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The noradrenaline–dopamine interaction in the rat medial prefrontal cortex studied by multi-probe microdialysis

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Abstract

Multi-probe microdialysis was used to investigate the interaction between the release of noradrenaline and dopamine in the medial prefrontal cortex. Retrograde microdialysis was used to stimulate or inhibit the activity of the locus coeruleus for a restricted period of time, and the response of extracellular noradrenaline and dopamine in the ipsilateral and contralateral medial prefrontal cortex was recorded with microdialysis probes. Infusion of clonidine into the locus coeruleus (100 μM for 45 min) suppressed noradrenaline release and slightly inhibited dopamine release in the ipsilateral medial prefrontal cortex. Application of carbachol to the locus coeruleus (100 μM for 45 min) stimulated both the noradrenaline and dopamine release in the ipsilateral medial prefrontal cortex. No changes were seen in the contralateral medial prefrontal cortex. In the ipsilateral nucleus accumbens, extracellular noradrenaline levels increased, but dopamine levels remained unchanged. Application to the locus coeruleus (during 10 min) of the glutamate receptor agonists *N*-methyl-D-aspartate (NMDA) (300 μM) or kainate (100 μM) strongly increased extracellular noradrenaline and dopamine levels in the ipsilateral medial prefrontal cortex. However, in the contralateral probe the release of dopamine (but not of noradrenaline) was also stimulated. Application of carbachol to the locus coeruleus was used as a model to further investigate the presumed noradrenaline–dopamine interaction. In a series of dual-probe experiments, α_1-, α_2-, and β-adrenoceptor antagonists (prazosin, idazoxan, propranolol) or a reuptake-inhibitor (nomifensine) was administered during carbachol stimulation of the locus coeruleus. Prazosin and propranolol were administered systemically in a dose of 3 mg/kg, whereas idazoxan (10 μM) and nomifensine (100 μM) were infused into the medial prefrontal cortex. However, none of these pretreatments modified the effects of the control carbachol-infusions. The results did not identify a receptor-interaction or a common reuptake site that explained the presumed interaction between dopamine and noradrenaline in the medial prefrontal cortex. Therefore, the noradrenaline–dopamine interaction hypothesis could not be confirmed or refuted.

Keywords: Noradrenaline; Dopamine; Cortex, prefrontal; Microdialysis

1. Introduction

Dopamine and noradrenaline neurons that project to the frontal cortex are implicated in various complex types of behavior such as cognitive processes, arousal, stress responses, anxiety and fear (Foote et al., 1983; Aston Jones et al., 1991; Berridge and Foote, 1991; Tassin, 1992). The noradrenaline and dopamine neurons converge in the medial prefrontal cortex, and there is growing evidence for an interaction between the release of various catecholamines in this brain region. An anatomical connection at the level of the ventral tegmental area has been proposed (Tassin et al., 1986, Tassin, 1992; Grenhoff et al., 1993), whereas others have emphasized that a common reuptake site for noradrenaline and dopamine could explain the interaction (Carboni et al., 1990; Gresch et al., 1995; Yamamoto and Novotney, 1998). An example of the supposed interaction is the recent observation that a number of typical and atypical antipsychotics induced similar changes in the release of noradrenaline and dopamine in the medial prefrontal cortex (Li et al., 1998; Westerink et al., 1998).

The nature of the noradrenaline–dopamine interaction in the cortex is not fully understood. In this study, we used

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multi-probe microdialysis to further investigate this issue. To this end, a microdialysis probe was implanted in the vicinity of the locus coeruleus. The probe was used to infuse compounds that stimulate or inhibit locus coeruleus activity. A second probe, which was used to record extracellular dopamine and noradrenaline, was implanted in the ipsilateral medial prefrontal cortex. In additional experiments, a third probe was placed in the contralateral medial prefrontal cortex.

The noradrenaline–dopamine interaction hypothesis was further investigated by stimulating or inhibiting the activity of the locus coeruleus, for a restricted period of time, and by recording the response of extracellular noradrenaline and dopamine in the ipsilateral medial prefrontal cortex.

Various pharmacological agents known to affect the electrical activity of noradrenergic locus coeruleus neurons (clonidine, carbachol, *N*-methyl-D-aspartate (NMDA) and kainate) were infused in the vicinity of this nucleus and bilateral changes in extracellular noradrenaline and dopamine were recorded in the medial prefrontal cortex.

Infusion of carbachol into the locus coeruleus stimulated the release of noradrenaline as well as dopamine in the medial prefrontal cortex. No contralateral effects were seen. The noradrenaline–dopamine interaction was specific for the cortical area and was not observed in the nucleus accumbens.

Next, infusion of carbachol (for 45 min) into the locus coeruleus was used as a model to further investigate the mechanism of the noradrenaline–dopamine interaction. In a series of dual-probe experiments, α_1- and α_2- and β-adrenoceptor antagonists or a reuptake-inhibitor was administered, during concurrent stimulation of the locus coeruleus with carbachol.

2. Material and methods

2.1. Animals, drug treatment and doses

Male albino rats of a Wistar-derived strain (285–320 g; Harlan, Zeist, The Netherlands) were used for the experiments. The rats were housed in plastic cages ($35 \times 35 \times 40$ cm) and had free access to food and water.

The following drugs were used: clonidine HCl, carbachol, NMDA, nomifensine maleate, prazosin HCl, (±)-propranolol HCl, kainate (all purchased from Research Biochemicals, Natick, MA). Idazoxan was purchased from Sigma (St. Louis, MO, USA). Except prazosin and propranolol (which were administered subcutaneously), the drugs were dissolved in the perfusion fluid and were infused via retrograde microdialysis into the locus coeruleus or medial prefrontal cortex (in the case of nomifensine and idazoxan). Infused concentrations were based on previous studies with related experiments (Van Gaalen et al., 1997; Kawahara et al., 1999a,b)

The experiments were approved by the Animal Care Committee of the Faculty of Mathematics and Natural Science of the University of Groningen.

