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European Journal of Pharmacology 445 (2002) 37–42

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The interaction between the locus coeruleus and dorsal raphe nucleus studied with dual-probe microdialysis

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Received 3 January 2002; received in revised form 18 April 2002; accepted 23 April 2002

Abstract

The interaction between the locus coeruleus and dorsal raphe nucleus was investigated by means of dual-probe microdialysis in conscious rats. The release of noradrenaline and 5-hydroxytryptamine (5-HT) after inhibition or stimulation of locus coeruleus and dorsal raphe activity was sampled in both nuclei and analysed by high-pressure liquid chromatography (HPLC). The inhibition of locus coeruleus activity by the infusion of the α_2-adrenoceptor agonist clonidine (100 μM) decreased the release of noradrenaline to 20% in the locus coeruleus and 30% in the dorsal raphe, whilst the release of 5-HT decreased to 80% of control in the two brain areas. The excitation of locus coeruleus activity by the muscarinic receptor agonist carbachol (100 μM) led to an increase in the release of noradrenaline to 240% and 220% of control in the locus coeruleus and dorsal raphe, respectively. The release of 5-HT in both nuclei did not respond to the carbachol infusion into the locus coeruleus. Infusion of the 5-HT_{1A} receptor agonist flesinoxan into the dorsal raphe (1 μM) significantly decreased the release of 5-HT in the dorsal raphe and locus coeruleus to 45% and 65% of control, respectively. The release of noradrenaline was decreased in the dorsal raphe to 45% by flesinoxan, whereas no changes were seen in the release of noradrenaline in the locus coeruleus. In conclusion, the innervation of the dorsal raphe by the locus coeruleus has a slight excitatory effect on the release of 5-HT in the dorsal raphe. The dorsal raphe does not exert a direct inhibitory influence on the release of noradrenaline in the locus coeruleus. Finally, the release of noradrenaline in the dorsal raphe may be locally regulated by 5-HT_{1A} receptors. © 2002 Published by Elsevier Science B.V.

Keywords: Locus coeruleus; Dorsal raphe nucleus; Noradrenaline; 5-HT (5-hydroxytryptamine, serotonin); Microdialysis

1. Introduction

Much attention has been paid to the interaction between noradrenergic and serotonergic (5-HT) systems of the brain, as both systems are involved in the control of complex behaviour and implicated in the actions of drugs used to treat depression (Aston-Jones et al., 1991; Jacobs and Azmitia, 1992; Rueter et al., 1997; Anttila and Leinonen, 2001; Blier, 2001). The specific role that these neuronal systems play in the control of physiological processes and in the mechanisms of drugs action is not fully clear at the present time. The investigation of this matter is complicated by the fact that the noradrenaline and 5-HT systems interact with each other, via direct as well as indirect pathways. To further elucidate the interaction between the noradrenaline and 5-HT systems in the brain, we used dual-probe microdialysis. We implanted probes in the locus coeruleus and dorsal raphe nucleus because these nuclei represent the main sources of noradrenaline and 5-HT in the brain, respectively.

A serotonergic projection to the locus coeruleus has been shown to arise, among other sources, from the dorsal raphe nucleus (Palkovits et al., 1977; Cedarbaum and Aghajanian, 1978; Morgane and Jacobs, 1979; Segal, 1979; Vertes and Kocsis, 1994). A number of investigations have shown that 5-HT contributes to the regulation of locus coeruleus activity and the release of noradrenaline. It was reported that selective lesions of the serotonergic system increased tyrosine hydroxylase activity in the locus coeruleus and the firing rate of noradrenaline neurons (Renaud et al., 1975; Crespi et al., 1980; McRae-Degueurce et al., 1982). Another example of the impact of the 5-HT system on noradrenaline neurons is their participation in the response to antidepressants. During chronic treatment with the specific serotonin reuptake inhibitor paroxetine, the firing activity of the locus coeruleus was lowered (Szabo et al., 1999).

