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The role of afferents to the locus coeruleus in the handling stress-induced increase in the release of noradrenaline in the medial prefrontal cortex: a dual-probe microdialysis study in the rat brain

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Abstract

This study was aimed to identify the neuronal pathways that mediate the handling stress-induced increase in the release of noradrenaline in the medial prefrontal cortex of the rat brain. For that purpose a microdialysis probe was implanted in the vicinity of the locus coeruleus and a second probe was placed in the ipsilateral medial prefrontal cortex. Receptor specific antagonists acting on the α_2-adrenoceptor (50 μM idazoxan), $GABA_A$ (50 μM bicuculline), $GABA_B$ (100 μM (3,4-Dichlorophenyl)methyl]propyl](diethoxymethyl) phosphonic acid; CGP 52432), acetylcholine (10 μM atropine), corticotropin releasing factor (CRF) (100 μM butyl-ethyl-[2,5-dimethyl-7-(2,4,6-trimethyl-phenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl]-amine; CP-154,526), NMDA glutamate (300 μM (±)-3(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid; CPP) and non-NMDA glutamate receptors (500 μM 6,7-dinitroquinoxaline-2,3-dione; DNQX) were infused into the locus coeruleus by retrograde dialysis, whereas extracellular noradrenaline was recorded in the ipsilateral medial prefrontal cortex. During infusion of the various compounds rats were gently handled for 10 min. Infusion of idazoxan potentiates the handling-induced increase in the release of noradrenaline in the medial prefrontal cortex. The infusions of, atropine, bicuculline, CGP 52432 and DNQX were without effect on the handling response. Infusion of the NMDA receptor antagonist CPP or the non-peptide CRF receptor antagonist CP-154,526 suppressed the stimulation of noradrenaline during stress. It is concluded that α_2-adrenoceptors, NMDA glutamate receptors and CRF receptors modify the handling stress response of locus coeruleus neurones. The data suggest no major role for glutamatergic, GABAergic, or cholinergic afferents to the locus coeruleus in mediating the stress response.

Keywords: Locus coeruleus; Noradrenaline; Cortex, prefrontal; Stress; NMDA receptor; GABA receptor; CRF receptor; Microdialysis

1. Introduction

Numerous anatomical, electrophysiological and behavioural studies indicate that the noradrenergic pathway that originates in locus coeruleus is involved in the regulation of transfer of information through sensory circuits during periods of stress, arousal and selective or sustained attention (Foote et al., 1983; Abercrombie and Jacobs, 1987; Abercrombie et al., 1988; Aston-Jones et al., 1991, Page and Valentino, 1994).

A range of arousing and stressful stimuli have been reported to phasically activate locus coeruleus noradrenergic neurones and to increase noradrenaline metabolism and turnover in brain areas, such as the hippocampus, cortex and hypothalamus. Recently a series of microdialysis studies have confirmed that the release of noradrenaline in these structures is increased during stress (for review, Westerink, 1995).

There is anatomical and electrophysiological evidence that excitatory and inhibitory pathways originating in the nucleus paragigantocellularis, and inhibitory projections originating in the nucleus prepositus hypoglossi, play a major role in the regulation of locus coeruleus activity (Aston-Jones et al., 1991; Chiang and Aston-Jones, 1993).

In the afferental pathways to the locus coeruleus a variety of neurotransmitters have been identified. Several studies were performed to identify whether these transmit-

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ters participate in the stress-induced activation of locus coeruleus neurons. For that purpose receptor-specific compounds have been administered systemically to animals during stressful conditions (Ida et al., 1985, 1990; Rosetti et al., 1990; Tanaka et al., 1991; Murase et al. 1992). This approach has provided evidence for participation of $GABA_A$ and NMDA glutamate receptors in controlling locus coeruleus activity during stress. However, because of the systemic approach, the location of these receptor sites remained unknown.

A second approach was followed by local application of compounds directly to the locus coeruleus, whereas stressors or noxious stimuli were applied. The results of these experiments provided evidence for participation of the α_2-adrenoceptor (Astier and Aston-Jones, 1989), the non-NMDA glutamate receptor (Ennis et al., 1992), the $GABA_A$ receptor (Aston-Jones et al., 1991) and the corticotropin releasing factor (CRF) receptor (Valentino et al., 1991; Emoto et al., 1993; Smagin et al., 1997) in the activation of the locus coeruleus by sensory and stressful stimuli.