2.2. Surgery and brain dialysis

Microdialysis was performed with two (dual-probe) or three (triple-probe) I-shaped cannulas. The dialysis tube was prepared from polyacrylonitrile/sodium methalyl sulfonate copolymer (inner diameter 0.22 mm; outer diameter 0.31 mm; AN 69, Hospal, Bologna, Italy). One probe (exposed length 1.5 mm) was implanted in the vicinity of the locus coeruleus, the second probe (exposed length 4.0 mm) was implanted in the ipsilateral medial prefrontal cortex (or nucleus accumbens) and the third probe (exposed length 4.0 mm) was implanted (in the experiments depicted in Figs. 1–4) in the contralateral medial prefrontal cortex. The probe implanted in the locus coeruleus was used to deliver drugs and the probes implanted in the medial prefrontal cortex were used to record extracellular noradrenaline and dopamine. In two experiments (idazoxan and a high nomifensine concentration), drugs were delivered via the cortical probe. Coordinates of the implantation were as follows: locus coeruleus, A/P −3.3 mm, L/M 1.3 mm, V/D 8.3 mm, implanted under an angle 15° from lambda and dura medial; prefrontal cortex, A/P 3.3 mm, L/M 1.2 mm, V/D 5.0 mm from bregma and dura; nucleus accumbens, AP +2.5 mm, LM 1.3 mm, VP 7.3 mm. The probes and cannulas were implanted under chloral hydrate anesthesia (400 mg/kg, i.p.) and local application of lidocaine (10%).

Microdialysis experiments were carried out in conscious animals 24–48 h after probe implantation. The probes were perfused with a Ringer's solution at a flow rate of 2.0 μl/min (Beehave infusion pump, BAS, West Lafayette, IN, USA). The composition of the Ringer's solution was (in mM): NaCl 140.0, KCl 4.0, $CaCl_2$ 1.2, $MgCl_2$ 1.0. Fractions of 15 min were on-line collected in the sample loop of a high-performance liquid chromatography (HPLC) system. Connections to the infusion-pump and HPLC valve were made with flexible tubing (Peek, ID 0.12 mm). To favor the detection of noradrenaline and dopamine, the reuptake inhibitor nomifensine (10 μM) was added to the perfusion fluid (of the medial prefrontal cortex probe). Two experiments were performed without the addition of nomifensine: during recordings in the nucleus accumbens (Fig. 5) and when idazoxan was infused into the medial prefrontal cortex (Fig. 7).

Before the experiments were finished, implantation of the locus coeruleus probe was functionally evaluated by infusion of 100 μM clonidine (during 45 min) into the locus coeruleus. A decrease in extracellular noradrenaline in the ipsilateral medial prefrontal cortex to at least 30% of control was considered to reflect as an appropriate implantation. When the experiment was terminated, the rat was given an overdose of chloral hydrate and the brain was

fixed with 4% paraformaldehyde via intracardiac infusion. Coronal sections (40-μm thick) were made, and dialysis probe placement was localized according to the atlas of Paxinos and Watson (1982).

2.3. Chemical assays

Noradrenaline and dopamine were quantified by HPLC with electrochemical detection. A Shimadzu LC-10AD pump (Kyoto, Japan) was used in conjunction with an electrochemical detector (ESA, Bedford, MA). The first cell was set to a potential of +175 mV; the second cell to −300 mV. A reverse-phase column (150 × 4.6 mm; Supelco LC, Belleofonte, PA) was used. The mobile phase consisted of a mixture of 2 g citric acid monoanhydrate, 5 g sodium acetate and 620 mg heptanesulfonic acid in 900 ml H_20, and 100 ml/l methanol. The flow rate was 1.2 ml/min.

2.4. Expression of results and statistics

All values given are expressed as percentages of control. The average concentration of three stable baseline samples was defined as 100%. Statistical analysis was performed using one-way analysis of variance (ANOVA) with repeated measures and Dunnett's multiple comparison test for post-hoc determination of significant differences. The level of significance was set at $P < 0.05$.

3. Results

3.1. Basal values

The basal values of noradrenaline in the left and right prefrontal cortex did not differ. Separate control experiments (saline injections) were not included. Mean basal values were (±S.E.M.), in the presence of 10 μM nomifensine, noradrenaline 4.95 ± 0.33 fmol/min ($n = 43$) and dopamine 4.86 ± 0.57 fmol/min ($n = 43$). The basal values of the various experimental groups were not statistically different; therefore, there were grouped together. Basal values in the absence of nomifensine were noradrenaline 1.85 ± 0.46 fmol/min ($n = 4$) and dopamine 0.31 ± 0.12 fmol/min ($n = 4$). Basal values in the nucleus accumbens (without nomifensine) were noradrenaline 0.19 ± 0.05 fmol/min ($n = 4$) and dopamine 1.43 ± 0.48 fmol/min ($n = 4$).

3.2. Effects of clonidine, infused into the locus coeruleus, on the dialysate content of noradrenaline and dopamine in the ipsilateral and contralateral medial prefrontal cortex

Infusion of the α_2-adrenoceptor agonist clonidine (100 μM, 45 min) into the locus coeruleus caused a decrease in extracellular noradrenaline in the ipsilateral medial prefrontal cortex to about 25% of basal levels (Fig. 1). Extracellular concentrations of dopamine decreased to 70% of controls. These changes were statistically significant between 15 and 90 min (noradrenaline) and 30 and 90 min (dopamine) after the start of the infusion. No significant changes were seen in dialysate values of noradrenaline and dopamine in the contralateral medial prefrontal cortex. Clonidine infusion had no behavioral effect.