The 5-HT effects on the locus coeruleus are primarily mediated by 5-HT_{1A} receptors. It was found that the

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0014-2999/02/$ - see front matter © 2002 Published by Elsevier Science B.V.
PII: S0014-2999(02)01663-1

activation of 5-HT_{1A} receptors in the locus coeruleus attenuated the response of noradrenergic neurons to glutamate (Charlety et al., 1991). A recent study from our laboratory revealed that the 5-HT_{1A} receptor agonist flesinoxan caused a profound decrease in the release of noradrenaline in the locus coeruleus when applied locally via retrograde microdialysis (Pudovkina et al., 2001).

In turn, the dorsal raphe receives noradrenergic innervation from the locus coeruleus (Sim and Joseph, 1993; Peyron et al., 1996). There is ample evidence that the firing activity of the dorsal raphe can be increased or decreased by excitation of α_1- and α_2-adrenoceptors, respectively (Svensson et al., 1975; Baraban and Aghajanian, 1980; Garratt et al., 1991; Clement et al., 1992), but there is a lack of knowledge about the changes in the release of 5-HT in the dorsal raphe upon alteration of the activity of noradrenergic inputs.

In the present study, we further analysed the direct mutual interaction between the locus coeruleus and dorsal raphe by measuring the release of noradrenaline and 5-HT in both nuclei, using the method of dual-probe microdialysis in conscious rats. To that end, the activity of the locus coeruleus and dorsal raphe was modified (inhibited or excited) by infusing receptor specific compounds—via retrodialysis—into the two nuclei, respectively. The following questions were studied: (1) Does inhibition of the activity of dorsal raphe 5-HT neurons influence the release of noradrenaline in the locus coeruleus? (2) Does activation or inhibition of the activity of locus coeruleus noradrenergic neurons influence the release of 5-HT in the dorsal raphe nucleus?

Locus coeruleus activity was inhibited by infusing the α_2-adrenoceptor agonist clonidine or stimulated by infusing the muscarinic receptor agonist carbachol (Kawahara et al., 2001). The dorsal raphe was inhibited by infusion of the 5-HT_{1A} receptor agonist flesinoxan. Preliminary experiments indicated that the stimulation of dorsal raphe activity (for example, by GABA receptor antagonist or glutamate receptor agonist) resulted in a vigorous behavioral activation of the animal. We, therefore, decided not to include an experiment with stimulation of dorsal raphe activity in this study.

2. Materials and methods

2.1. Animals and drug treatment and doses

Male albino rats of a Wistar-derived strain (285–340 g; Harlan, Zeist, The Netherlands) were used for the experiments. The rats were housed in plastic cages (20 × 30 × 70 cm), with lights on from 7 a.m. until 7 p.m. and had free access to food and water. After probe implantation and during the experiments, the rats were individually housed in a plastic cage (25 × 25 × 30 cm). Experiments were carried out in the light cycle.

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

The following drugs were used: clonidine HCl, carbachol (Research Biochemicals International, Natick, MA, USA) and flesinoxan (Solvay Pharmaceuticals, Weesp, The Netherlands). All drugs were dissolved in Ringer's solution and were applied to the locus coeruleus and dorsal raphe via retrograde microdialysis. Concentrations of clonidine, carbachol and flesinoxan were based on earlier microdialysis experiments (Bosker et al., 1997; Van Gaalen et al., 1997; Kawahara et al., 2001).

2.2. Surgery and brain dialysis

Microdialysis was performed with two I-shaped homemade probes. The dialysis membrane used was polyacrylonitrile/sodium methalyl sulphonate 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 right locus coeruleus at an angle of 15°. Coordinates of the implantation were: A/P −3.3 mm, L/M 1.3 mm and V/D 8.3 mm from lambda and surface of the skull, respectively. Another probe (exposed length 2 mm) was placed in the dorsal raphe nucleus at an angle of 10°. Coordinates of implantation were: A/P −7.8 mm, L/M 1.4 mm and V/D 7.0 mm from bregma. The probes were implanted under ketamine/xylazine anaesthesia (50/8 mg/kg, i.p.) with pretreatment with midazolam (5 mg/kg, s.c.) and atropine nitrate (0.1 mg/kg, s.c.). Finadyne (flunixinum), 1 mg/kg, i.m., was used as a postoperative analgesia.

