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Influence of AMPA/kainate receptors on extracellular 5-hydroxytryptamine in rat midbrain raphe and forebrain

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1 The regulation of 5-hydroxytryptamine (5-HT) release by excitatory amino acid (EAA) receptors was examined by use of microdialysis in the CNS of freely behaving rats. Extracellular 5-HT was measured in the dorsal raphe nucleus (DRN), median raphe nucleus (MRN), nucleus accumbens, hypothalamus, frontal cortex, dorsal and ventral hippocampus.

2 Local infusion of kainate produced increases in extracellular 5-HT in the DRN and MRN. Kainate infusion into forebrain sites had a less potent effect.

3 In further studies of the DRN and nucleus accumbens, kainate-induced increases in extracellular 5-HT were blocked by the EAA receptor antagonists, kynurenate and 6,7-dinitroquinoxaline-2,3-dione (DNQX).

4 The effect of infusing kainate into the DRN or nucleus accumbens was attenuated or abolished by tetrodotoxin (TTX), suggesting that the increase in extracellular 5-HT is dependent on 5-HT neuronal activity. In contrast, ibotenate-induced lesion of intrinsic neurones did not attenuate the effect of infusing kainate into the nucleus accumbens. Thus, the effect of kainate in the nucleus accumbens does not depend on intrinsic neurones.

5 Infusion of α-amino-3-hydroxy-5-methyl-4-isoxazolaproprionate (AMPA) into the DRN and nucleus accumbens induced nonsignificant changes in extracellular 5-HT. Cyclothiazide and diazoxide, which attenuate receptor desensitization, greatly enhanced the effect of AMPA on 5-HT in the DRN, but not in the nucleus accumbens.

6 In conclusion, AMPA/kainate receptors regulate 5-HT in the raphe and in forebrain sites.

Keywords: 5-Hydroxytryptamine (5-HT, serotonin); kainate receptors, AMPA receptors; dorsal raphe nucleus (DRN); median raphe nucleus (MRN); microdialysis

Introduction

The activity of 5-hydroxytryptamine (5-HT) neurones in the mammalian central nervous system (CNS) is influenced by a number of different neurotransmitters including the excitatory amino acids (EAAs). Iontophoretic application of glutamate, a major EAA in the forebrain (reviewed by Dingledine & McBain, 1994), stimulated 5-HT neuronal discharge in the dorsal raphe nucleus (DRN) of anaesthetized rats (Vander-Maelen *et al*., 1986) and head-restrained cats (Levine & Jacobs, 1992). EAA-accumulating afferents are present in the median raphe nucleus (MRN), another site of 5-HT cell bodies (Kalén *et al*., 1986). Behavioural effects are produced in response to microinjection of EAA receptor agonists and antagonists into the MRN (Wirtshafter *et al*., 1989; Wirtshafter & Krebs, 1990). However, it has not been determined if these effects are the result of EAA-induced changes in MRN 5-HT neuronal activity. Also, 5-HT release may be regulated by EAA receptors on nerve terminals in the forebrain, but both inhibitory (Becquet *et al*., 1990; Whitton *et al*., 1994) and excitatory (Ohta *et al*., 1994; Whitton *et al*., 1994; Fink *et al*., 1996) effects have been observed. This may be due to differences in local circuitry in specific forebrain sites.

The ionotropic effects of glutamate on 5-HT neurones are mediated by *N*-methyl-D-aspartate (NMDA) and non-NMDA EAA receptors. Thus, evoked excitatory postsynaptic potentials recorded from 5-HT neurones in DRN slices were completely blocked only by a combination of NMDA and non-NMDA receptor antagonists (Pan & Williams, 1989). Non-NMDA receptors are further subdivided into kainate- and α-amino-3-hydroxy-5-methyl-4-isoxazolapropionate (AMPA)-preferring subtypes. No selective receptor agonists or antagonists clearly distinguish kainate from AMPA receptors, and both receptor subtypes mediate voltage-independent excitatory responses involving increased permeability to Na^+ and K^+ (Bettler & Mulle, 1995). However, there are several ways that kainate and AMPA receptors can be distinguished. Kainate produces a rapid and completely desensitizing response when applied to cloned receptors consisting of the kainate-preferring subunits GluR-5 to -7 and KA-1,-2 (Sommer *et al*., 1992). Concanavalin A selectively potentiates the response to kainate (Partin *et al*., 1993). AMPA application to the receptor subunits GluR-1 to -4 subunits produces a response that partially desensitizes, but has little effect on kainate-preferring subunits (Partin *et al*., 1993; Patneau *et al*., 1993). The desensitization in response to AMPA can be selectively attenuated by diazoxide and cyclothiazide, but not concanavalin A (Partin *et al*., 1993; Wong & Mayer, 1993). Hybrid receptors between the kainate and AMPA receptor subunits are unlikely to be present in the CNS, supporting the distinction between AMPA- and kainate-preferring subtypes (Partin *et al*., 1993).

