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Influence of excitatory amino acids on basal and sensory stimuli-induced release of 5-HT in the locus coeruleus

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1 The interactions between 5-hydroxytryptaminergic neurones and excitatory amino acid utilizing neurones were studied in the locus coeruleus of conscious, freely moving rats. The locus coeruleus was superfused with artificial cerebrospinal fluid through a push-pull cannula and 5-hydroxytryptamine (5-HT) was determined in the superfusate that was continuously collected in time periods of 10 min.

2 Superfusion of the locus coeruleus with the NMDA receptor antagonist AP5 (10 μM), kynurenic acid (1 mM), or the AMPA/kainate receptor antagonist DNQX (10 μM) reduced the 5-HT release in the locus coeruleus.

3 Superfusion with the agonists NMDA (50 μM), kainic acid (50 μM) or AMPA (10 μM) enhanced the release rate of 5-HT. AP5 (10 μM) blocked the stimulant effect of NMDA, while tetrodotoxin (1 μM) failed to influence the NMDA-induced release of 5-HT. In the presence of 10 μM DNQX, the releasing effect of 50 μM kainic acid was abolished.

4 Pain elicited by tail pinch, as well as noise-induced stress, increased the release of 5-HT. Superfusion of the locus coeruleus with 10 μM AP5 reduced the tail pinch-induced 5-HT release. AP5 (10 μM) did not affect the noise-induced release of 5-HT which was reduced, when the locus coeruleus was superfused simultaneously with this concentration of AP5 and 1 μM kynurenic acid. DNQX (10 mM) failed to influence the release of 5-HT induced by tail pinch or noise.

5 The findings suggest that 5-hydroxytryptaminergic neurones of the locus coeruleus are tonically modulated by excitatory amino acids via NMDA and AMPA/kainate receptors. The release of 5-HT elicited by tail pinch and noise is mediated to a considerable extent through endogenous excitatory amino acids acting on NMDA receptors, while AMPA/kainate receptors are not involved in this process.

Keywords: 5-Hydroxytryptamine (5-HT) release; locus coeruleus; NMDA; kainic acid; AP5; kynurenic acid; DNQX; glutamate; tail pinch; noise stress; push-pull superfusion

Introduction

The locus coeruleus, the principal noradrenergic nucleus in the brain (Foote *et al*., 1983), contains 5-hydroxytryptaminergic axon terminals and perikarya (Pickel *et al*., 1977; Sladek & Walker, 1977; Léger & Descarries, 1978; Léger *et al*., 1979; Steinbusch, 1984; Iijima, 1993). 5-Hydroxytryptaminergic input to the locus coeruleus (LC) has been shown to arise from the dorsal raphe (Cedarbaum & Aghajanian, 1978; Morgane & Jacobs, 1979; Imai *et al*., 1986; Maeda *et al*., 1991; Vertes & Kocsis, 1994). Both the rostral and caudal regions of the dorsal raphe nucleus seem to contribute to this innervation (Vertes & Kocsis, 1994). The main targets of 5-hydroxytryptamine (5-HT) fibres arising from the dorsal raphe seem to be extranuclear dendrites localized rostromedial at the confines of the locus coeruleus proper (Aston-Jones *et al*., 1991; Maeda *et al*., 1991). Apart from the dorsal raphe, several other 5-HT cell body areas including the median raphe nucleus (Cedarbaum & Aghajanian, 1978; Maeda *et al*., 1991; Luppi *et al*., 1995) and the medial lemnicus including the B9 cell group (Maeda *et al*., 1991; Luppi *et al*., 1995) contribute to the 5-hydroxytryptaminergic innervation of the LC. There is some controversy as to the main source of the 5-HT fibres that impinge on the locus coeruleus, since it was also suggested that the major innervation derives from the median raphe nucleus and the B9 5-hydroxytryptaminergic cell group (Luppi *et al*., 1995), or from the rostroventral pericoerulear region (Aston-Jones *et al*., 1991). Although electrophysiological studies have provided evidence that 5-HT modulates the activity of locus coeruleus neurones (for review see Haddjeri *et al*., 1997), studies concerning the *in vivo* release of 5-HT in the locus coeruleus are scarce. Very recently, we showed that the release of 5-HT in the locus coeruleus is enhanced by noxious and stress stimuli and suggested that 5-hydroxytryptaminergic neurones of the locus coeruleus play a functional role in the modulation by sensory stimuli of locus coeruleus neurones (Singewald *et al*., 1997).

The locus coeruleus also receives a prominent excitatory amino acid input that originates from the nucleus paragigantocellularis and is implicated in the activation of locus coeruleus neurones in response to polymodal stimuli (Aston-Jones *et al*., 1991; Ennis *et al*., 1992). It has been shown that noxious and stress stimuli enhance the release of the excitatory amino acids glutamate and aspartate in the locus coeruleus (Singewald *et al*., 1994; 1995; 1996b) and excite noradrenergic locus coeruleus neurones (Cedarbaum *et al*., 1978; Ennis *et al*., 1992) via non-N-methyl-D-aspartate (NMDA) (Aston-Jones *et al*., 1991) and NMDA receptors (for review see Van Bockstaele & Colago, 1996a). In addition to a postsynaptic effect of excitatory amino acids on noradrenergic neurones, interactions of excitatory amino acids with neurotransmitter systems afferent to the locus coeruleus have to be taken into account.