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 Ringer's solution at a flow rate of 2.0 μl/min (CMA/102 microdialysis pump, Sweden).The composition of the Ringer's solution was (in mM): NaCl 140.0, KCl 3.0, $CaCl_2$ 1.2, $MgCl_2$ 1.0. Noradrenaline was determined in 15-min fractions that were collected on-line in the sample loop of a high-pressure liquid chromatography (HPLC) system. Connections to the infusion pump and HPLC were made with flexible tubing (Peek, ID 0.12 mm). 5-HT was determined in 15-min fractions that were collected off-line using a microfraction collector (CMA/142, Sweden). Noradrenaline and 5-HT were determined in separate experiments.

When the experiment was terminated, the rat was given an overdose of chloral hydrate and the brain was fixed with 4% paraformaldehyde via intracardiac perfusion. Coronal sections (40 μm thick) were made, and dialysis probe placement was localised according to the atlas of Paxinos and Watson (1982).

2.3. Chemical assays of noradrenaline

Noradrenaline was 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 oxidising potential was set at +175 mV; the reduction potential was set at −200

mV. A reversed-phase column (150 × 4.6 mm, Supelco LC18: Belleofonte, PA, USA) was used. The mobile phase consisted of a mixture of 4.1 g sodium acetate, 140 mg 1-octanesulphonic acid, 50 mg EDTA and 7% of methanol in 1000 ml H_2O (pH 4.45). The flow rate was 1 ml/min. The detection limit of the assay was 1.5 fmol noradrenaline per sample (on-column).

2.4. Chemical assays of 5-hydroxytryptamine

5-HT was analysed using an HPLC/auto-injector (CMA, Sweden) and a Shimadzu LC-10AD pump (Kyoto, Japan), connected to a reversed-phase column (Phenomenex hypersyl 3: C18, 3 μm, 100 × 2.0 mm, Bester, Amstelveen, The Netherlands) and an electrochemical detector (Antec Leyden, Leyden, The Netherlands) working at a potential setting of 500 mV vs. Ag/AgCl reference. The mobile phase consisted of 5 g/l diammonium sulphate, 500 mg/l EDTA, 50 mg/l heptane sulphonic acid, 30 μl/l of triethylamine and 4.5% v/v methanol, at a pH of 4.65. The flow rate was 0.4 ml/min. The detection limit was 0.5 fmol 5-HT per sample.

2.5. Expression of results and statistics

All values given are expressed as percentages of control ± S.E.M. The average concentration of four stable baseline samples before drug administration was considered as a control and was defined as 100%. A statistical program (Sigmastat 1.0) was used to calculate the statistics. Data were analysed by a 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 dialysate values

Basal dialysate 5-HT values were 5.34 ± 0.6 fmol per 15-min sample in the locus coeruleus and 12.76 ± 0.9 fmol per 15-min sample in the dorsal raphe ($n = 23$). Basal dialysate noradrenaline values were 10.04 ± 0.3 fmol per 15-min sample in the locus coeruleus and 6.98 ± 0.8 fmol per 15-min sample in the dorsal raphe ($n = 23$).

Basal dialysate values were not corrected for in vitro recovery.

3.2. Infusions into the locus coeruleus: effect of clonidine infused into the locus coeruleus on the release of noradrenaline and 5-HT in the locus coeruleus and dorsal raphe

Infusion of the α_2-adrenoceptor agonist clonidine into the locus coeruleus, for 60 min at a concentration of 100 μM, decreased the level of noradrenaline in the locus coeruleus and dorsal raphe to 20% and 35%, respectively (Fig. 1A). In the locus coeruleus, the decrease was statistically significant between 30 and 120 min after the start of the infusion. In the dorsal raphe, the level of noradrenaline returned to the basal level within 60 min after cessation of the clonidine infusion. The decrease in noradrenaline in the dorsal raphe was statistically significant between 30 and 90 min after the start of the infusion. At the same time, the 5-HT output (Fig. 1B) was slightly decreased to about 80% of the control in the locus coeruleus as well as in the dorsal raphe. Both decreases were statistically significant between 30 and 120 min after the start of the infusion.