In the experiments in the present study microdialysis was used to investigate kainate- and AMPA-elicited increases in extracellular 5-HT in the rat brain. The majority of forebrain 5-HT projections arise from the midbrain raphe. Thus, we were interested in determining if AMPA/kainate receptors are present in the midbrain raphe and if these receptors are tonically active in regulating 5-HT release in forebrain sites. Based on evidence that the DRN may be more sensitive than the MRN to the excitatory effects of NMDA (Tao & Auerbach, 1996), we compared the effects of EAA receptor ligands infused into these two midbrain raphe nuclei. The evidence cited above suggests that AMPA/kainate receptors in the forebrain regulate 5-HT release through effects on nerve endings. Thus, we compared the effect of local infusion of kainate into several forebrain sites innervated by terminals from the DRN or

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MRN. Pretreatment with ibotenic acid was used to destroy intrinsic neurones in the nucleus accumbens and thus determine if these were involved in the effects of infusing EAA receptor ligands into this forebrain site of 5-HT terminals.

Methods

Animals

Male Sprague-Dawley rats (Harlan Sprague Dawley Inc., Indianapolis, IN) were individually housed in cages with food and water available *ad libitum*. The animals were kept at least two weeks on a reversed light-dark cycle (lights off from 9h 30min to 21h 30min) and were briefly handled three to four times a week.

All animal use procedures were in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Rutgers University Institutional Review Board. Rats weighing 300–350 g were anaesthetized with a combination of xylazine (4 mg kg^{-1}, intraperitoneally, i.p.) and ketamine (80 mg kg^{-1}, i.p.), and then mounted in a Kopf stereotaxic frame in the flat skull position. Guide cannulae (22 gauge stainless steel tubing) were implanted above dura (0.9 mm ventral to the skull surface). According to a rat brain atlas (Paxinos & Watson, 1986), the coordinates for the guide cannulae relative to interaural zero were: ventral hypothalamus, AP 6.2, ML 1.0; frontal cortex, AP 12.2, ML 3.2; dorsal hippocampus, AP 4.5, ML −4.4 at a 64° angle lateral to midline; ventral hippocampus, AP 3.0, ML 4.6; nucleus accumbens, AP 10.7, ML 1.4; DRN, AP 1.2, ML 4.0, at a 32° angle lateral to midline; and MRN, AP 1.2, ML 4.0, at an angle of 25° lateral to midline. Experiments were begun no sooner than one week after surgery.

Microdialysis

Microdialysis was performed with an I-shaped probe constructed from 26 gauge stainless steel tubing and glass silica as previously described in detail (Auerbach *et al*., 1989). The dialysis tubing was hollow nitrocellulose fibre (0.2 mm o.d., 6000 MW cut-off; Spectrum Medical Industries, Los Angeles, CA). The length of the steel shaft was adjusted to place 2.5 mm long segments of dialysis tubing in nucleus accumbens (DV 6.0–8.5), frontal cortex (DV 2.0–4.5), ventral hypothalamus (DV 7.2–9.7), ventral hippocampus (DV 6.0–8.5), dorsal hippocampus (DV 3.1–4.2, 64° angle). Similarly, the length of the probe was adjusted to place a 1.0 mm long segment of dialysis tubing in DRN (DV 5.5–6.4, 32° angle), or MRN (DV 7.7–8.6, 25° angle).

The night before an experiment, rats were briefly anaesthetized with methoxyflurane, and dialysis probes were inserted through the guide cannulae and secured with dental cement. Animals were attached to a fluid swivel, allowing unrestricted behaviour within the testing chamber. Before collecting samples, dialysis probes were perfused overnight with a modified, buffered Ringer solution containing (mM): NaCl 140, KCl 30, $CaCl_2$ 1.5, $MgCl_2$ 1.0, NaH_2PO_4 0.27, Na_2HPO_4 1.2 and citalopram 1 μM, pH 7.4. This Ringer solution was pumped at a rate of 1.0 μl min^{-1}. Sample collection began at the beginning of the lights-off period under dim red light conditions. Samples were collected every 30 min and analysed within 30 min of collection by high performance liquid chromatography (h.p.l.c.) with electrochemical detection (e.d.) as previously described in detail (Auerbach *et al*., 1989; h.p.l.c.-e.d., mobile phase composition: 0.15 M chloroacetic acid, 0.12 M NaOH, 0.18 mM EDTA, 1.0 mM sodium octane sulphonic acid and 56 ml l^{-1} acetonitrile; h.p.l.c. pump rate was 0.90 ml min^{-1}).