In vitro and *in vivo* experiments have shown that in various brain regions agonists and antagonists of excitatory amino acid receptors influence the release of 5-HT (Becquet *et al*., 1993; Whitton *et al*., 1994; Tao & Auerbach, 1996; Fink *et al*.,

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1995). Ionotropic excitatory amino acid receptors are present in the locus coeruleus (Luque *et al*., 1995; Van Bockstaele & Colago, 1996a, b), and there is evidence that these receptors are also localized presynaptically on axon terminals in the locus coeruleus (Van Bockstaele & Colago, 1996a).

The aim of the present work was to investigate whether excitatory amino acids modulate 5-HT release in the locus coeruleus under *in vivo* conditions. The push-pull superfusion technique (Philippu, 1984; 1985a, b; Philippu *et al*., 1996) was used to examine the effects of local application of excitatory amino acid receptor agonists and antagonists on the release of 5-HT in the locus coeruleus. These experiments were carried out in conscious rats, since it has been shown that significant differences exist in 5-HT neurotransmission in anaesthetized compared to conscious rats (Done & Sharp, 1994). Furthermore, we investigated the influence of amino acid receptor ligands on the 5-HT release evoked by tail pinch and noise to reveal whether a functional interaction exists between 5-HT and excitatory amino acids, in the mediation of sensory impulses within the locus coeruleus.

Methods

Experimental procedures

Male Sprague-Dawley rats (230–280 g), housed in a light, temperature- and humidity-controlled environment were used. Protocols of experiments were approved by the Bundesministerium für Wissenschaft und Verkehr, Kommission für Tierversuchsangelegenheiten, Austria. Rats were anaesthetized with sodium pentobarbitone (40 mg kg^{-1}, i.p.) and ketamine (50 mg kg^{-1}, i.p.) and the head was fixed in a stereotaxic frame. A guide cannula (o.d. 0.9 mm, i.d. 0.6 mm) with its stylet was stereotaxically inserted until the tip of the cannula was 2 mm above the locus coeruleus (Singewald *et al*., 1995; 1997). The stereotaxic coordinates were (mm): AP 0.8 posterior to interaural line, L 1.3, DV 2.8 mm above the interaural zero plane (Paxinos & Watson, 1986). The guide cannula was fixed on the skull with stainless steel screws and dental cement. PE 50 tubings were inserted into iliac artery and jugular vein, for recording blood pressure (Recomed Hellige, Freiburg, Germany) and intravenous infusions of drugs, respectively. At least two days after surgery, the stylet of the guide cannula was replaced by a push-pull cannula with the following diameters (mm): outer needle o.d. 0.5, i.d. 0.3; inner needle o.d. 0.2, i.d. 0.1. The push-pull cannula was 2 mm longer than the guide cannula, thus reaching the locus coeruleus. The rat was placed in a large cage and, after an adaptation period of two hours, the locus coeruleus of the conscious, freely moving animals was superfused with artificial cerebrospinal fluid (CSF), pH 7.2, at a flow rate of 14 μl min^{-1}. CSF consisted of (mM): NaCl 140, KCl 3.0, $CaCl_2$ 1.25, $MgCl_2$ 1.0, Na_2HPO_4 1.2, $NaHPO_4$ 0.3, glucose 3.0 and pargyline 0.4.

Sensory stimuli were applied for 10 min as previously described (Singewald *et al*., 1995; 1997); for untraumatic tail pinch, a clamp was attached to the rat tail approximately 2 cm from its tip. Noise stress was elicited with 95 dB, frequency band 0.7–20 kHz and positioning the loudspeakers above the animal cage. Noise and tail pinch were applied only once in each animal. Superfusions with tetrodotoxin (TTX) were carried out at the end of the experiments. The interval between two adjacent performances was at least 80 min.

The superfusate was continuously collected for time periods of 10 min at 0°C. Tubes used to collect the superfusate contained 4 μl of the following solution (mmol l^{-1}, final concentrations): $HClO_4$ 140, HCl 1.6, $Na_2S_2O_5$ 0.05, EDTA 0.9 and EGTA 1.4. The samples were stored at −80°C until biochemical analysis was carried out. At the end of the superfusion experiment, the brain was removed and the localization of the cannula was verified histologically. Experiments with cannula localizations outside the locus coeruleus were discarded.