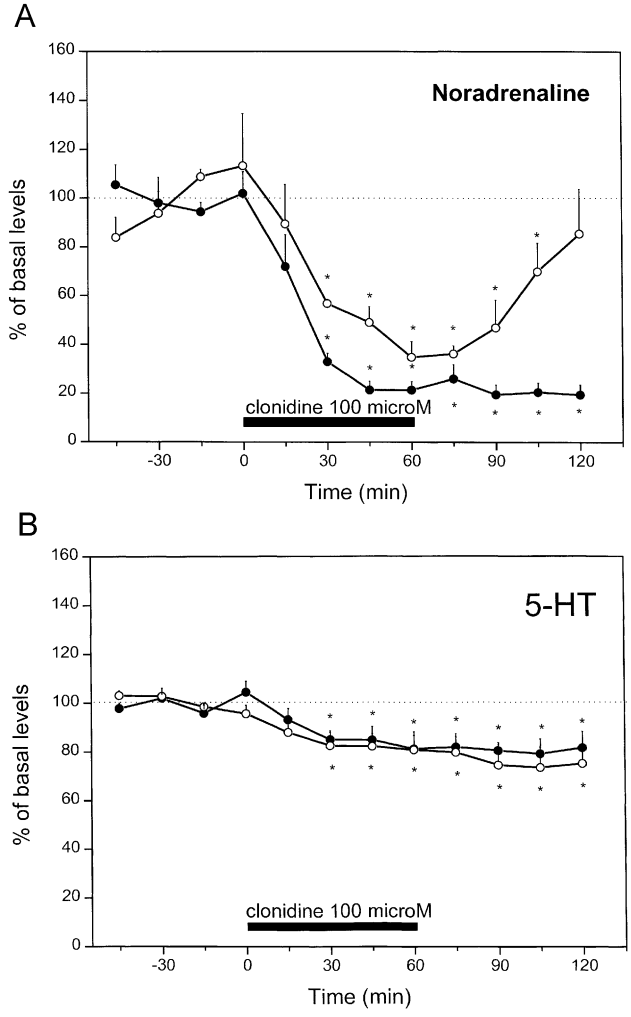

Fig. 1. (A) Effect of clonidine infusion (100 μM, black bar) into the locus coeruleus, for 60 min, on extracellular noradrenaline in the locus coeruleus (closed circles, $n = 9$) and the dorsal raphe (open circles, $n = 8$). Data are given as percentage of basal levels ± S.E.M. * $P < 0.05$ compared to basal levels. (B) Effect of clonidine infusion (100 μM, black bar) in the locus coeruleus, for 60 min, on extracellular serotonin in the locus coeruleus (closed circles, $n = 9$) and the dorsal raphe (open circles, $n = 8$). Data are given as percentage of basal levels ± S.E.M. * $P < 0.05$ compared to basal levels.

3.3. Infusions into the locus coeruleus: effect of carbachol infused into the locus coeruleus on the release of noradrenaline and 5-HT in the locus coeruleus and dorsal raphe

Infusion of the muscarinic receptor agonist carbachol into the locus coeruleus, at a concentration of 100 μM for 60 min, caused an increase of the release of noradrenaline in the locus coeruleus and dorsal raphe to 240% and 220% of the basal levels, respectively (Fig. 2A). The increases were statistically significant from 30 to 90 min after the start of the infusion. In contrast, the extracellular levels of 5-HT in the both nuclei remained unchanged (Fig. 2B).

