex, prefrontal cortex.

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Abbreviations used: DA, dopamine; DMI, desipramine; DOPAC, dihydroxyphenylacetic acid; mPFC, medial prefrontal cortex; OCC, occipital cortex; NA, noradrenaline.
To explain the concomitant changes in the extracellular levels of the two catecholamines in the mPFC, it has been suggested that most DA in the extracellular fluid is taken up by NA terminals, so that DA would compete with NA for the same NA transporter, for which the two catecholamines have similar affinity (Carboni et al. 1990; Pozzi et al. 1994). Consistent with this hypothesis (termed here ‘hetero-transport hypothesis’), while the DA transporter inhibitor GBR 12909 was found to produce no effect on DA reuptake in the mPFC, both in vitro (Moron et al. 2002) and in vivo (Mazei et al. 2002), the inhibition of NA transporter by desipramine (DMI) produced a concomitant increase in both catecholamines (Gresch et al. 1995); furthermore, DA uptake was greatly reduced in synaptosomes from NA transporter knockout mice (Moron et al. 2002).

These data have not precluded other interpretations, such as that the locus coeruleus NA neurons would activate the electrophysiological activity of meso-cortical DA neurons and elicit an action potential dependent release of DA in the mPFC (Herve et al. 1982; Linner et al. 2001). However, this hypothesis is inconsistent with the observation that the electrical stimulation of the locus coeruleus NA neurons elicits a brief stimulation followed by a long-lasting inhibition of the electrical activity of midbrain DA neurons (Grenhoff et al. 1993). Moreover, previous results indicate that a profound decrease and a large increase of both NA and DA was produced in the prefrontal cortex after local perfusion of the α2 agonist clonidine and the α2 antagonist idazoxan, respectively (Hertel et al. 1999; Devoto et al. 2001). This indicates that the coupling between DA and NA release is primarily regulated by α2-adrenoceptors at the nerve terminals level rather than via the electrical activation of dopaminergic neurons.

The alternative possibility that extracellular DA in the mPFC may derive in part from its release from noradrenergic terminals, where it resides as a precursor of NA, has been dismissed on the reasoning that, being less than 10% of tissue DA contained in noradrenergic terminals, this relatively small pool of DA could unlikely account for the large changes in extracellular DA accompanying NA changes in the above mentioned pharmacological or physiological conditions (Garris et al. 1993). Moreover, the possibility that DA from noradrenergic neurons might contribute to the extracellular DA content was ruled out by the observation that lesions of noradrenergic neurons with 6-hydroxydopamine produced no reduction in extracellular DA in the mPFC (Pozzi et al. 1994). However, against the latter conclusion it might be argued that an actual fall in DA from noradrenergic terminals might have been concealed either by a compensatory increase in DA output from dopaminergic terminals and spared noradrenergic ones and/or by the reduced retrieval of the amine from extracellular space due to the loss of NA transporter.

In spite of these contradictions, growing evidence from our laboratory supports the hypothesis (termed here ‘co-transmission hypothesis’) that extracellular DA in the mPFC, as well as in other cortices, may indeed originate other than from dopaminergic nerve terminals also from noradrenergic ones, where DA acts not only as NA precursor but as co-transmitter as well (Devoto et al. 2001, 2002a,b, 2003a,b).

This hypothesis is mainly based on the following arguments:

(i) Although tissue NA concentrations exceed those of DA in different cortices including the mPFC, the rate of DA synthesis is much faster than that of NA.

(ii) Extracellular DA concentrations in areas with scarce dopaminergic projections, such as the OCC and parietal cortices, were found to be similar, or a little smaller, than in cortices receiving dense dopaminergic projections, suggesting that DA is released from noradrenergic rather than dopaminergic neurons.

(iii) Treatments that modify noradrenergic activity were found to modify not only, as expected, extracellular NA, but also extracellular DA, both in the mPFC as in the OCC, receiving scarce dopaminergic afferents (Devoto et al. 2001, 2002a,b, 2003a,b).