In these studies different types of stress were studied, ranging from immobilisation, colon distension and hemodynamic stress (hypotension), which makes the results not directly comparable. It is evident that additional information is needed for a better understanding of the neurochemical interactions that participate in the activation of the locus coeruleus.

Here, we have applied the dual-probe microdialysis technique to identify receptor subtypes involved in the effect of stress on noradrenaline release in the medial prefrontal cortex. For that aim one microdialysis probe was implanted in the vicinity of the locus coeruleus and the second probe was implanted the medial prefrontal cortex. In this approach extracellular noradrenaline in the medial prefrontal cortex is considered as index of locus coeruleus activity. Receptor specific drugs were applied to the locus coeruleus by retrograde microdialysis, whereas extracellular noradrenaline was recorded in the medial prefrontal cortex. During the microdialysis session a stressful condition was applied to the rat. Ten min handling was chosen to activate the locus coeruleus, as it represents a mild and reproducible condition that includes elements of novelty, fear and stress.

2. Materials and methods

2.1. Animals, drug treatment, and doses

Male albino rats of a Wistar-derived strain (275–320 g; Harlan, Zeist, The Netherlands) were used for the experiments. The rats were housed in plastic cages ($20 \times 40 \times 55$ cm) with light from 0700 till 1900 h and had free access to food and water. Experiments were carried out in the light cycle. After probe implantation and during the experiments the rats were individually housed in a plastic cage ($35 \times 35 \times 40$ cm).

The following drugs were used: atropine, (−)-bicuculline, (±)-3(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP), 6,7-dinitroquinoxaline-2,3-dione (DNQX), clonidine · HCl, idazoxan, (Research Biochemicals International, Natick, USA). [[(3,4-Dichlorophenyl) methyl]propyl] (diethoxymethyl) phosphonic acid (CGP 52432) was a generous gift from Ciba Geigy (Basel, Switzerland). Butyl-ethyl-[2,5-dimethyl-7-(2,4,6-trimethyl-phenyl)-7*H*-yrrolo[2,3-*d*]pyrimidin-4-yl]-amine (CP-154,526) was a generous gift from Pfizer (Groton, USA). All drugs were dissolved in the perfusion fluid and infused via retrograde microdialysis into the locus coeruleus.

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

2.2. Choice of the infused concentrations

One of the conditions of the dual-probe microdialysis technique is that infused concentrations of drugs should be sufficiently high to block or stimulate the appropriate receptors in the locus coeruleus area. Infused doses used in this study were based on earlier studies on related experiments. The doses of atropine, bicuculline, DNQX, CPP and CGP 52432, that were used in the present study, have been shown in earlier dual-probe experiments to diffuse well into cell body areas such as the substantia nigra, ventral tegmental area and septum, and to induce specific pharmacological effects in the corresponding nerve terminal areas (Moor et al., 1995, 1996; Westerink et al., 1996; Van Gaalen et al., 1997). Concentrations of idazoxan were based on literature data of comparable microdialysis experiments (Dennis et al., 1987; Van Veldhuizen et al., 1993).

2.3. Surgery and brain dialysis

Microdialysis was performed with two I-shaped cannulas. The dialysis tube was prepared from polyacrylonitrile/sodium methyl 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 and the second probe (exposed length 4 = 5 mm) was implanted in the ipsilateral medial prefrontal cortex. The probe implanted in the locus coeruleus was used to deliver drugs, the probe implanted in the medial prefrontal cortex was used to record extracellular noradrenaline. Co-ordinates of the implantation were as follows, locus coeruleus: A/P −3.3 mm, L/M 1.3 mm, V/D 8.3 mm, 15° from vertical; and medial prefrontal cortex: A/P 3.3 mm, L/M 1.2 mm, V/D 5.0 mm (Paxinos and Watson, 1982). The probes were implanted during chloral hydrate anesthesia (400 mg/kg, i.p.) and local application of lidocaine (10%).