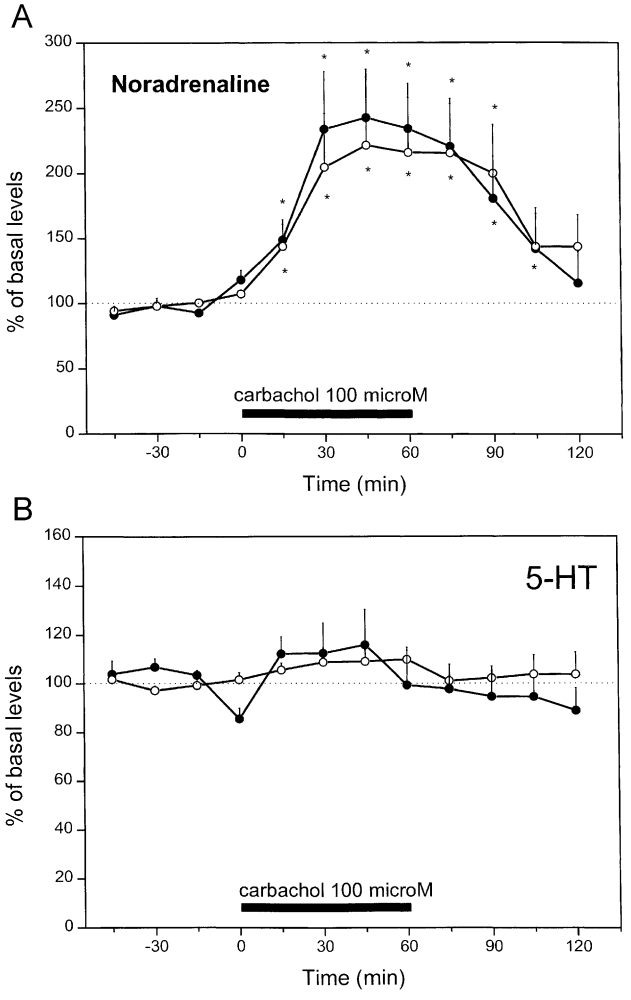

Fig. 2. (A) Effect of carbachol infusion (100 μM, black bar) in the locus coeruleus, for 60 min, on extracellular noradrenaline in the locus coeruleus (closed circles, $n=7$) and the dorsal raphe (open circles, $n=7$). Data are given as percentage of basal levels ± S.E.M. * $P<0.05$ compared to basal levels. (B) Effect of carbachol infusion (100 μM, black bar) in the locus coeruleus, for 60 min, on extracellular serotonin in the locus coeruleus (closed circles, $n=7$) and the dorsal raphe (open circles, $n=7$). Data are given as percentage of basal levels ± S.E.M. * $P<0.05$ compared to basal levels.