Determination of 5-HT

5-HT was determined by high performance liquid chromatography (h.p.l.c.) with electrochemical detection as previously described (Singewald *et al*., 1997). Briefly, a flow splitter was used to produce a flow rate through the analytical column (SepStik microbore column 150 × 1 mm, 5 μm C18, BAS, West Lafayette, U.S.A.) of 70 μl min^{-1}. The analytical column was protected by a guard column (SepStik 14 × 1 mm, 5 μm C8, BAS) that was directly coupled to a BAS Unijet 3 mm glassy carbon electrode MF-1003 and the electrochemical detector BAS LC-4B. Samples (50 μl) were automatically injected by a CMA 200 (CMA, Stockholm, Sweden) refrigerated microsampler. Injection port and guard column were interconnected by a short piece of peek tubing with an i.d. of 0.005 inches. The mobile phase consisted of 88% phosphate buffer (0.1 M NaH_2PO_4, 1 mM sodium octanesulphonic acid, 10 mM NaCl, 0.5 mM Na_2EDTA, pH was adjusted to 3.5 with o-phosphoric acid), 6% acetonitrile and 6% methanol. Evaluation of data was carried out by comparing peak heights of samples with external standard solutions that contained various concentrations of 5-HT by using an integrator (SIC Chromatocorder 12, System Instruments, Tokyo, Japan). Retention time of 5-HT was 8.5 min, the minimum detection limit amounted to 0.3 pg/sample at a signal: noise ratio of 3.

Drugs

Drugs used were of highest grade available. N-methyl-D-aspartic acid, kainic acid hydrate, kynurenic acid, 5-HT creatinine-sulphate were obtained from Sigma Chemical (Munich, Germany), 6,7-Dinitroquinoxaline-2,3-dione (DNQX), (±)-2-amino-5-phosphonopentanoic acid (AP5) and (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) hydrobromide from Research Biochemical International (RBI, Natick, MA, U.S.A.).

Statistical analysis

Results are presented as mean ± s.e.mean. The release rate of 5-HT in each 10 min sample of superfusate was expressed as a relative value, whereby the mean release rates in the 3 samples preceding the administration of a drug or presentation of a sensory stimulus were taken as 1. Data were analysed by Friedman's test followed by Wilcoxon's signed rank test for paired data.

Results

Basal release of 5-HT in the locus coeruleus; effects of excitatory amino acid receptor ligands

Superfusion of the locus coeruleus with CSF was started immediately after insertion of the push-pull cannula. Since a steady-state in the release of 5-HT is reached approximately after one hour (Singewald *et al*., 1997), collection of super-

fusate started at least 80 min after onset of superfusion. The basal release rate of 5-HT amounted to 6.7 ± 0.7 fmol min^{-1} (mean value $\pm$ s.e.mean, $n = 47$) and was comparable with that found previously (Singewald *et al*., 1997).

Superfusion of the locus coeruleus with the selective NMDA receptor antagonist (±)-2-amino-5-phosphonopentanoic acid (AP5, 10 μM) for 160 min led to an immediate (by 30–40%) and sustained decrease in the release rate of 5-HT which lasted to the end of superfusion with the drug (Figure 1a). The antagonist of NMDA/glycine receptors kynurenic acid (1 mM) diminished 5-HT release by 50% after 20 min. When the locus coeruleus was simultaneously superfused with 10 μM AP5 and kynurenic acid (1 mM) 5-HT was immediately decreased by more than 50% (Figure 1b). However, the decrease in 5-HT release under these conditions did not differ significantly from that obtained with AP5 alone.

In contrast to the antagonists, superfusion with 50 μM N-methyl-D-aspartic acid (NMDA) for 10 min enhanced the release of 5-HT in the locus coeruleus. Presuperfusion with 10 μM AP5 and subsequent superfusion with AP5 and 50 μM NMDA abolished the NMDA-induced release of 5-HT (Figure 2a).

Superfusion with 1 μM TTX decreased 5-HT release by 60% (not shown), as previously demonstrated (Singewald *et al*., 1997). To investigate the effect of TTX on the NMDA-induced release of 5-HT, the locus coeruleus was presuperfused with the neurotoxin (1 μM) for 40 min and subsequently with 50 μM NMDA in the presence of 1 μM TTX. TTX did not really affect the NMDA-induced release of 5-HT (Figure 2b).

The release of 5-HT was also substantially decreased, when the locus coeruleus was superfused with the AMPA/kainate receptor antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX, 10 μM), while the agonists α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA, 10 μM) and kainic acid (50 μM) exerted the opposite effect (Figure 3). Presuperfusion of the locus coeruleus with 10 μM DNQX and, subsequently, with DNQX and 50 μM kainic acid, abolished the effect of kainic acid on the release of 5-HT in the locus coeruleus (Figure 3c).

Although behavioural effects were not recorded, it might be of interest to mention that application of NMDA, AMPA and kainic acid led to alertness, which seemed to be reduced when the agonists were combined with antagonists of excitatory amino acid receptors.