Microdialysis experiments were carried out 24–48 h after implantation of the probes. An on-line approach was used in which 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) and 15-min fractions were collected. The composition of the Ringer's solution was (in mM): NaCl 140.0, KCl 4.0, $CaCl_2$ 1.2, $MgCl_2$ 1.0.

Before the experiments were terminated the implantation of the locus coeruleus probe was functionally evaluated by infusion of 100 μM clonidine (45 min) into the locus coeruleus probe. A decrease in extracellular noradrenaline in the medial prefrontal cortex to at least 30% of controls was considered as an appropriate implantation. Then the rat was given an overdose of chloral hydrate and the brain was fixed with 4% paraformaldehyde via intracardiac infusion. Coronal section (40 μm thick) were made, and the probe placement was localised according to the atlas of Paxinos and Watson (1982). A photomicrograph of the implantation is shown in Van Gaalen et al. (1997).

2.4. Chemical assays

Noradrenaline was quantified by high performance liquid chromatography with electrochemical detection. A Shimadzu LC-10AD pump (Kyoto, Japan) was used in conjunction with an electrochemical detector (ESA, Bedford, MA). The potential of the first cell was +175 mV and of the second cell −300 mV. A reverse-phase column (150 × 4.6 mm; Supelco LC18, 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.

2.5. Protocol and statistics

On the experimental day the rat was handled twice. Two handling sessions on the same day produces a similar increases in the release of noradrenaline (data not shown). When rats were handled, the animals were picked up from the home cage and were gently held in the hands for 10 min. The first handling was considered as control, the second handling was applied several hours later during infusion of a receptor-specific compound. In this way each rat served as its own control.

All values given are expressed as percent of controls. The average concentration of three stable baseline samples was defined as 100%. Of the seven infused drugs only bicuculline modified the baseline values of extracellular noradrenaline in the medial prefrontal cortex. In the case of bicuculline the baseline was reset at 100% before the second handling.

Statistical analysis (SuperANOVA, Abacus Concepts, Berkeley, CA, 1989) 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. Two-way ANOVA and Scheffé's multiple comparison test for post-hoc determination was used for comparison between the control and drug-infused handled rats. The level of significance was set at $P < 0.05$.

3. Results

3.1. Basal values

The basal values (±S.E.M.) of extracellular noradrenaline in the medial prefrontal cortex were 1.35 ± 0.18 fmol/min ($n = 33$).

3.2. Effect of handling on the release of noradrenaline in the medial prefrontal cortex

Gentle handling during 10 min, when a Ringer solution was perfused through the locus coeruleus probe, induced a short-lasting (15–30 min) increase in extracellular noradrenaline in the medial prefrontal cortex to about 150–180% of controls (Figs. 1–5).

3.3. Effect of idazoxan, infused into the locus coeruleus, on the handling-induced increase of the noradrenaline content in dialysates of the ipsilateral medial prefrontal cortex

The application by retrograde dialysis to the locus coeruleus of the α_2-adrenoceptor antagonist idazoxan, in a concentration of 50 μM ($n = 6$), did not modify the extracellular levels of noradrenaline in the medial prefrontal cortex. During idazoxan infusion, the handling-induced increase in noradrenaline in the medial prefrontal cortex rose to about 205% of controls (Fig. 1). This

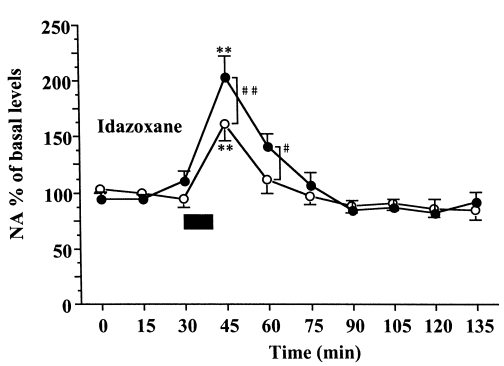

Fig. 1. Effect of application of the α_2-adrenoceptor antagonist idazoxan (50 μM) by retrograde dialysis to the locus coeruleus, on the handling-induced increase in extracellular noradrenaline in the medial prefrontal cortex. The handling period (10 min) is represented by the black bar. Open symbols refer to the handling during infusion of Ringer solution. $^{**}P < 0.01$ vs. basal values. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs. control handling.