3.3. Effects of carbachol, infused into the locus coeruleus, on the dialysate content of noradrenaline and dopamine in the ipsilateral and contralateral medial prefrontal cortex

Infusion of the muscarinic receptor agonist carbachol (100 μM , 45 min) into the locus coeruleus caused an increase in extracellular noradrenaline in the ipsilateral medial prefrontal cortex to about 155% of basal levels, and an increase in extracellular dopamine to about 175% of control values (Fig. 2). These increases were statistically significant between 15 and 45 min (noradrenaline) and 30 and 60 min (dopamine) after the start of the carbachol

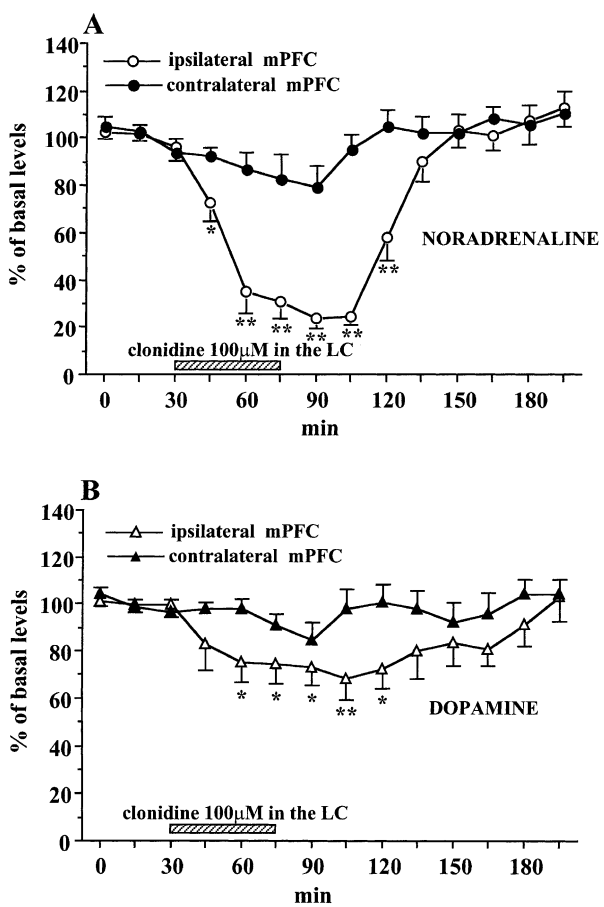

Fig. 1. Effect of infusion of clonidine (100 μM) into the locus coeruleus for 45 min (hatched bar) on the extracellular concentration of noradrenaline (A) and dopamine (B) in the ipsilateral (open symbols) and contralateral (closed symbols) medial prefrontal cortex of the conscious rat. Data are given as percentages of basal values ± E.M. and are the average of 11 experiments. $^{*}P < 0.05$; $^{**}P < 0.01$, compared to basal values.

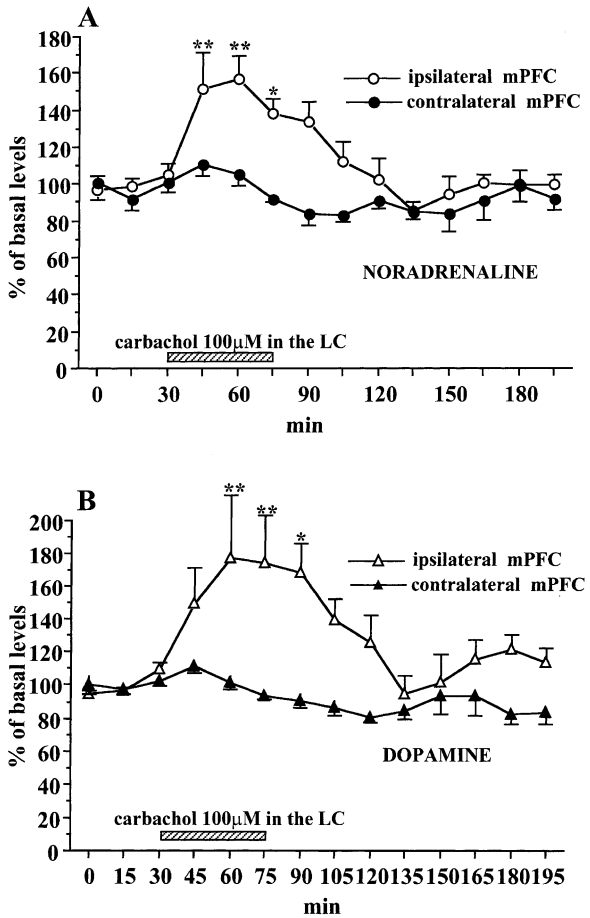

Fig. 2. Effect of infusion of carbachol (100 μM) into the locus coeruleus for 45 min (hatched bar) on the extracellular concentration of noradrenaline (A) and dopamine (B) in the ipsilateral (open symbols) and contralateral (closed symbols) medial prefrontal cortex of the conscious rat. Data are given as percentages of basal values ± S.E.M. and are the average of six experiments. $^{*}P < 0.05$; $^{**}P < 0.01$, compared to basal values.

infusion. No effects were seen during carbachol infusion on extracellular noradrenaline and dopamine in the contralateral medial prefrontal cortex. The infusion of carbachol had no behavioral effect.