Effects of excitatory amino acid receptor antagonists on the sensory stimuli-induced release of 5-HT

In agreement with previous findings (Singewald *et al*., 1997), tail pinch led to an enhanced release of 5-HT in the locus coeruleus and to a slight rise in mean arterial blood pressure (Figure 4). Presuperfusion of the locus coeruleus with 10 μM AP5 for 40 min before and during tail pinch, reduced the release of 5-HT elicited by tail pinch. Presuperfusion with 1 mM kynurenic acid in the presence of 10 μM AP5 diminished the tail pinch-induced 5-HT release to about the same extent,

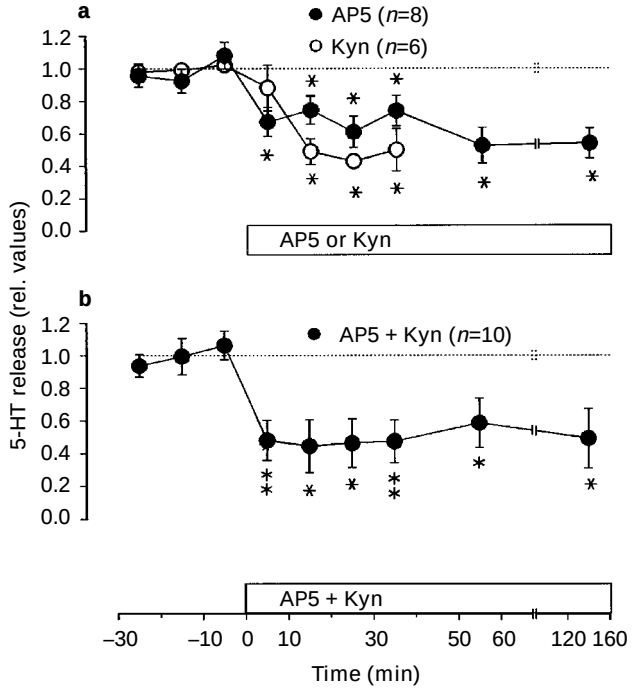

Figure 1 Effects of AP5 (10 μM) and kynurenic acid (1 mM) on the release of 5-HT in the locus coeruleus. (a) Superfusion with AP5 or kynurenic acid, (b) superfusion with AP5 and kynurenic acid (Kyn). Data represent mean values and vertical lines show s.e.mean. The mean release rates of 5-HT in the three samples preceding superfusion with drug were taken as 1.0. Horizontal bars denote start and duration of superfusion with drugs. $*P<0.05$, $**P<0.01$.

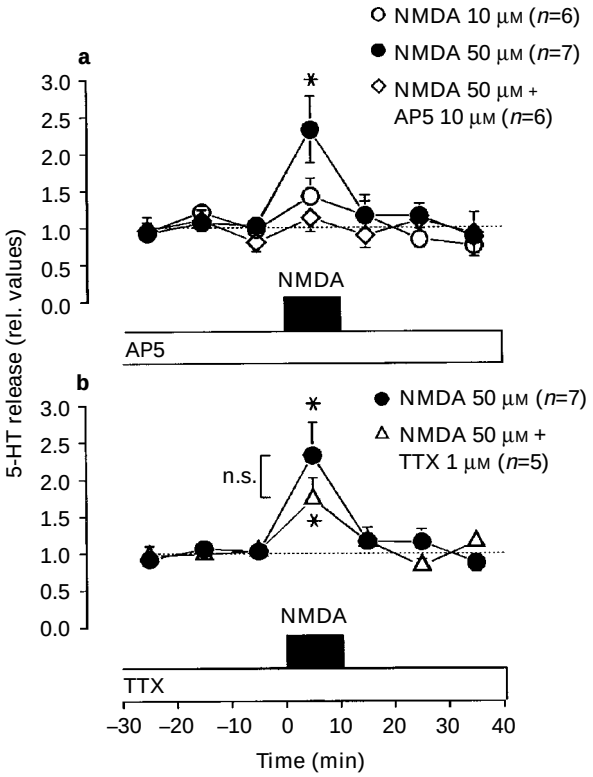

Figure 2 Effects of AP5 and TTX on the NMDA-induced release of 5-HT. Data represent mean values and vertical lines show s.e.mean. The mean release rates in the three samples preceding superfusion with drugs were taken as 1.0. Horizontal bars denote the start and duration of superfusion with drugs. (a) Superfusion with AP5 was started 40 min before the superfusion with 50 μM NMDA and continued to the end of the experiment. Basal release rates preceding NMDA were (fmol min^{-1}) 7.79 ± 1.58 (superfusion with CSF) and 2.14 ± 0.29 (superfusion with AP5). (b) Superfusion with TTX was started 40 min before the superfusion with NMDA and continued to the end of the experiment. Basal release rates preceding NMDA were 7.79 ± 1.58 (superfusion with CSF) and 2.89 ± 0.52 (superfusion with TTX). $*P<0.05$ (Wilcoxon's signed rank test), n.s. not significantly different (Mann-Whitney U-test).

as did superfusion with AP5 alone (Figure 4a, b). Superfusion with 10 μM DNQX failed to influence the pinch-induced rise in the 5-HT release rate (Figure 4c). The excitatory amino acid antagonists did not influence the tail pinch-induced rise in blood pressure (Figure 4d).