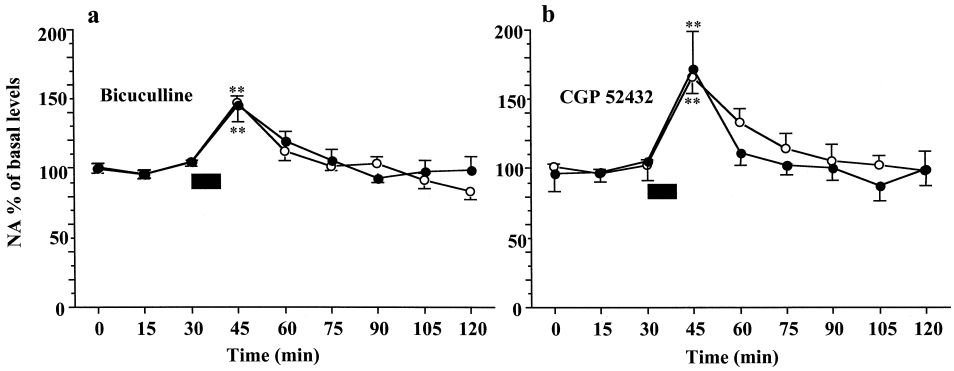

Fig. 2. Effect of application of the $GABA_A$ receptor antagonist bicuculline (50 μM) (A) or the $GABA_B$ receptor antagonist CGP 52432 (100 μM) (B) by retrograde dialysis to the locus coeruleus, on the handling-induced increase in extracellular noradrenaline in the medial prefrontal cortex. The handling period (10 min) is represented by the black bar. Open symbols refer to the handling during infusion of Ringer solution. $^{**}P < 0.01$ vs. basal values.

increase was statistically significant when compared to the effect of control handling (165% of controls).

3.4. Effect of bicuculline or CGP 52432, infused into the locus coeruleus, on the handling-induced increase of the noradrenaline content in dialysates of the ipsilateral medial prefrontal cortex

The application by retrograde dialysis to the locus coeruleus of the $GABA_A$ receptor antagonist bicuculline, in a concentration of 50 μM ($n = 5$), increased the extracellular levels of noradrenaline in the medial prefrontal cortex rose to about 170% of controls (data not shown). The three samples that preceded the handling stress were reset as 100% (Fig. 2a). The handling-induced increase in noradrenaline in the medial prefrontal cortex during bicuculline infusion, rose to about 150% of controls. This increase did not differ from the effect of the control handling (145% of controls).

The application by retrograde dialysis to the locus coeruleus of the $GABA_B$ receptor antagonist CGP 52432, in a concentration of 100 μM ($n = 4$), did not modify the extracellular levels of noradrenaline in the medial prefrontal cortex. During CGP 52432 infusion, the handling-induced increase in noradrenaline in the medial prefrontal cortex rose to about 170% of controls (Fig. 2b). This increase did not differ from the effect of a control handling (165% of controls).

3.5. Effect of CPP and DNQX, infused into the locus coeruleus, on the handling-induced increase of the noradrenaline content in dialysates of the ipsilateral medial prefrontal cortex

The application by retrograde dialysis to the locus coeruleus of the NMDA glutamate receptor antagonist CPP, infused in a concentration of 300 μM ($n = 4$), did not modify the extracellular levels of noradrenaline in the medial prefrontal cortex. During CPP infusion, the handling-induced increase in noradrenaline in the medial prefrontal cortex rose to about 125% of controls (Fig. 3a). This increase was significantly different when compared to control handling (175%). During the application of CPP some turning behaviour was observed.