3.4. Effects of NMDA, infused into the locus coeruleus, on the dialysate content of noradrenaline and dopamine in the ipsilateral and contralateral prefrontal cortex

Infusion of the glutamate receptor agonist NMDA (300 μM, 10 min) into the locus coeruleus caused an increase in extracellular noradrenaline in the ipsilateral medial prefrontal cortex to about 165% of controls and an increase in extracellular dopamine to about 160% of controls (Fig. 3). These increases were statistically significant 15 min (noradrenaline) and between 15 and 30 min (dopamine) after the start of the infusion. NMDA caused no change in extracellular noradrenaline in the contralateral medial prefrontal cortex, but dopamine values increased to 140% of control in this brain area. The latter effect was statistically significant 15 min after infusion. The rats showed some behavioral activation during the NMDA infusion. The rats displayed chewing and grooming, and occasionally some locomotion and turning was observed. The activation lasted for about 10 min, after which the animals returned to their usual resting state.

3.5. Effects of kainate, infused into the locus coeruleus, on the dialysate content of noradrenaline and dopamine in the ipsilateral and contralateral medial prefrontal cortex

Infusion of the glutamate receptor agonist kainate (100 μM, 10 min) into the locus coeruleus caused a strong increase in extracellular noradrenaline in the ipsilateral medial prefrontal cortex to about 215% of control and a more pronounced increase in extracellular dopamine to about 320% of control (Fig. 4). These increases were statistically significant between 15 and 45 min (noradrenaline) and 15 and 60 min (dopamine) after the start of the infusion of kainate. Kainate caused no change in extracellular noradrenaline in the contralateral medial prefrontal cortex, but dopamine values increased to 140% of control

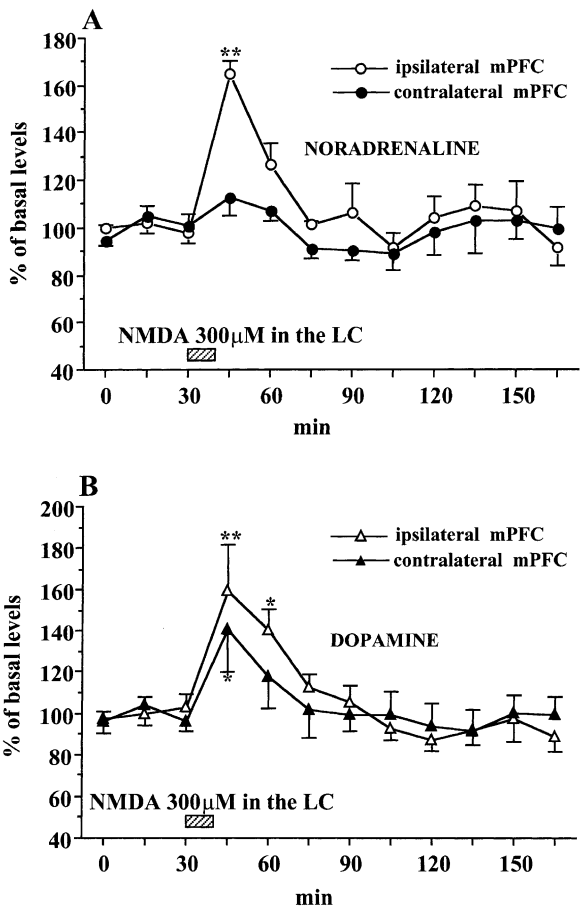

Fig. 3. Effect of infusion of NMDA (300 μM) into the locus coeruleus for 10 min (hatched bar) on the extracellular concentration of noradrenaline (A) and dopamine (B) in the ipsilateral (open symbols) and contralateral (closed symbols) medial prefrontal cortex of the conscious rat. Data are given as percentages of basal values ± S.E.M. and are the average of seven experiments. $^{*}P < 0.05$; $^{**}P < 0.01$, compared to basal values.

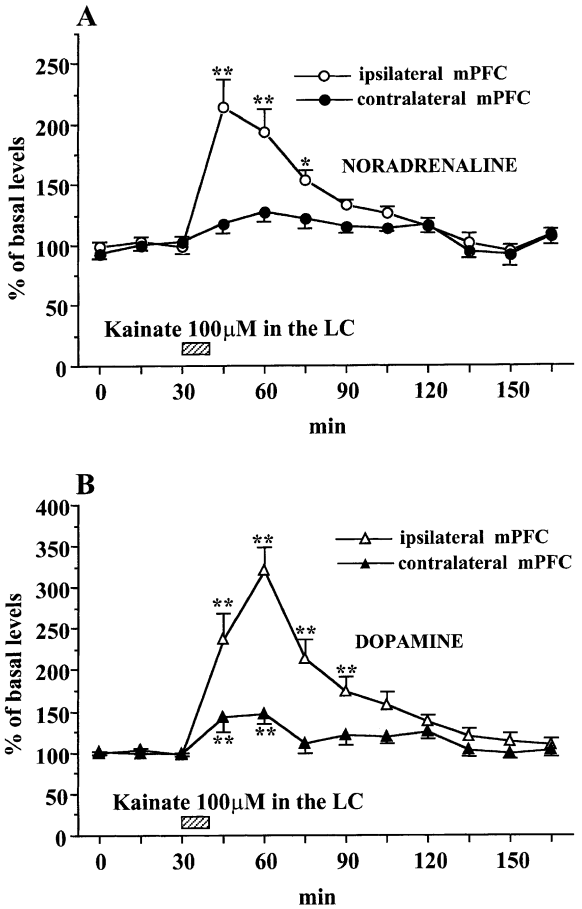

Fig. 4. Effect of infusion of kainate (100 μM) into the locus coeruleus for 10 min (hatched bar) on the extracellular concentration of noradrenaline (A) and dopamine (B) in the ipsilateral (open symbols) and contralateral (closed symbols) medial prefrontal cortex of the conscious rat. Data are given as percentages of basal values ± S.E.M. and are the average of 11 experiments. $^{*}P < 0.05$; $^{**}P < 0.01$, compared to basal values.

in this brain area. The latter effect was statistically significant between 15 and 30 min after the start of the infusion. The rats showed some behavioral activation during the kainate infusion. The rats displayed chewing and grooming, and occasionally some locomotion and turning was observed. The activation lasted about 10 min, after which the animals returned to their usual resting state.