The aim of this study was to further verify the hypothesis that DA may act as co-transmitter for noradrenergic neurons. Accordingly, extracellular DA, its deaminated metabolite dihydroxyphenylacetic acid (DOPAC) and NA in the mPFC, with dense dopaminergic innervation, and in the OCC, which is equally innervated by noradrenergic neurons but receives scarce dopaminergic terminals, were compared. Moreover, the effect of the D2-receptor antagonist haloperidol and the selective α2-adrenoceptor antagonist RS 79948, which should differently activate DA and NA neuronal activity, on extracellular DA, DOPAC and NA in both cortices was studied.

Finally, the relative contribution of the DA and NA transporter in the clearance of extracellular DA and NA from extracellular space in the mPFC and OCC was analysed.

Materials and methods

Animals

The experiments were performed on male Sprague-Dawley rats (Harlan Italy, S. Pietro al Natisone, Italy) weighing 250–280 g, housed in groups of five per cage prior to surgery and singly afterwards, at standard conditions of temperature and humidity and artificial light from 8.00 a.m. to 8.00 p.m.; food and water were available ad libitum. Experiments were approved by the local ethical committee and performed according to the Guidelines for Care and Use of Experimental Animals of the UE (EEC Council 86/609; D.L. 27/01/1992, no. 116).

Microdialysis

Each animal, anaesthetized with Equithesin (0.97 g pentobarbital, 2.1 g MgSO4, 4.25 g chloral hydrate, 42.8 mL propylene glycol,
11.5 mL 90% ethanol, distilled water up to 100 mL), was stereotaxically implanted, with one vertical microdialysis probe in the mPFC cortex (A +3.0, L ±7.0, V −6.5, 4 mm active membrane length), or one transversal microdialysis probe in the OCC (A −5.0, V −1.8 from bregma, 9 mm active membrane length), according to the rat brain atlas by Paxinos and Watson (1986). At the end of experiments, probe positions were histologically verified, and no rats with incorrect probe position were found. The day after surgery, artificial CSF (147 mM NaCl, 4 mM KCl, 1.5 mM CaCl2, pH 6–6.5) was pumped through the dialysis probes at a constant rate of 2.2 µL/min via a CMA/100 microinjection pump (Carnegie Medicine, Stockholm, Sweden). Dialysates were collected every 20 min, and NA, DA and DOPAC simultaneously analysed by HPLC, equipped with a 3.0 × 150 mm C18 (5 µ) Symmetry column (Waters, Milan, Italy) and an ESA Coulochem II detector (Chelmford, MA, USA). The mobile phase consisted of 80 mM Na2HPO4, 0.27 mM EDTA, 0.58 mM sodium octyl sulfate, 12% methanol, pH 2.8 with H3PO4, delivered by a model 307 Gilson pump (Gilson Italia, Milan, Italy) at 0.4 mL/min; the Coulochem analytical cell first electrode was set at +200 mV, the second at −300 mV. In these conditions, the detection limits (signal to noise ratio 3 : 1) were 0.5 pg of NA and DA and 2 pg of DOPAC on column.

Expression of results and statistics
In microdialysis experiments, the average concentration of three stable samples (less than 15% variation) before treatment was considered as the control and was defined as 100%. Thus, values are expressed as percentages of controls ± SEM. Statistical significance was evaluated by Student’s t-test or analysis of variance (ANOVA) for repeated measures, with Dunnett multiple comparison as post hoc test.

Drugs and treatments
RS 79948 [(2aR,2aS,13aS)-5,8,9a,10,11,12a,13a-decahydro-3-methoxy-12-(ethylsulfonyl)-6H-isooquinoline[2,1 g]1,6]naphthyridine hydrochloride and GBR 12909 [1-(2-((4-fluorophenyl)methoxy)ethyl)-4-(3-phenylpropyl)piperazine] dihydrochloride were purchased from Tocris Cookson (Bristol, UK), and desipramine hydrochloride (DMI) and haloperidol were purchased from Sigma-Aldrich (Milan, Italy). DMI and GBR 12909 were dissolved in artificial CSF and administered via reverse dialysis, RS 79948 was dissolved in saline, haloperidol was dissolved in a few microlitres of acetic acid, diluted in saline and buffered to pH 6.0–6.5 with NaOH.