Stress elicited by 95 dB noise also enhances the release of 5-HT in the locus coeruleus and elicits a slight increase in blood pressure (Singewald *et al.*, 1997). Presuperfusion with 10 μM AP5 did not affect the noise-induced release of 5-HT (Figure 5a). However, simultaneous presuperfusion of the locus coeruleus with 10 μM AP5 and 1 mM kynurenic acid decreased the release of 5-HT elicited by noise (Figure 5b). DNQX (10 μM) was ineffective (Figure 5c). The antagonists did not influence the noise-induced rise in blood pressure (Figure 5d).

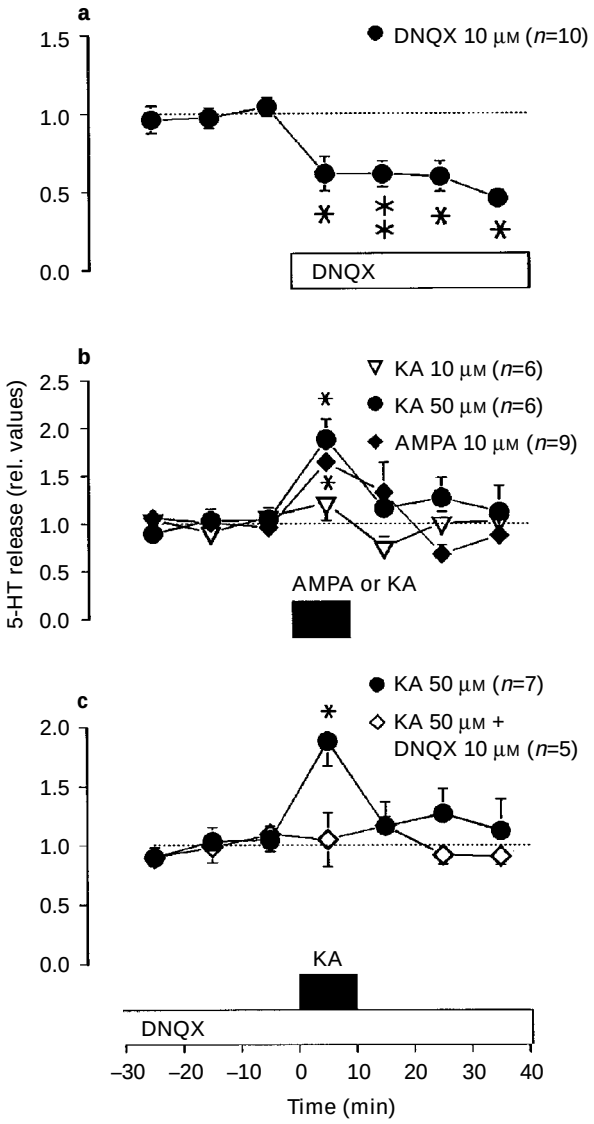

Figure 3 Effects of DNQX, AMPA and kainic acid (KA) on the release of 5-HT. Data represent mean values and vertical lines show s.e.mean. The mean release rates in the three samples preceding superfusion with drugs were taken as 1.0. Horizontal bars denote the start and duration of superfusion with drugs. (c) Superfusion with DNQX was started 40 min before the superfusion with kainic acid and continued to the end of the experiment. Basal release rates preceding kainic acid were (fmol min^{-1}) 5.56 ± 0.66 (superfusion with CSF) and 3.75 ± 0.85 (superfusion with DNQX). $^{*}P<0.05$, $^{**}P<0.01$ (Wilcoxon's signed rank test).

Discussion

This study presents evidence that excitatory amino acids modulate tonically the activity of 5-hydroxytryptaminergic neurones and that the interaction between excitatory amino acids and 5-HT is of functional significance for transmission of sensory stimuli within the locus coeruleus.