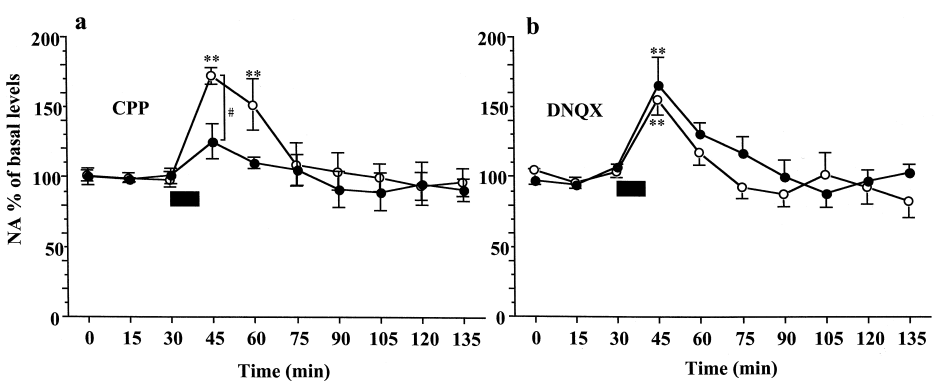

Fig. 3. Effect of application of the NMDA glutamate receptor antagonist CPP (300 μM) (A) or the non-NMDA glutamate receptor antagonist DNQX (500 μM) (B) by retrograde dialysis to the locus coeruleus, on the handling-induced increase in extracellular noradrenaline in the medial prefrontal cortex. The handling period (10 min) is represented by the black bar. Open symbols refer to the handling during infusion of Ringer solution. $^{**}P < 0.01$ vs. basal values. $^{\#}P < 0.05$ vs. control handling.

The application by retrograde dialysis to the locus coeruleus of the non-NMDA glutamate receptor antagonist DNQX, in a concentration of 500 μM ($n = 5$), did not modify the extracellular levels of noradrenaline in the medial prefrontal cortex. During DNQX infusion, the handling-induced increase in noradrenaline in the medial prefrontal cortex rose to about 165% of controls (Fig. 3b). This increase did not differ when compared to control handling (155% of controls).

3.6. Effect of atropine, infused into the locus coeruleus, on the handling-induced increase of the noradrenaline content in dialysates of the ipsilateral medial prefrontal cortex

The application by retrograde dialysis to the locus coeruleus of the muscarinic receptor antagonist atropine infused in a concentration of 10 μM ($n = 4$) did not modify the extracellular levels of noradrenaline in the medial prefrontal cortex. During atropine infusion, the handling-induced increase in noradrenaline in the medial prefrontal cortex rose to about 190% of controls (Fig. 4). This increase was not statistically significant when compared to control handling (180% of controls).

3.7. Effect of the CRF-1 receptor antagonist CP-154,526, infused into the locus coeruleus, on the handling-induced increase of noradrenaline content in dialysates of the ipsilateral medial prefrontal cortex

The application by retrograde dialysis to the locus coeruleus of the non-peptide CRF-1 receptor antagonist CP-154,526, in a concentration of 100 μM ($n = 5$), did not modify the extracellular levels of noradrenaline in the medial prefrontal cortex (data not shown). During CP-154,526 infusion, the handling-induced increase in noradrenaline in the medial prefrontal cortex rose to about

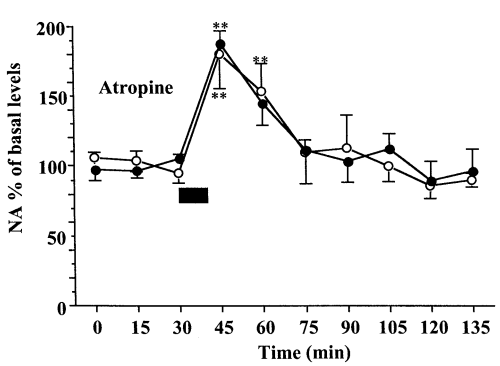

Fig. 4. Effect of application of the muscarinic receptor antagonist atropine (10 μM) by retrograde dialysis to the locus coeruleus, on the handling-induced increase in extracellular noradrenaline in the medial prefrontal cortex. The handling period (10 min) is represented by the black bar. Open symbols refer to the handling during infusion of Ringer solution. $^{**}P < 0.01$ vs. basal values.