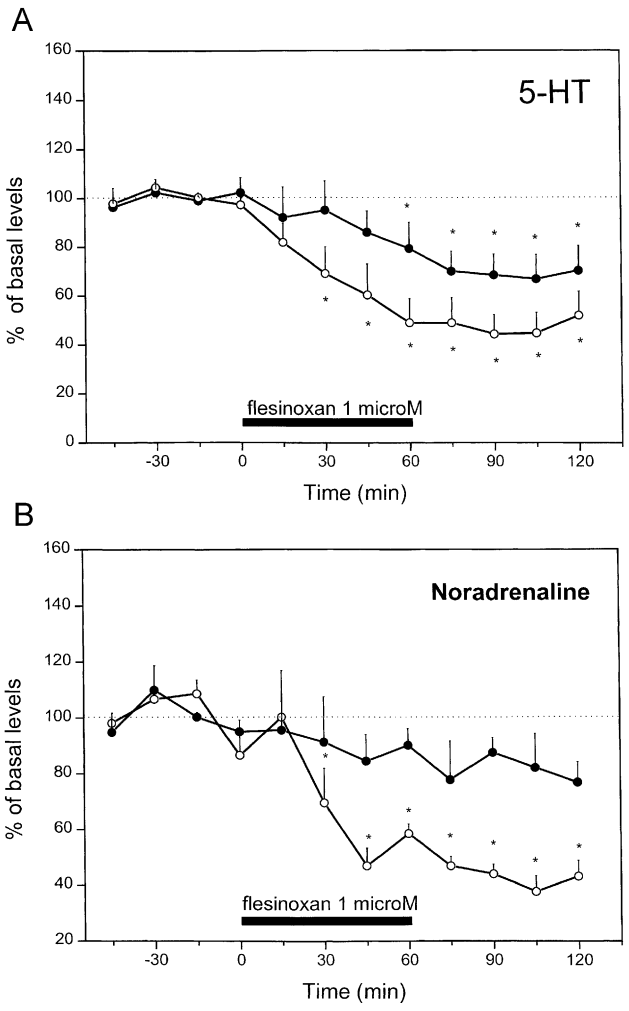

Fig. 3. (A) Effect of flesinoxan infusion (1 μM, black bar) into the dorsal raphe, for 60 min, on extracellular serotonin in the dorsal raphe (open circles, $n=7$) and the locus coeruleus (closed circles, $n=7$). Data are given as percentage of basal levels ± S.E.M. * $P<0.05$ compared to basal levels. (B) Effect of flesinoxan infusion (1 μM, black bar) into the dorsal raphe, for 60 min, on the extracellular noradrenaline in the dorsal raphe (open circles, $n=7$) and the locus coeruleus (closed circles, $n=7$). Data are given as percentage of basal levels ± S.E.M. * $P<0.05$ compared to basal levels.

3.4. Infusion into the dorsal raphe: effect of flesinoxan infused into the dorsal raphe on the release of 5-HT and noradrenaline in the dorsal raphe and locus coeruleus

Infusion of the 5-HT_{1A} receptor agonist flesinoxan into the dorsal raphe, at a concentration of 1 μM for 60 min, decreased the levels of 5-HT in the dorsal raphe and locus coeruleus to 65% and 45% of control, respectively (Fig. 3A). The decreases were significant between 30 and 120 min in case of the dorsal raphe and between 60 and 120 min in case of the locus coeruleus. The levels of noradrenaline in the dorsal raphe decreased to 45% of the basal levels (Fig. 3B). This decrease was statistically significant between 30 and 120 min after the start of the infusion. The level of noradrenaline in the locus coeruleus did not change significantly.

4. Discussion

4.1. Noradrenergic modulation of the dorsal raphe nucleus

Inhibition of locus coeruleus activity by clonidine infusion resulted in a profound decrease in extracellular noradrenaline in the locus coeruleus as well as in the dorsal raphe. The decrease in the locus coeruleus was expected and confirms earlier observations about the ability of clonidine to block the firing of locus coeruleus neurons (Svensson et al., 1975). Although the morphological data revealed that the noradrenergic inputs to the dorsal raphe arise from all the catecholaminergic cell groups of the lower brainstem, except the A7 noradrenergic group (Peyron et al., 1996), the pronounced decrease in noradrenaline in the dorsal raphe indicates that the major part of noradrenaline released in the dorsal raphe is indeed derived from locus coeruleus neurons.

The release of 5-HT in the dorsal raphe was slightly but significantly decreased to about 80% of control after inhibition of locus coeruleus activity. This effect demonstrates that under resting conditions the locus coeruleus exerts only a minor excitatory influence on the release of 5-HT in the dorsal raphe. It is not fully clear how the small decrease in the level of 5-HT should be interpreted. The origin of 5-HT in the dorsal raphe is not unequivocal. It has been proposed that 5-HT in the dorsal raphe may derive from dendrites (Bagdy and Harsing, 1995). Alternatively, 5-HT might originate from serotonergic afferents, as it was shown that serotonergic nuclei send projections to one another (Mosko et al., 1977). Finally, collaterals as a possible source of 5-HT also cannot be excluded.

Data in the literature revealed that the noradrenergic input exerts a tonic excitatory influence on the spontaneous firing of the dorsal raphe via α_1-adrenoceptors (Baraban and Aghajanian, 1980; Vandermaelen and Aghajanian, 1983). One could assume that the decrease in noradrenaline release in the dorsal raphe after application of clonidine to the locus coeruleus resulted in a lack of stimulation of α_1-adrenoceptors, which in turn produced the decrease in dorsal raphe activity followed by the decrease in the release of 5-HT. From the present results, it is impossible to conclude whether clonidine infusion has only a presynaptic effect on the release of 5-HT or whether the activity of the dorsal raphe is also changed. Experiments with simultaneous measurement of the release of 5-HT from the terminal area of the dorsal raphe or direct measurement of firing activity are necessary to clarify this.