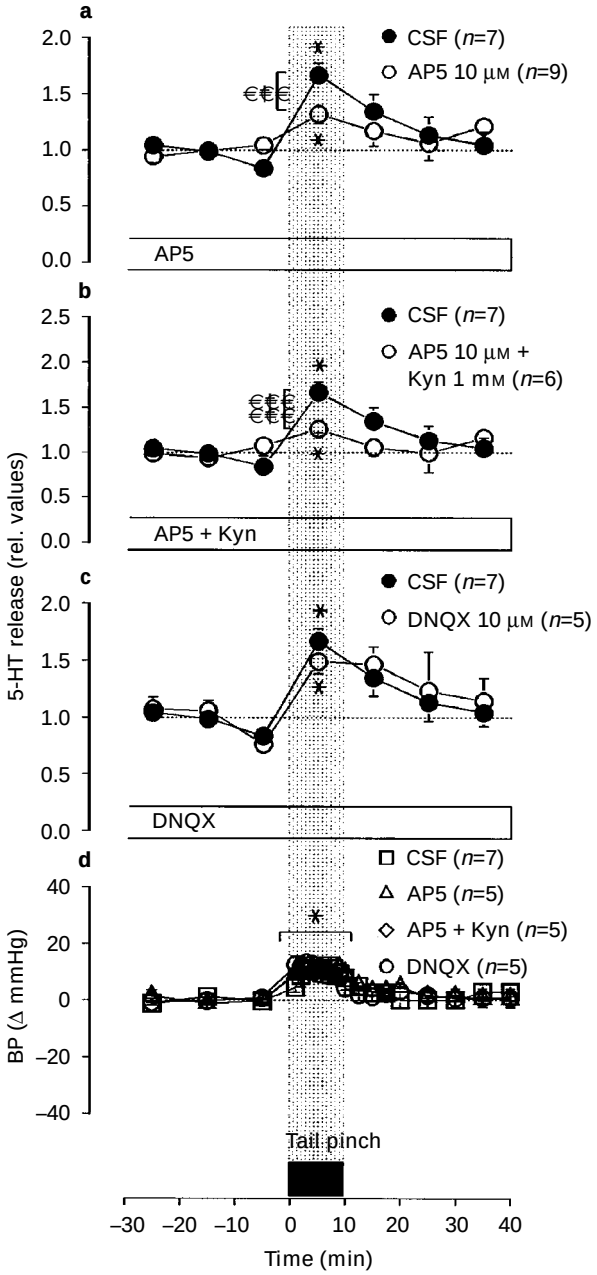

Figure 4 Effects of excitatory amino acid receptor antagonists on the tail pinch-induced release of 5-HT. (a,b,c). Data represent mean values and vertical lines show s.e.mean. The mean release rates in the three samples preceding superfusion with drugs were taken as 1.0. Horizontal bars denote the start and duration of tail pinch (solid bar) or superfusion with excitatory amino acid antagonists (open bars) which was started 40 min before the tail pinch and continued to the end of the experiment. Basal release rates preceding tail pinch were (fmol min^{-1}) 6.59 ± 1.49 (superfusion with CSF, a,b,c), 2.07 ± 0.31 (superfusion with AP5, a), 4.44 ± 1.07 (superfusion with AP5 and kynurenic acid, (Kyn) b), and 2.45 ± 0.39 (superfusion with DNQX, c). (d) Changes in mean arterial blood pressure in mmHg. Basal blood pressure values in the three 10 min periods preceding tail pinch were taken as zero. $^{*}P<0.05$ (Wilcoxon's signed rank test), $\dagger P<0.05$, $\ddagger P<0.01$ (Mann-Whitney U-test).