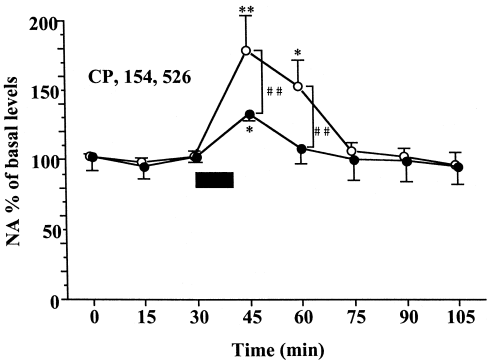

Fig. 5. Effect of application of the CRF receptor antagonist CP-154,526 (100 μM) by retrograde dialysis to the locus coeruleus, on the handling-induced increase in extracellular noradrenaline in the medial prefrontal cortex. The handling period (10 min) is represented by the black bar. Open symbols refer to the handling during infusion of Ringer solution. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. basal values. $^{\#\#}P < 0.01$ vs. control handling.

130% of controls (Fig. 5). This increase was significantly different from control handling (180% of controls).

4. Discussion

4.1. Adrenoreceptors

Simson and Weiss (1989) have demonstrated that α_2-adrenoceptors play a major role in regulating the responsiveness of locus coeruleus neurones to excitatory influences. An adrenergic projection (referred to as C1) originating in the nucleus paragigantocellularis that innervates the locus coeruleus, has been implicated in this response (Aston-Jones et al., 1991). The adrenergic projection inhibits locus coeruleus cells during sensory stimulation. Blockade of α_2-adrenoceptors increased the response of neurons in the locus coeruleus to an physiological excitatory stimulus (compression of the hind paw) at doses well below those necessary to increase the spontaneous activity of these neurons.

The present findings elaborated this hypothesis as application of idazoxan to the locus coeruleus by retrograde dialysis, during conditions in which idazoxan itself was not effective in activating locus coeruleus neurons, clearly stimulated the handling-induced increase in noradrenaline in the medial prefrontal cortex. An alternative explanation for the effects of idazoxan can be given by inhibition of impulse-flow controlling α_2 autoreceptors localised on locus coeruleus noradrenergic neurones (Ivanov and Aston-Jones, 1995).

4.2. GABA receptors

Locus coeruleus neurons are potently inhibited by iontophoretic application of GABA receptor agonists (Olpe et al., 1988; Osmanovic and Shefner, 1990). The prepositus

hypoglossi provides a direct inhibitory synaptic input to the locus coeruleus, for which GABA acting at $GABA_A$ receptors is proposed as transmitter (Ennis and Aston-Jones, 1989; Aston-Jones et al., 1991).

Infusion of the $GABA_A$ receptor antagonist bicuculline, in the vicinity of the locus coeruleus, clearly stimulated the basal release of noradrenaline in the medial prefrontal cortex (Kawahara et al., 1999), indicating that $GABA_A$ receptors participate in tonic inhibition of the locus coeruleus neurones. Handling stress during bicuculline infusion increased the stimulation of the release of noradrenaline in the ipsilateral medial prefrontal cortex when compared with a control handling. However, when the noradrenaline output during bicuculline infusion was reset as 100%, the effect did not differ from control handling. Apparently the degree of inhibition of locus coeruleus neurones by $GABA_A$ receptors does not change during handling stress, suggesting that these receptors do not mediate the stress response.

The present results are consistent with literature data showing that blockade of $GABA_A$ receptors did not affect the stimulation of locus coeruleus activity by paw compression (Simson and Weiss, 1989) or foodpad stimulation (Ennis and Aston-Jones, 1989). They are at variance with the study of Shiekhattar and Aston-Jones (1992), who showed that direct application of bicuculline to the locus coeruleus potently enhanced the sensory responsiveness of these neurons. However, in the latter study effects were only seen when a high stimulus intensity was used. It is unlikely that a similar sensory stimulation was experienced in the present mild behavioural activation.

The absence of significant effects on the handling stress-induced increase in extracellular noradrenaline in the medial prefrontal cortex, during infusion of the potent $GABA_B$ receptor antagonist CGP 52432, indicates that $GABA_B$ receptors play no major role in the handling stress response.

Taken together, these findings indicate that GABAergic neurons projecting from the prepositus hypoglossi to the locus coeruleus play no major role in the activation of the locus coeruleus during handling.