3.6. Effect of carbachol, infused into the locus coeruleus, on the dialysate content of noradrenaline and dopamine in the ipsilateral nucleus accumbens

To investigate whether the noradrenaline–dopamine interaction was also detectable outside the cortex, a probe was implanted into the ipsilateral nucleus accumbens. Carbachol infusions into the locus coeruleus clearly stimulated extracellular noradrenaline in the ipsilateral accumbens to about 200% of control, but no effect was seen on extracellular dopamine (Fig. 5). The effect on noradrenaline was statistically significant between 30 and 60 min after the start of the carbachol infusion.

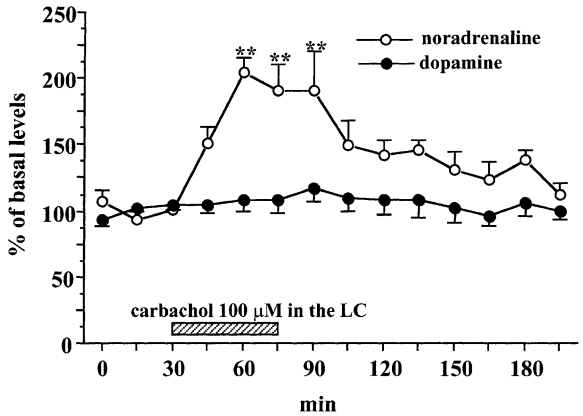

Fig. 5. Effect of infusion of carbachol (100 μM) into the locus coeruleus for 45 min (hatched bar) on the extracellular concentration of noradrenaline (open circle) and dopamine (closed circle) in the nucleus accumbens of the conscious rat. Data are given as percentages of basal values ± S.E.M. and are the average of six experiments. $^{**}P < 0.01$, compared to basal values.

3.7. Effect of carbachol, infused into the locus coeruleus, on the dialysate content of noradrenaline and dopamine in the ipsilateral medial prefrontal cortex: effect of prazosin pretreatment

Pretreatment with the α_1-adrenoceptor antagonist prazosin (3 mg/kg s.c.) increased the extracellular levels of noradrenaline to about 125% of basal levels. This increase did not reach the level of statistical significance (Fig. 6). Dopamine levels in the medial prefrontal cortex were unchanged. Stimulation of the locus coeruleus by carbachol infusion induced a further increase in extracellular levels of noradrenaline as well as dopamine in the ipsilateral medial prefrontal cortex to about 220% of control. The

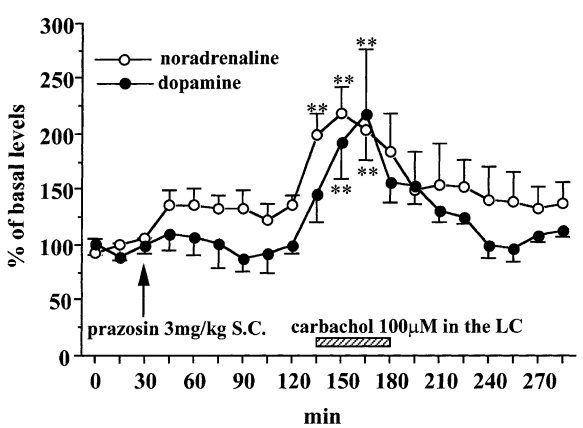

Fig. 6. Effect of infusion of carbachol (100 μM) into the locus coeruleus for 45 min (hatched bar) on the extracellular concentration of noradrenaline (open circle) and dopamine (closed circle) in the ipsilateral medial prefrontal cortex of the conscious rat, during systemic administration of prazosin (3 mg/kg s.c.). Data are given as percentages of basal values ± S.E.M. and are the average of four experiments. $^{**}P < 0.01$, compared to $t = 120$.

increase in noradrenaline was statistically significant between 15 and 45 min after the start of the carbachol infusion. The increase in dopamine was statistically significant between 30 and 45 min after the start of the infusion. The area under the curve of the effects of carbachol on the release of dopamine and noradrenaline is shown in Fig. 10. The prazosin-modified noradrenaline levels were reset to 100% at $t = 120$ min. The effect of carbachol after prazosin pretreatment did not differ from that in the control experiments (Fig. 10).

3.8. Effect of carbachol, infused into the locus coeruleus, on the dialysate content of noradrenaline and dopamine in the ipsilateral medial prefrontal cortex: effect of idazoxan infused via the medial prefrontal cortex probe

Infusion of the α_2-adrenoceptor antagonist idazoxan via the medial prefrontal cortex probe, in a concentration of 10 μM, increased the extracellular level of noradrenaline and dopamine to 175% and 150% of basal levels, respectively. The increase did not reach the level of statistical significance. Stimulation of the locus coeruleus by carbachol infusion (Fig. 7) induced a further rise in extracellular levels of noradrenaline and dopamine in the ipsilateral medial prefrontal cortex to about 325% of controls for both transmitters. The increase in noradrenaline was statistically significant between 15 and 45 min after the start of the carbachol infusion. The increase in dopamine was statistically significant between 30 min after the start of the infusion. The area under the curve of the effects of carbachol is shown in Fig. 10. The idazoxan-modified noradrenaline and dopamine levels were reset to 100% at $t = 150$ min. The effect of carbachol on the release of dopamine and noradrenaline, during idazoxan infusion, did not differ from that in the control experiments (Fig. 10).