Results
As reported in Table 1, extracellular NA concentrations in the mPFC and OCC were alike, moreover extracellular DA concentration in the mPFC was similar to that of NA, whereas in the OCC it was approximately one half that of NA. Notably, in the OCC, in spite of the scarce dopaminergic terminals, extracellular DA concentration was nearly one half that in the densely DA innervated mPFC. On the other hand, extracellular DOPAC in the OCC was less than 10% that in the mPFC. The D2 antagonist haloperidol (0.1 mg/kg i.p.) produced a modest increase in extracellular DA (35%) and a remarkable increase (150%) in DOPAC in the mPFC, but no significant changes in the OCC (Fig. 1).

On the other hand, the α2 antagonist RS 79948 (1.5 mg/kg i.p.) not only increased extracellular NA concentrations by approximately 50% in both cortices, as expected from the ability of α2 antagonists to activate noradrenergic neurons, but also increased DA and DOPAC both in the mPFC (by 60% and 130%, respectively) and in the OCC (by 80 and 200%, respectively) (Fig. 1).

In order to determine if the weak haloperidol effect on extracellular DA in the mPFC, or its lack of effect in the OCC, was due to the fact that most of DA released from dopaminergic terminals had been actually recaptured from extracellular fluid, the effect of haloperidol on extracellular DA was tested after inhibition of the DA transporter with GBR 12909 or of both DA and NA transporters with DMI. Indeed, it has been suggested that the NA transporter removes most DA from extracellular space (Carboni et al. 1990; Pozzi et al. 1994; Moron et al. 2002).

Table 1 Basal extracellular NA, DA and DOPAC concentrations in the rat medial prefrontal cortex and occipital cortex

| Cerebral area | NA     | DA     | DOPAC  |
|---------------|--------|--------|--------|
| mPFC (14)     | 0.77 ± 0.09 | 0.84 ± 0.11 | 44.90 ± 6.94 |
| OCC (11)      | 0.72 ± 0.05 | 0.36 ± 0.05 | 4.12 ± 0.38  |
| mPFC + DMI (12)| 8.33 ± 0.74a | 2.05 ± 0.17a | 31.05 ± 5.96 |
| OCC + DMI (12)| 7.26 ± 0.71a | 2.17 ± 0.32a | 2.81 ± 0.18a |
| mPFC + GBR (8)  | 1.09 ± 0.18 | 1.02 ± 0.15 | 29.76 ± 7.24 |
| OCC + GBR (6)  | 0.72 ± 0.16 | 0.46 ± 0.06 | 4.06 ± 0.44  |

Values are the means ± SEM of data obtained in the number of animals indicated in parenthesis, and are expressed as pg/dialysate injected on column (40 µL) normalized for unitary membrane length. DMI (100 µM DMI) or GBR 12909 (10 µM) were locally perfused via reverse dialysis, and basal values were obtained at the peak effect, 3–4 h after the start of local perfusion.

*p < 0.005 vs. corresponding value in the absence of uptake inhibitor (two-tailed unpaired Student’s t-test).

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Neither inhibitor significantly modified extracellular DOPAC in the mPFC, while DMI reduced DOPAC in the OCC by 30% (Table 1).

During GBR 12909 perfusion, haloperidol (0.1 mg/kg i.p.) produced 38% and 140% increase in extracellular DA and DOPAC, respectively, in the mPFC, but produced no significant changes in the OCC, and no effect on extracellular NA concentration in both cortices (Fig. 2) as observed after haloperidol alone.

On the other hand, during DMI perfusion, at the time extracellular DA and NA attained the highest plateau levels, the administration of haloperidol (0.1 mg/kg i.p.) increased DA by approximately 20%, i.e. less than in control conditions, failed to modify NA and increased DOPAC by approximately 100% in the mPFC. As in the control condition, haloperidol had no effect on DA, NA and DOPAC in the OCC of DMI-perfused rats (Fig. 3). In contrast, the administration of RS 79948 (1.5 mg/kg i.p.) to DMI-perfused rats increased not only NA efflux by approximately 100% in both cortices, but also produced a far greater increase in DA and DOPAC both in the mPFC (of 240% and 230%, respectively) and in the OCC (of 350% and 470%, respectively) (Fig. 3).