The slight decrease in 5-HT in the locus coeruleus after clonidine application is difficult to consider because data in the literature describe not only the presence of serotonergic afferents to the locus coeruleus but also serotonergic pericaria in the pericoerulear region (Pickel et al., 1977; Iijima, 1993). The results are most likely explained by an α_2-inhibiting interaction—probably at an axon–axon level—with serotonergic nerve terminals in the locus coeruleus.

During stimulation of locus coeruleus activity with carbachol, the release of noradrenaline was strongly increased (to about 200% and 250% of control) in the locus coeruleus and dorsal raphe, respectively. This confirms the previous conclusion that the dorsal raphe receives a pronounced noradrenaline input from the locus coeruleus. In spite of the large and long-lasting increase in noradrenaline release, the release of 5-HT was not modified in the dorsal raphe. A possible explanation for this observation is that the excitatory influence of noradrenergic innervation is already maximal under resting conditions. As another explanation, it is suggested that the excitatory effect of noradrenaline on the dorsal raphe mediated by α_1-adrenoceptor is counterbalanced by the inhibitory action of α_2-adrenoceptors. The present result is comparable with the observation that noradrenergic reuptake inhibitors, when locally applied to the dorsal raphe, do not change the firing rate of dorsal raphe neurons (Blier et al., 2000). Further investigation is needed to clarify the impact and ratio of α_1- and α_2-adrenoceptors in the regulation of dorsal raphe neuron activity by noradrenaline neurons.

4.2. Serotonergic modulation of the locus coeruleus

Infusion of the 5-HT_{1A} receptor agonist flesinoxan into the dorsal raphe clearly decreased the levels of serotonin in this brain area, indicating that the compound acted as an autoreceptor agonist. A decrease in extracellular 5-HT, to about 70% of control, was seen in the locus coeruleus, thereby indicating that a certain amount of 5-HT released in the locus coeruleus is indeed derived from dorsal raphe innervation. This result confirms the finding of dual-probe push–pull experiments (in anaesthetised rats) that 50% of extracellular serotonin in the locus coeruleus originates from the dorsal raphe (Kaehler et al., 1999). The decrease of activity of the dorsal raphe had no significant effect on the level of noradrenaline in the locus coeruleus. This lack of response suggests that the dorsal raphe does not exert a direct inhibitory influence on the release of noradrenaline in the locus coeruleus under resting conditions. These results are in line with an electrophysiological study demonstrating that serotonin and related compounds failed to modify spontaneous firing activity of noradrenaline neurons when applied locally into the locus coeruleus or by microiontophoresis (Gorea et al., 1991; Haddjeri et al., 1997).

Unexpectedly, infusion of flesinoxan caused a strong decrease in extracellular noradrenaline in the dorsal raphe to about 40% of basal levels. This result illustrates the complexity of the noradrenaline–serotonin interaction at the level of the dorsal raphe. This observation points to an inhibition of noradrenaline release in the dorsal raphe by serotonergic neurons. Presynaptic 5-HT_{1A} receptors located on noradrenergic terminals in the dorsal raphe might be responsible for this effect. The latter could be a more general property of noradrenergic neurons, as we have recently shown that the infusion of flesinoxan into the prefrontal cortex as well as into the locus coeruleus causes a profound

decrease in extracellular noradrenaline in these two different brain areas (Pudovkina et al., 2001).

In conclusion, the present study demonstrated that manipulation of the noradrenergic innervation of the dorsal raphe—by infusing excitatory or inhibitory compounds into the locus coeruleus—had only a minor effect on the release of serotonin in the dorsal raphe.

In turn, evidence was provided that the dorsal raphe does not exert a direct inhibitory influence on the release of noradrenaline in the locus coeruleus. Finally, it was shown that the release of noradrenaline in the dorsal raphe might be locally regulated by 5-HT_{1A} receptors.

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