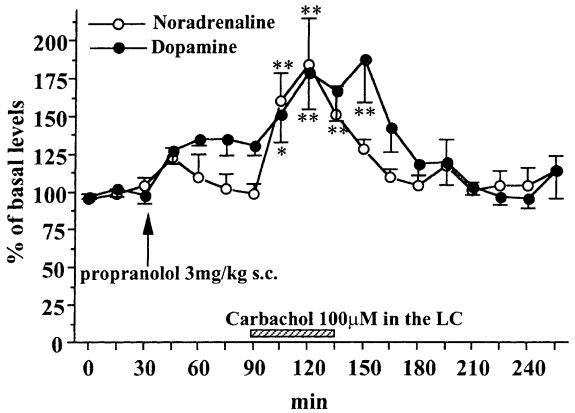

Fig. 8. Effect of infusion of carbachol (100 μM) into the locus coeruleus for 45 min (hatched bar) on the extracellular concentration of noradrenaline (open circle) and dopamine (closed circle) in the ipsilateral medial prefrontal cortex of the conscious rat, during systemic administration of propranolol (3 mg/kg s.c.). Data are given as percentages of basal values ± S.E.M. and are the average of four experiments. $^{**}P < 0.01$, compared to $t = 150$.

The experiment was repeated with an idazoxan concentration of 50 μM. Similar results were obtained (data not shown).

To prevent a direct interaction with the reuptake sites, the idazoxan experiments were carried out without addition of nomifensine to the perfusion fluid.

3.9. Effects of carbachol, applied to the locus coeruleus, on extracellular levels of noradrenaline and dopamine in the ipsilateral medial prefrontal cortex: effect of β-adrenoceptor blockade.

Pretreatment with the β-adrenoceptor antagonist (±)-propranolol (3 mg/kg s.c.) increased the extracellular levels of dopamine to about 135% of basal levels, but this

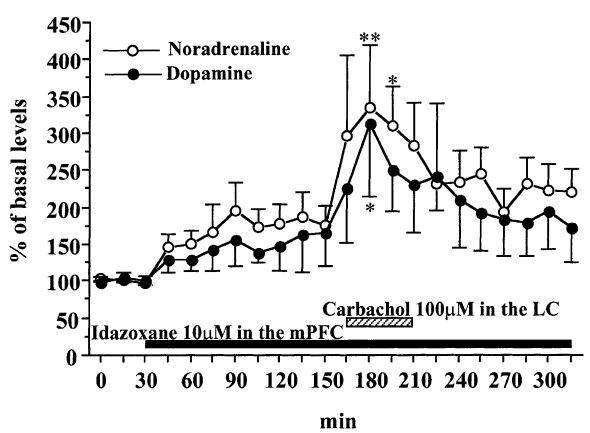

Fig. 7. Effect of infusion of carbachol (100 μM) into the locus coeruleus for 45 min (hatched bar) on the extracellular concentration of noradrenaline (open circle) and dopamine (closed circle) in the ipsilateral medial prefrontal cortex of the conscious rat, during local application of idazoxan (10 μM; black bar). Data are given as percentages of basal values ± S.E.M. and are the average of four experiments. $^{*}P < 0.05$; $^{**}P < 0.01$, compared to $t = 120$.

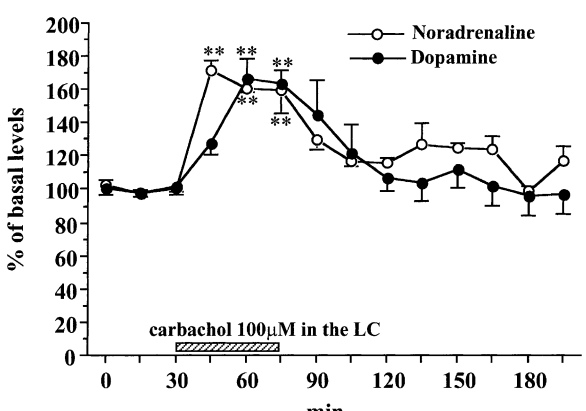

Fig. 9. Effect of infusion of carbachol (100 μM) into the locus coeruleus for 45 min (hatched bar) on the extracellular concentration of noradrenaline (open circle) and dopamine (closed circle) in the ipsilateral medial prefrontal cortex of the conscious rat, during local application of nomifensine (100 μM; continuously added). Data are given as percentages of basal values ± S.E.M. and are the average of four experiments. $^{**}P < 0.01$, compared to basal values.

increase did not reach the level of statistical significance (Fig. 8). Noradrenaline levels in the medial prefrontal cortex were unchanged. Stimulation of the locus coeruleus by carbachol infusion induced a further increase in extracellular levels of noradrenaline and dopamine in the ipsilateral medial prefrontal cortex to 180% and 185% of control, respectively. The increase in noradrenaline was statistically significant between 15 and 45 min after the start of the carbachol infusion. The increase in dopamine was statistically significant between 15 and 60 min after the start of the infusion.

The area under the curve of the effects of carbachol is shown in Fig. 10. The propranolol-modified dopamine levels were reset to 100% at $t = 90$ min. The effect of carbachol on the release of dopamine and noradrenaline, after propranolol pretreatment, did not differ from that in the control experiments (Fig. 10).

3.10. Effects of carbachol, applied to the locus coeruleus, on extracellular levels of noradrenaline and dopamine in the ipsilateral medial prefrontal cortex: effect of reuptake blockade

High levels of nomifensine (100 μM) were infused via the medial prefrontal cortex probe. Basal values ± S.E.M. ($n = 4$) of noradrenaline and dopamine in the medial prefrontal cortex dialysates during nomifensine rose to 27.1 ± 3.5 fmol/min ($n = 4$) and 9.5 ± 3.3 fmol/min, respectively (Fig. 9). These values were defined as 100%. Stimulation of the locus coeruleus by carbachol infusion increased extracellular levels of noradrenaline and dopamine in the ipsilateral medial prefrontal cortex to about 160% of control. The increase in noradrenaline was statistically significant between 15 and 45 min after the start of the carbachol infusion. The increase in dopamine was statistically significant between 30 and 45 min after the start of the infusion.