**Discussion**

Extracellular NA concentrations in the mPFC and in the OCC were approximately the same, consistent with the homogeneous distribution of noradrenergic projections in the cerebral cortex. On the other hand, extracellular DA concentrations in the OCC were higher than expected from the scarce dopaminergic innervation, being approximately 40% the respective DA concentrations in the mPFC.

To explain the discrepancy between the paucity of dopaminergic terminals and the relatively high concentration of extracellular DA in the OCC, we previously suggested that most DA and DOPAC in this area is formed, stored and released from noradrenergic neurons. We also suggested that a significant portion of extracellular DA and DOPAC in the
prefrontal cortex originates from noradrenergic neurons (Devoto et al. 2001). The finding that the DOPAC/DA ratio is higher in the mPFC than in the OCC might be explained with a greater amount of DOPAC formed in dopaminergic than in noradrenergic neurons.

The results of the experiments with the \( \alpha_2 \)- and \( D_2 \)-receptor antagonists, that distinctly activate noradrenergic and dopaminergic neurotransmission, support the hypothesis that DA may be co-released with NA from noradrenergic neurons and that such co-release is controlled by \( \alpha_2 \)-adrenoceptors.

Thus, the \( D_2 \)-receptor antagonist haloperidol produced a modest increase in DA and a remarkable increase in DOPAC in the mPFC but had no effect in the OCC, consistent with the idea that DA and DOPAC in the latter area do not reflect dopaminergic but noradrenergic innervation and activity. On the other hand, the \( \alpha_2 \)-adrenergic antagonist RS 79948 increased not only extracellular NA in both cortices, as expected from the ability of \( \alpha_2 \) inhibitors to activate noradrenergic transmission, but also increased extracellular DA and DOPAC both in the mPFC and in the ‘noradrenergic’ OCC. These results are in accord with the hypothesis that most of DA and DOPAC in the OCC and a significant portion in the mPFC may originate from noradrenergic terminals.

To explain the modest effect of haloperidol on extracellular DA in the mPFC we previously suggested that the majority of DA released from dopaminergic nerve terminals is actually recaptured by dopaminergic nerve terminals and deaminated to DOPAC (Devoto et al. 2003a). This possibility was conceivable since basal extracellular DOPAC concentration in the mPFC is approximately 50 times higher than that of DA and is markedly increased by haloperidol, even if one concedes that most DOPAC reflects the synthesis and not the reuptake of DA (Zetterstrom et al. 1988).

However, this hypothesis has to be rejected because haloperidol effect was not enhanced after the blockade of the DA transporter with GBR 12909 or the combined blockade of the NA and DA transporter with DMI. Therefore, these findings suggest that haloperidol is rather ineffective in releasing DA from dopaminergic nerve terminals, in spite of being potent and effective in activating both meso-cortical dopaminergic neurons (Gessa et al. 2000), and DA synthesis in the mPFC (Fadda et al. 1984).

Conceivably, the modest effect of haloperidol on DA release might depend, rather than on the pharmacological features of the drug, on the limited capacity of cortical dopaminergic neurons, at difference from noradrenergic ones, to release DA. Consistent with this possibility is the observation that even large increases in firing of dopaminergic neurons (Gessa et al. 2000) do not elicit changes in extracellular DA as large and long-lasting as those produced by RS 79948 (this study) or clozapine (Devoto et al. 2003a).

The weak haloperidol effect on DA release contrasts with that of clozapine, the prototype atypical antipsychotic, which markedly increases both NA and DA not only in the mPFC but also, and to a greater extent, in the OCC (Devoto et al. 2003a).