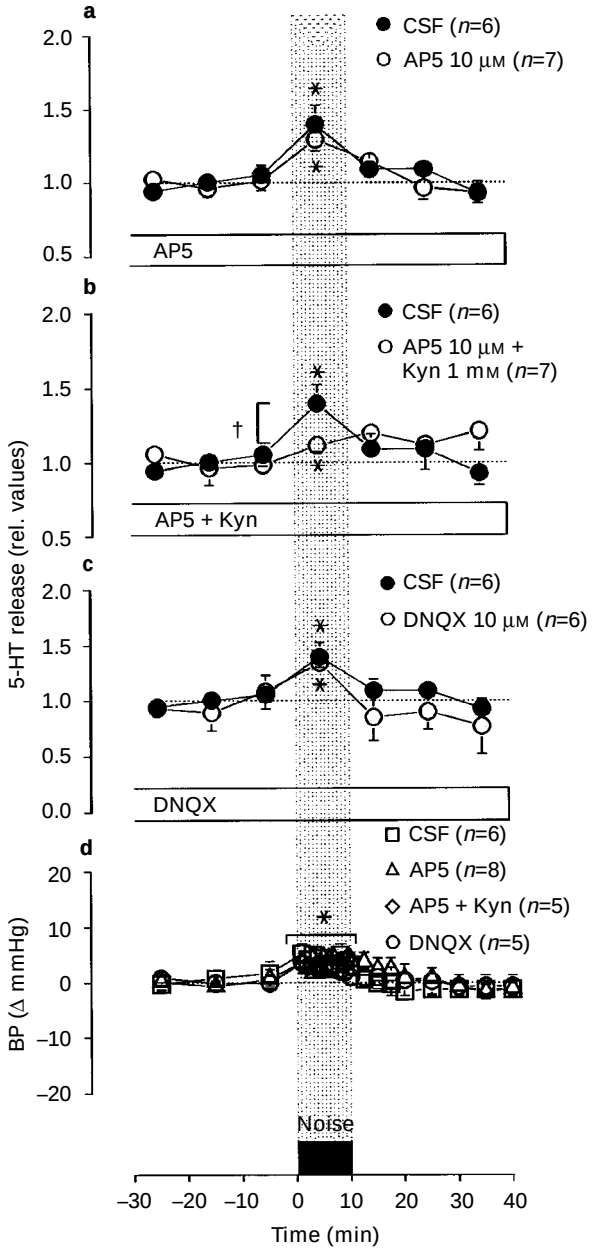

Figure 5 Effects of excitatory amino acid receptor antagonists on the noise-induced release of 5-HT. (a,b,c) Data represent mean values and vertical lines show s.e.mean. The mean release rates in the three samples preceding superfusion with drugs were taken as 1.0. Horizontal bars denote the start and duration of noise (solid bar) or superfusion with excitatory amino acid antagonists (open bars) which was started 40 min before the noise and continued to the end of the experiment. Basal release rates preceding noise were (fmol min^{-1}) 8.43 ± 1.62 (superfusion with CSF, a,b,c,), 3.38 ± 1.43 (superfusion with AP5, a), 3.18 ± 0.72 (superfusion with AP5 and kynurenic acid (Kyn), b), and 2.67 ± 0.50 (superfusion with DNQX, c). (d) Changes in mean arterial blood pressure in mmHg. Basal blood pressure values in the three 10 min periods preceding noise were taken as zero. $^*P < 0.05$ (Wilcoxon's signed rank test), $\dagger P < 0.05$ (Mann-Whitney U-test).

Very recently we demonstrated that, in the conscious rat, 5-HT appears in the superfusate of the locus coeruleus superfused with aCSF through a push-pull cannula. The release rate of 5-HT is enhanced by veratridine and strongly inhibited by TTX. Moreover, the 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-n-propylamino)tetralin applied systemically decreases the release of 5-HT in the locus coeruleus. Thus, 5-HT released in the superfusate derives to a considerable extent from neuronal sites (Singewald *et al*., 1997).

The selective NMDA receptor antagonist AP5 decreased the basal release of 5-HT, suggesting that 5-HT neurones in the locus coeruleus are tonically stimulated by excitatory amino acids via NMDA receptors. Addition of kynurenic acid, a broad-spectrum antagonist of ionotropic excitatory amino acid receptors acting preferentially at the glycine site of the NMDA receptor (Birch *et al*., 1988), did not further decrease basal 5-HT release. Superfusion of the locus coeruleus with NMDA enhanced the release of 5-HT and this effect was antagonized by AP5, demonstrating that the NMDA-induced release is mediated selectively through activation of NMDA receptors. On the other hand, presuperfusion with TTX did not significantly influence the increase in 5-HT release by NMDA. The failure of TTX to block the NMDA-induced rise in 5-HT suggests that these receptors are not located on interneurones, but might be presynaptic receptors on 5-hydroxytryptaminergic nerve terminals. Nevertheless, the possibility that 5-HT release evoked by NMDA also derives from non-neuronal sites cannot be excluded. Under *in vitro* conditions, the stimulating effect of NMDA on the release of 5-HT has also been shown to be partly resistant to the neurotoxin. By using ligands of NMDA subunits it has been suggested that NMDA receptors mediating activation of 5-hydroxytryptaminergic neurones by NMDA contain the NR_{2B} subunit (Fink *et al*., 1995). It is worthwhile to note that this subunit has been shown recently to be expressed in the locus coeruleus (Luque *et al*., 1995).

Like NMDA, AMPA and kainate also enhanced the release of 5-HT. The kainate-induced 5-HT release was abolished by DNQX, an antagonist of AMPA/kainate receptors. Since DNQX also reduced the basal 5-HT release rate, it seems that both NMDA and AMPA/kainate receptors mediate the tonic activation of 5-HT release by endogenous excitatory amino acids, released from excitatory amino acid-containing neurones located in the vicinity (Singewald *et al*., 1994; 1995; 1996b). Enhanced 5-HT release by excitatory amino acids has been observed under *in vivo* conditions in the striatum (Ohta *et al*., 1994; Whitton *et al*., 1994) and raphe (Tao & Auerbach, 1996). However, in other brain areas such as hippocampus (Whitton *et al*., 1994; Tao & Auerbach, 1996), nucleus caudatus (Bequet *et al*., 1990), nucleus accumbens, frontal cortex (Tao & Auerbach, 1996), NMDA seems to decrease the release of 5-HT.

To establish whether the modulation of 5-hydroxytryptaminergic neurones by excitatory amino acids has functional relevance for mediation of sensory stimuli in the locus coeruleus, we investigated the influence of excitatory amino acid antagonists on 5-HT release evoked by pain (tail pinch) and noise stress. Since preliminary experiments have shown that excitatory amino acid antagonists decrease basal 5-HT release rate down to levels not reliably detectable (unpublished observation), pargyline was used to enhance the basal release rate from approximately 2 fmol min^{-1} to 7 fmol min^{-1}. The presence of pargyline does not qualitatively change the effects of tail pinch and noise stress on 5-HT release in the LC (Kaehler *et al*., 1997). We have also shown (Kaehler *et al*., 1996; Singewald *et al*., 1997) that the 5-HT release evoked by both sensory stimuli is abolished by TTX, demonstrating that the stimulus-induced increase in 5-HT release is due to neuronal activation.