The area under the curve of these effects is shown in Fig. 10. The increases in noradrenaline and dopamine in the medial prefrontal cortex did not differ from those in the control carbachol experiments (Fig. 10), in which 10 μM nomifensine was added to the perfusion fluid (Fig. 2).

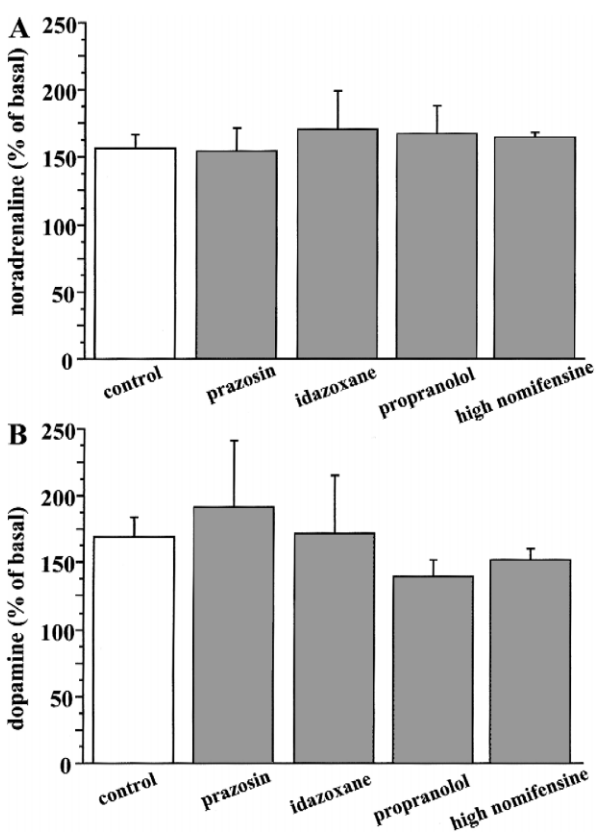

Fig. 10. Comparison of areas under the curve (± S.E.M.) of the carbachol (100 μM infused into the locus coeruleus)-induced increases in extracellular noradrenaline (A) and dopamine (B) in the ipsilateral medial prefrontal cortex. Data are obtained from the experiments shown in Figs. 6–9. There was no significant change compared to that in the control carbachol-infusion group.

4. Discussion

Various authors have speculated about a coupling between the release of noradrenaline and dopamine in the medial prefrontal cortex (Tassin et al., 1986; Carboni et al., 1990; Tassin, 1992; Grenhoff et al., 1993; Gresch et al., 1995; Yamamoto and Novotney, 1998). A possible direct interaction between these neurons has important pharmacological implications, because it means that psychotropic drugs that modify cortical noradrenaline release will also modify dopamine activity. Here, we used multiprobe microdialysis to further investigate this issue. The technique of retrograde microdialysis was used to stimulate or inhibit the activity of the locus coeruleus for a restricted period of time, and the response of extracellular noradrenaline and dopamine in the medial prefrontal cortex was recorded.

4.1. Changes in noradrenaline

Inhibition of locus coeruleus activity by local application of clonidine resulted in a pronounced decrease in the release of noradrenaline in the medial prefrontal cortex, whereas stimulation of the locus coeruleus by carbachol, NMDA or kainate increased extracellular noradrenaline in the cortical area (Van Gaalen et al., 1997). These effects are explained by the presence of α_2-adrenoceptors, muscarinic receptors and NMDA as well as non-NMDA glutamate receptors on locus coeruleus neurons (Freedman and Aghajanian, 1984; Engberg and Svensson, 1980; Olpe et al., 1989; Aston Jones et al., 1991; Luque et al., 1995).

All these manipulations had no statistically significant effects on the release of noradrenaline in the contralateral medial prefrontal cortex, although there was a tendency to follow the changes in extracellular noradrenaline in the ipsilateral cortex. Two conclusions can be drawn from these findings. First, the data provide direct evidence that

the two locus coeruleus noradrenergic pathways are not directly linked. This is in good agreement with an anatomical study (Waterhouse et al., 1983) describing that locus coeruleus neurons labeled by cerebrocortical injections of horseradish peroxidase are primarily located in the ipsilateral and, to a lesser extent, (fewer than 5% of total labeled cells) in the contralateral locus coeruleus. The tendency of extracellular noradrenaline in the contralateral medial prefrontal cortex to follow the ipsilateral changes could well be explained by the small overlap between the two pathways that was observed in the study of Waterhouse et al. (1983).

Secondly, kainate and NMDA, when applied to the locus coeruleus, induced some behavioral activation of the rat. These behavioral changes might increase the levels of noradrenaline in the cortex. However, the finding that no effects were seen on noradrenaline levels in the contralateral medial prefrontal cortex does not support such indirect effects.

4.2. Is the release of noradrenaline and dopamine in the medial prefrontal cortex coupled?

The similarity in both the time and effect of changes in extracellular noradrenaline and dopamine in the ipsilateral medial prefrontal cortex, during carbachol application to the locus coeruleus, suggest a close coupling between the two catecholamines (Fig. 2). The stimulation was restricted to the unilateral part of the brain and also to the cortical areas because extracellular dopamine in the ipsilateral nucleus accumbens was not modified (Fig. 5).