Consistent with our interpretation, clozapine effect on DA release does not depend on the activation of dopaminergic neuronal activity, but appears to be mediated by the inhibition of \( \alpha_2 \)-adrenoceptors, and the consequent co-release of DA and NA from activated noradrenergic neurons, as suggested in the present study for RS 79948 and previously for the \( \alpha_2 \) antagonist idazoxan (Devoto et al. 2001, 2003a).

The results of the experiments aimed at clarifying the relative role of DA and NA transporter in the clearance of DA from extracellular fluid indicated that blockade of DA transporter with GBR 12909 produced no significant increase in extracellular DA in either cortex, whereas the blockade of NA transporter with DMI increased DA both in the mPFC and OCC. These results provide apparent support to the hypothesis that most of extracellular DA in the mPFC, as well as in the OCC, is taken up by NA rather than DA nerve terminals (Carboni et al. 1990; Pozzi et al. 1994; Gresch et al. 1995; Moron et al. 2002). However, in contrast to the heterotransport hypothesis, we contend that DA taken up by noradrenergic neurons is that which actually originates from these same neurons – and not from dopaminergic ones. Consistent with this possibility, GBR 12909 did not enhance haloperidol effect on DA efflux, whereas after administration of DMI, RS 79948 not only further increased extracellular NA, as expected from the ability of \( \alpha_2 \) antagonists to stimulate NA neuronal activity, but also markedly increased extracellular DA and DOPAC in both cortices. Incidentally, the heterotransporter regulation hypothesis of extracellular DA presumes that extracellular DA, once released into the synapse, should diffuse from layers V and VI, where dopaminergic afferents prevalently terminate (Descarries et al. 1987), to cortical layer II and III, where most noradrenergic endings terminate (Seguela et al. 1990).

At variance from the co-transmission hypothesis, others have interpreted the changes in extracellular DA produced by \( \alpha_2 \)-adrenoceptor ligands as being mediated by \( \alpha_2 \)-heteroreceptors located on dopaminergic terminals (Yavich et al. 1997; Ihalainen and Tanila 2002). However, several considerations argue against this interpretation.

(i) \( \alpha_2 \)-Adrenoceptor antagonists increase DA efflux not only in the mPFC but also, and to a greater extent, in the ‘noradrenergic’ OCC.
(ii) \( \alpha_2 \) antagonists have no effect in two brain regions with dense dopaminergic innervation, the nucleus accumbens and the caudate nucleus, in spite of the presence of \( \alpha_2 \)-adrenoceptors (Hertel et al. 1999).
(iii) Lesions of the locus coeruleus noradrenergic neurons have been shown to suppress the effect of \( \alpha_2 \)-adrenocep-
tor antagonists and NA uptake blockers on DA release in the mPFC (G. Di Chiara, personal communication).

(iv) The local perfusion of the $\alpha_2$-adrenoceptor agonist clonidine into the locus coeruleus has been shown to reduce both extracellular NA and DA not only in the mPFC (Kawahara et al. 2001; Devoto et al. 2002a, 2003b) but in the OCC as well (Devoto et al. 2002a, 2003b).

These considerations do not negate the existence of $\alpha_2$-heteroreceptors on dopaminergic terminals, but they question the possibility that they might play a relevant role in controlling DA release, at least under the experimental conditions of our study.

The $\alpha_2$-adrenoceptor antagonist RS 79948 used in our study is not selective for $\alpha_2A$- or $\alpha_2C$-adrenoceptor subtypes, therefore our results do not allow to discriminate which $\alpha_2$-adrenoceptor subtype mediates the effect of the compound on DA and NA efflux. Therefore, although the $\alpha_2A$-adrenoceptor has been considered the predominant subtype in the brain and the major regulator of presynaptic autoinhibition of NA release in the CNS (Starke 2001; Lähdesmäki et al. 2003), additional work is needed to clarify this point.

Experiments in progress in our laboratory indicate that treatments that have been shown to produce concomitant increases in DA and NA in the mPFC (such as foot-shock, the atypical antipsychotic olanzapine, the antidepressant mirtazapine) do also produce the same increases in shock, the atypical antipsychotic olanzapine, the antidepressant mirtazapine) do also produce the same increases in

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