4.3. Glutamate receptors

There is increasing evidence for the presence of NMDA as well as non-NMDA glutamate receptors on somatodendritic sites of noradrenergic locus coeruleus neurons (Olpe et al., 1989; Williams et al., 1991; Ennis et al., 1992; Van Gaalen et al., 1997). The major excitatory input to the locus coeruleus originates in the paragigantocellularis and glutamatergic neurons probably play a major role in this pathway, although this notion has been disputed (Rasmussen and Aghajanian, 1989). Activation of locus coeruleus neurons by stimulation of the paragigantocellularis or by noxious sensory stimulation was prevented by local application of CNQX to the locus coeruleus (Hajos and Engberg, 1990; Ennis et al., 1992). Based on these findings, it was hypothesised that activation of locus coeruleus neurons from paragigantocellularis during sensory stimulation is mediated by non-NMDA glutamate receptors; in addition a NMDA component of this activation was demonstrated in electrophysiological recordings of the locus coeruleus during which normal cerebrospinal fluid was substituted for a Mg^{2+}-free medium in the locus coeruleus region (Shiekhattar and Aston-Jones, 1992).

Infusion by retrograde dialysis of the competitive NMDA glutamate receptor antagonist CPP, markedly inhibited the handling stress-induced increase in extracellular noradrenaline in the medial prefrontal cortex. These data support that NMDA glutamate receptors play a major role in activation noradrenergic neurons in the locus coeruleus during behavioural activation. In contrast the results of the DNQX experiments, do not confirm that non-NMDA glutamate receptors participate in the activation of the locus coeruleus during handling.

4.4. Acetylcholine receptors

Electrophysiological studies have demonstrated that cholinomimetics of the muscarinic type stimulate locus coeruleus activity (Svensson and Engberg, 1980; Egan and North, 1985; Ennis and Shipley, 1992). Nevertheless, the presence of acetylcholine receptors on noradrenergic neurones in the locus coeruleus is questioned. Cholinergic fibres are densely located in the peri-locus coeruleus, but there is doubt that they directly innervate the noradrenergic neurones (Ruggiero et al., 1990; Aston-Jones et al., 1991).

In a recent microdialysis study, it was shown that the infusion of carbachol or oxotremorine to the locus coeruleus clearly stimulated the release of noradrenaline in the ipsilateral medial prefrontal cortex (Van Gaalen et al., 1997; Kawahara et al., 1999). Based on these experiments we investigated the role of cholinergic afferents in activation of the locus coeruleus during handling, by retrograde dialysis of atropine to the locus coeruleus. The muscarinic antagonist did not affect basal release of noradrenaline, nor did it modify the handling stress-induced increase in noradrenaline. Apparently, acetylcholine receptors play no major role in the handling-induced activation of locus coeruleus neurones.

4.5. CRF-receptors

There is accumulating evidence that an excitatory projection to the locus coeruleus, that uses CRF as neurotransmitter, participates in the activation of the locus during various types of stress such as bladder distension, hypotension and immobilisation (Valentino et al., 1991; Emoto et al., 1993; Page and Valentino, 1994; Smagin et al., 1997). In these studies — based on electrophysiological recordings — intracerebral administration of the peptide CRF receptor antagonist αhCRF was able to attenuate the stress-induced activation of the locus coeruleus.

The present findings confirm these data as application of the non-peptide CRF-1 receptor antagonist CP-154,526 (Schultz et al., 1996) by retrograde microdialysis to the locus coeruleus, significantly reduced the handling-induced release of noradrenaline in the medial prefrontal cortex.

4.6. Conclusion

In the present study, a natural stimulus was given to conscious animals, whereas locus coeruleus activity was monitored and manipulated by dual-probe microdialysis. The results provide evidence that the NMDA glutamate receptor as well as the CRF-receptor participate in the handling-induced stimulation of locus coeruleus activity. In addition an inhibitory modulation via α_2-adrenoceptor receptors could be established. No evidence was found for a role of the non-NMDA glutamate receptor of for GABAergic or cholinergic afferents to the locus coeruleus, in the studied stress response.

It is realised that the applied handling stress was mild. Evidence based on c-*fos* expression indicated that the pontine nuclei are fully activated during immobilisation stress (Krukoff and Khalili, 1997). The present protocol is suitable to further investigate the neurotransmitter pathways involved in different activating conditions (such as hypotension, noxious stimuli and immobilisation).

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