The effects of inhibiting locus coeruleus activity with clonidine were less clearcut with respect to extracellular noradrenaline and dopamine in the medial prefrontal cortex. The finding that noradrenaline was more strongly suppressed than dopamine suggests that the presumed coupling between noradrenaline and dopamine is limited during stimulatory conditions. A low tonic activation of the mesocortical dopaminergic neurons by the locus coeruleus neurons might explain the observation that inactivation of the locus coeruleus is less effective than activation.

The results with carbachol were mimicked by stimulating the locus coeruleus with the specific muscarinic agonist oxotremorine (data not shown). In addition, when the locus coeruleus was stimulated with the $GABA_A$ receptor antagonist bicuculline, extracellular levels of noradrenaline and dopamine in the ipsilateral medial prefrontal cortex were enhanced to the same extent (data not shown).

However, when the locus coeruleus was stimulated by infusion of NMDA or kainate, the data on extracellular dopamine in the cortical areas were more difficult to interpret. During application of carbachol or NMDA to the locus coeruleus, the rise in extracellular noradrenaline and dopamine levels in the medial prefrontal cortex was very similar in both time and effect. However, during kainate infusion the dopamine content in dialysates of the medial prefrontal cortex rose more (to 320% of controls) than did the noradrenaline levels (to 215% of controls), indicating that another mechanism might participate. This different mechanism might be responsible for the observation that stimulation of the locus coeruleus with kainate or NMDA enhanced the release of dopamine in the contralateral medial prefrontal cortex as well .

The increase in the release of dopamine in the contralateral medial prefrontal cortex was not the result of a noradrenaline–dopamine interaction, because noradrenaline levels were unchanged in the contralateral medial prefrontal cortex. Kainate as well as NMDA induced a mild behavioral activation, which might complicate the interpretation of changes in dopamine release in the medial prefrontal cortex. It is speculated that some of the bilateral effects on dopamine are secondary to the behavioral activation. The results from the kainate and NMDA experiments indicate that the dopamine–noradrenaline interaction in the medial prefrontal cortex is not as tight as suggested by the results of the carbachol experiments.

4.3. Investigating the mechanism of action of the noradrenaline–dopamine coupling

Two hypotheses have been put forward to explain the interaction between noradrenaline and dopamine in the medial prefrontal cortex. First, it has been suggested that anatomical connections between the locus coeruleus and the ventral tegmental area, in which α_1-adrenoceptors participate at the level of the ventral tegmental area, are responsible for the noradrenaline–dopamine interaction in the medial prefrontal cortex (Tassin et al., 1986; Tassin, 1992; Grenhoff et al., 1993). Secondly, it has been suggested (Gresch et al., 1995; Yamamoto and Novotney, 1998) that (re)uptake mechanisms play a dominant role in the noradrenaline–dopamine interaction. The finding that some of the dopamine released in the medial prefrontal cortex is taken up by noradrenergic terminals is a possible explanation for the observed interaction (Carboni et al., 1990).

In the second part of this study, the two hypothesized mechanisms of action for the noradrenaline–dopamine interaction were further investigated using carbachol-infusions into the locus coeruleus as an experimental model. The first hypothesis was evaluated by pretreating rats with the α_1-adrenoceptor antagonist prazosin, but the effects of the carbachol-infusions were not modified by prazosin (Fig. 6). Next, we studied the effect of the α_2-adrenoceptor antagonist idazoxan. Idazoxan itself induces a strong increase in extracellular noradrenaline after systemic administration (Thomas and Holman, 1991), whereas infused idazoxan had few effects on the release of the catecholamines (Fig. 7). We therefore infused idazoxan into the medial prefrontal cortex during stimulation of the locus coeruleus with carbachol. However, idazoxan did not modify the effects of locus coeruleus stimulation (Fig. 7).

Likewise, administration of the β-adrenoceptor antagonist propranolol was not effective.

Also, no effects were seen when high concentrations of the reuptake inhibitor nomifensine were infused.

Taken together, the results mean that it was not possible to identify an adrenoceptor involved in a possible anatomical interaction between noradrenaline and dopamine neurons, although it cannot be excluded that α_2 adrenoceptors localized outside the medial prefrontal cortex might be responsible for the interaction. In addition, no support was found for the assumption that a common reuptake mechanism is responsible for the noradrenaline–dopamine interaction.

4.4. The noradrenaline–dopamine interaction questioned

The fact that no mechanism of action could be identified for the carbachol-induced simultaneous increase in extracellular noradrenaline and dopamine in the medial prefrontal cortex questions the existence of the assumed interaction. There are more data that contradict a noradrenaline–dopamine interaction in the medial prefrontal cortex. We noticed already that—based on the infusions with kainate or NMDA—dopamine increased contralaterally without a concomitant increase in noradrenaline. Another argument against a noradrenaline–dopamine interaction is found in the observation that the release of dopamine and noradrenaline in the medial prefrontal cortex often changes independently. For example, lesions of the locus coeruleus do not prevent the effect of handling stress or hypotension on dopamine release in the medial prefrontal cortex (Kawahara et al., 1999b). Moreover, certain pharmacological treatments induce different effects on catecholamine levels in the medial prefrontal cortex, e.g. prazosin pretreatment increased the release of noradrenaline, but not of dopamine (Fig. 6), whereas the reverse was true in the case of propranolol (Fig. 8). The observation that a variety of antipsychotics induced similar increases in the release of dopamine in the medial prefrontal cortex was recently explained by a common 5-HT_2 serotonin receptor (Westerink et al., 2001).

It is concluded that a direct coupling between the release of noradrenaline and dopamine in the medial prefrontal cortex could not unequivocally be established. Under certain conditions (e.g. during stimulation of the locus coeruleus) such an interaction might occur, but the adrenergic receptor that mediates this interaction could not be identified. Therefore, the noradrenaline–dopamine interaction hypothesis could not be confirmed or refuted.

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