Abstract The effects of two different types of stress (hypotension and handling) on the release of dopamine, noradrenaline and DOPAC in the locus coeruleus (LC) and medial prefrontal cortex (mPFC) was studied by means of the dual-probe microdialysis technique. One probe was implanted in the vicinity of the LC and a second probe was implanted in the mPFC. Both probes were used to record simultaneously noradrenaline, dopamine and DOPAC. Samples from the LC were collected in the presence of nomifensine, which was added to the perfusion fluid in a concentration of 50 μM. Hypotension (20 min) induced by intravenous administration of nitroprusside stimulated the release of noradrenaline in the LC and mPFC to about 190% and 150% of control values, respectively. Hypotension also strongly stimulated the release of dopamine in the mPFC (to 320% of control) and DOPAC in the LC (to 270% of control). The effect of hypotension on extracellular dopamine, noradrenaline and DOPAC, was decreased by halothane anaesthesia, and was blocked by chloral hydrate anaesthesia. Handling stress (10 min) stimulated the release of dopamine in the mPFC to about 160% of control. The effect of hypotension or handling stress was further evaluated in animals in which the LC was lesioned by an infusion of 6-OH-dopamine. Lesioning of the noradrenergic LC neurons did not prevent the hypotension-related stimulation of dopamine release, but shortened the time course of the effect dramatically. Lesioning of the noradrenergic neurons had no effect on the stimulatory effect of handling on the release of dopamine in the mPFC.

This study shows that mesocortical dopamine neurons, in contrast to noradrenaline neurons, respond much stronger to hemodynamic stress than to an emotional stress. During certain conditions like hypotension stress, but not during handling stress, the LC activity is able to modulate the release of dopamine from mesocortical neurons.

Key words Noradrenaline release · Dopamine release · Locus coeruleus · Prefrontal cortex · Hypotension · Stress · Microdialysis

Introduction

Noradrenaline neurons that originate in the locus coeruleus (LC) and dopamine neurons that originate in the ventral tegmental area (VTA) play an important role in the cascade of events that occur during complex behaviors such as arousal, stress, anxiety, fear or cognitive process (Thierry et al. 1976; Mantz et al. 1988; Berridge and Foote 1991; Berridge et al. 1993; Kalivas 1993; Aston-Jones et al. 1991, 1996). In addition, there is accumulating evidence that the VTA and LC are involved in the mechanism of action of various therapeutic drugs used in psychiatry. A strategic brain area in this respect is the medial prefrontal cortex (mPFC), as the projections of VTA dopamine neurons and LC noradrenaline neurons overlap in this region. There is evidence for a specific interaction between the release of dopamine and noradrenaline in the mPFC (Carboni et al. 1990; Tassin et al. 1992; Gresch et al. 1995).

The LC is activated during several types of stress. Numerous findings implicate the LC as an integral link in stress responses. In the present study we have compared the effects of two different stressors on the transmitter release from mesolimbic dopamine neurons and LC noradrenaline neurons: hemodynamic stress (hypotension) and emotional stress (handling); both types of stress have been reported to increase LC activity (Abercrombie et al. 1988; Valentino and Wehby 1988; Valentino et al. 1998). Hypotension was induced by nitroprusside infusion via a permanent catheter implanted in the jugular vein. Emotional stress was applied...
by gentle handling; the effect of this type of stress on the release of noradrenaline and dopamine in the mPFC has been documented in several microdialysis studies (Abercrombie et al. 1989; Imperato et al. 1990; Jedema and Moghaddam 1994; Nakane et al. 1994; Feenstra et al. 1995; Enrico et al. 1998). In most of the experiments neurotransmitter release was recorded by dual-probe microdialysis. With the help of two microdialysis probes extracellular levels of dopamine, DOPAC and noradrenaline in the mPFC, and noradrenaline and DOPAC in the LC were recorded simultaneously.

In addition, the effect of anaesthesia on the neurotransmitter response to hypotension was studied. Interactions between dopaminergic and noradrenergic neurons were investigated by lesioning the LC with 6-OH-dopamine (6-OH-DA).

**Materials and methods**

*Animals and drug treatment.* 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 × 35 × 40 cm) and had free access to food and water. The experiments were approved by the Animal Care Committee of the Faculty of Mathematics and Natural Science of the University of Groningen.

*Surgery and brain dialysis.* Microdialysis was performed with two I-shaped cannulas (Van Gaalen et al. 1997). 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 LC, and a second probe (exposed length 4.0 mm) was implanted in the ipsilateral mPFC. The probe implanted in the LC was used to deliver 6-OH-DA or to record noradrenaline and DOPAC. The probe in the PFC was used to record noradrenaline, dopamine and DOPAC. Coordinates of the implantation were as follows – LC: A/P –3.3 mm, L/M 1.3 mm, V/D 8.3 mm, implanted under an angle 15° from lambda and dura; mPFC: A/P 3.3 mm, L/M 1.2 mm, V/D 5.0 mm from bregma and dura. In the case of the hypothalamus experiments we polyethylene cannula (PE-10; Becton Dickinson, N.J., USA) was inserted into the external jugular vein. The probe and cannulas were implanted during chloral hydrate anaesthesia (400 mg/kg i.p.) and local application of lidocaine (10%).