Tail pinch-induced release of 5-HT was reduced by AP5. Addition of a high concentration of kynurenic acid did not further inhibit the tail pinch-evoked 5-HT release. Thus, a functionally intact NMDA receptor seems to be necessary for

the full response of 5-hydroxytryptaminergic neurones to pain. As mentioned above, there is strong evidence that these receptors are located presynaptically.

Although AP5 did not influence the noise-induced release of 5-HT, the amine release was diminished on superfusion with AP5 and kynurenic acid, suggesting the importance of NMDA/glycine site to the mediation of noise-induced 5-HT release. Indeed, NMDA/glycine interactions in the control of 5-HT release have been described *in vitro* (Bequet *et al*., 1993). It is known that kynurenic acid in higher concentrations also blocks non-NMDA receptors (Birch *et al*., 1988). However, as shown in the experiments with DNQX, these receptors are not involved in noise-induced 5-HT release. Indeed DNQX, at the same concentration that blocked the pronounced 5-HT increase by kainic acid, failed to influence either the tail pinch-induced, or the noise-provoked 5-HT output. Thus AMPA/kainate receptors are not implicated in the 5-HT release elicited by sensory stimuli.

These results indicate that the tail pinch- or noise-induced activation of 5-hydroxytryptaminergic neurones in the locus coeruleus is mediated via glutamate and aspartate released in response to these stimuli (Singewald *et al*., 1994; 1995; 1996b) and stimulation of presynaptic NMDA receptors, but not AMPA/kainate receptors. However, the excitatory amino acid receptor antagonist, even at high concentrations, diminished but did not abolish the effects of the sensory stimuli on the 5-HT release, thus indicating that the amine release is partially independent of the excitatory amino acid input into the locus coeruleus. This finding suggests that tail pinch and noise might also activate 5-HT neurones via additional, still unidentified excitatory neurotransmitters acting on receptors different to those investigated in the present study. Indeed, in preliminary experiments we obtained evidence that agonists of metabotropic excitatory amino acid receptors also stimulate 5-HT release in the locus coeruleus (unpublished observation).

Alternatively, tail pinch and noise stress might activate 5-HT neurones in the cell body areas contributing to the locus coeruleus 5-hydroxytryptaminergic input (see Introduction). This notion is supported by the finding that noxious pinch has been shown to excite approximately 50% of the neurones studied in the dorsal raphe nucleus of conscious rats (Montagne-Clavel *et al*., 1995, but see results in the cat, reviewed by Jacobs & Fornal, 1991). Moreover, inescapable noise stress seems to increase 5-HT turnover in 5-HT neurones of the median raphe nucleus but not those of the dorsal raphe or hindbrain (Dilts & Boadle-Biber, 1995).

Electrophysiological, biochemical and pharmacological studies have revealed a predominantly inhibitory role of 5-HT on the function of locus coeruleus noradrenergic neurones (for review see Haddjeri *et al*., 1997). For example, 5-HT has been shown to inhibit locus coeruleus neurones (Segal 1979), as well as the responsiveness of locus coeruleus neurones to stimuli that evoke an excitatory amino acid-mediated activation of the LC (Aston-Jones *et al*., 1991). In agreement with an *in vitro* study suggesting a presynaptic inhibitory effect of 5-HT on glutamate synaptic potentials (Bobker & Williams, 1989), we have shown in a preliminary *in vivo* study (Singewald *et al*., 1996a) that 5-HT, applied locally into the locus coeruleus, inhibits glutamate release evoked by tail pinch or noise. The data of the present study suggest that the inhibitory 5-HT modulation is recruited in response to activity of excitatory amino acid afferents. Hence, a possible function of 5-HT release in the locus coeruleus might be to restrain the action of excitatory amino acids released by pain and stress, thus preventing the overstimulation of locus coeruleus neurones in response to sensory stimuli.

This regulatory function of 5-HT might be of clinical relevance in conditions where 5-HT neurotransmission within the locus coeruleus is suspected to be altered, such as in uncontrollable (Weiss *et al*., 1981) or repeated stress (Culman *et al*., 1984). It has been proposed that disturbed 5-HT/noradrenaline interactions may play a role in the pathophysiology of anxiety, panic disorder and depression (Asnis *et al*., 1992; Goddard *et al*., 1996).

In conclusion, we have shown that the release of 5-HT in the locus coeruleus is tonically modulated by excitatory amino acids via NMDA and AMPA/kainate receptors. Furthermore, the release of 5-HT by tail pinch and noise is partly mediated indirectly via excitatory amino acids acting on NMDA receptors, but not on AMPA/kainate receptors.

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