Microdialysis experiments were carried out 24–48 h after implantation of the probes and cannulas. An on-line approach was used in which both probes were perfused simultaneously with a Ringer solution at a flow rate of 2.0 μl/min (Beehave infusion pump; BAS, USA) and 15-min fractions were collected. The composition of the Ringer solution was (in mM): NaCl 140.0, KCl 4.0, CaCl₂ 1.2, MgCl₂ 1.0. To improve the detection limit of dopamine and noradrenaline, 50 μM of the reuptake inhibitor nomifensine was added to the perfusion fluid of the PFC probes. Dopamine was not detectable in the LC, also not in the presence of reuptake blocker, therefore noradrenaline and DOPAC in the LC were recorded without the addition of nomifensine. Before the experiments were terminated, implantation of the cannulas was functionally evaluated by infusion of 100 μM clonidine (45 min) into the LC probe. A decrease in extracellular noradrenaline in the mPFC to at least 30% of control was considered as indicating an appropriate implantation. Rats were killed with an overdose of chloral hydrate and the brain was fixed with 4% paraformaldehyde via intracardiac infusion. Coronal sections (16 μm thick) were made, and dialysis probe placement was localised according to the atlas of Paxinos and Watson (1986).

*Chemical assays.* Noradrenaline, dopamine and DOPAC were quantified by HPLC with electrochemical detection. A Shimadzu LC-10AD pump (Kyoto, Japan) was used in conjunction with an electrochemical detector (ESA; potential first cell: +175 mV; potential second cell: −250 mV). A reverse-phase column (150 × 4.6 mm; Supelco LC18, Bellefonte, Pa., USA) was used. The mobile phase consisted of a mixture of 2 g citric acid, 5 g sodium acetate and 620 mg heptanesulfonic acid in 900 ml H₂O, and 100 ml/methanol. The flow rate was 1.0 ml/min. The detection limit of the catecholamines was about 3–4 fmol/sample (on-column).

**Hypotension experiments.** Hypotension was induced by sodium nitroprusside infused via a cannula that was implanted in the external jugular vein. Blood pressure was measured by tail cuff (TC; Life Science, USA). Systolic blood pressure (measured every 5 min) was recorded 15 min before sodium nitroprusside was infused, until 15 min after cessation of the infusion. We compared the effects of hypotension in conscious and anaesthetised rats. Two different types of anaesthesia (halothane and chloral hydrate) were studied. Halothane was applied at 5% concentration for induction and 1.0%–2.0% for maintenance in air through a face mask. Chloral hydrate was administered continuously into the jugular vein via the venous cannula. The initial dose was 150 mg/kg i.v., the maintenance dose 2.0–3.0 mg/kg min. The rectal temperature was maintained at 37.5°C. Sodium nitroprusside was infused in a dose of 30–40 μg/kg min for anaesthetised animals and 225–300 μg/kg min for conscious second animals. The doses of sodium nitroprusside were taken from Curtis et al. (1993). According to the latter study, using these doses, blood pressure in both conscious and anaesthetised rats was reduced to about 50% of control. To prevent additional stress, blood pressure was recorded only in anaesthetised animals; however, in a separate group (n = 4) we recorded the fall in blood pressure in conscious rats with an invasive method, for which aim intra-arterial and intravenous catheters (AP 641G; Nihon Kohden, Japan) were implanted.

*Handling stress.* When the rats were handled, they were picked up from the home cage and held in the hands for 10 min.

**6-OH-DA lesions.** Lesions of the noradrenergic neurons in the LC were produced by infusion of 6-OH-DA (50 mM) through the probe for 4 h. The microdialysis experiments were carried out 48 h later.

**Protocols.** Seven conditions were investigated: (1) hypotension in conscious rats; (2) hypotension in halothane-anaesthetised rats, including blood pressure measurements; (3) hypotension in chloral hydrate anaesthetised rats, including blood pressure measurements; (4) handling in conscious rats; (5) hypotension in conscious LC lesioned rats; (6) handling in conscious LC lesioned rats; (7) invasive blood pressure measurement in conscious rats, no microdialysis.

**Expression of results and statistics.** All values given are expressed as percent of control. The average concentration of three stable baseline samples was defined as 100%. Statistical analysis (SuperANOVA; Abacus Concepts, Berkeley, Calif., USA, 1989) was performed using one-way analysis of variance with repeated measures and Dunnett’s multiple comparison test for post-hoc determination of significant differences. Two-way analysis of variance and Scheffé’s multiple comparison test for post-hoc determination were used for comparison between conscious and anaesthetised rats. The level of significance was set at P < 0.05.

**Results**

Basal values of noradrenaline, dopamine and DOPAC

The mean basal values of noradrenaline, dopamine and DOPAC in the LC and PFC are shown in Table 1. Basal values of noradrenaline, dopamine and DOPAC in the mPFC were collected in the presence of nomifensine, which was added to the perfusion fluid at a concentration of 50 μM.