Experimental protocol

Drugs were administered to rats after 5-HT levels in four successive samples were stable (less than ±10% fluctuation of baseline). The basic experimental protocol involved a 60 min period of kainate or AMPA administration by reverse dialysis in DRN, MRN, or forebrain sites. In some experiments kainate was administered by reverse dialysis in the DRN or MRN while 5-HT was measured by a second dialysis probe in the nucleus accumbens or dorsal hippocampus, respectively. In other experiments, the EAA receptor antagonists kynurenate or 6,7-dinitroquinoxaline-2,3-dione (DNQX) were infused beginning 30 min before and continuing through the 60 min period of kainate treatment. Doses of these drugs were chosen based on comparable dialysis studies of the effects of EAA receptor ligands on dopamine (Keefe *et al*., 1992; Westerink *et al*., 1992). Drugs were dissolved in the phosphate buffered Ringer solution. The pH was not further adjusted before infusion. At 100 μM and 300 μM kainate, pH was reduced to 7.20 and 7.05, respectively. However, there was no significant effect of infusing Ringer solution without drugs and adjusted with HCl to pH values between 4.0–7.4 (data not shown).

The effects of pre-infusion of tetrodotoxin (TTX), cyclothiazide or diazoxide were also determined as described in detail below. For ibotenic acid-induced lesions, the drug was dissolved in Ringer solution at a concentration of 10 μg μl^{-1} and unilaterally infused at a rate of 1 μl min^{-1} for 1 min. The infusion was made via a microinjection cannula inserted into the nucleus accumbens, and ten days later, microdialysis measurements were made in the same site to determine the effect of kainate after lesioning of intrinsic neurones.

Histological procedure

At the end of an experiment, rats were deeply anaesthetized with chloral hydrate (400 mg kg^{-1}) and a 2% fast green solution was perfused through the dialysis probe to stain the surrounding tissue. The brain was removed, frozen, and sliced free hand with a razor blade. Data were excluded if the probe track was not in the targeted brain site. Also, some brains were stained according to a modified silver impregnation method (de Olmos *et al*., 1994). This was used to detect degenerating neurones in the nucleus accumbens after local infusion of kainate (300 μM), or after microinjection of ibotenic acid (10 μg). Briefly, the rats were deeply anaesthetized with chloral hydrate and intra-cardially perfused with 0.067 M cacodylate buffered paraformaldehyde solution (4%). Brains were coronally sectioned (30 μM) by a freezing microtome, and post-fixed for 2–4 days at 4°C. Sections were stained and mounted on slides for viewing with dark field illumination.

Statistics

The data were normalized and presented in figures as mean ± s.e.mean % change from the average of four sequential baseline measurements. Significance was determined by repeated measures ANOVA followed by Scheffe's *F*-test.

Materials

All chemicals were reagent grade or better. Kainate and kynurenate were purchased from Sigma (St. Louis, MO), and citalopram hydrobromide was provided courtesy of Dr C. Sanchez, H. Lundbeck A/S (Copenhagen-Valby). Ibotenic acid, cyclothiazide, diazoxide, (±)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid hydrobromide (AMPA) and 6,7-dinitroquinoxaline-2,3-dione (DNQX) were obtained from RBI (Natick, MA). TTX was obtained from Calbiochem-Novabiochem Corp. (La Jolla, CA).

Results

Infusion of kainate into the DRN and MRN

Effects in the DRN Kainate was dissolved in Ringer solution and locally administered by reverse dialysis in the DRN to

determine its effect in the site of 5-HT cell bodies. As shown in Figure 1a, kainate in the DRN evoked a sustained increase in DRN 5-HT. At a concentration of 10 μM, kainate caused nearly a 100% increase, and the maximal increase, about 150% above baseline, was obtained during infusion of 30 μM kainate. Also, 5-HT was measured in a DRN projection site, the nucleus accumbens, during reverse dialysis infusion of kainate into the DRN. As shown in Figure 1b, infusing kainate into the DRN at concentrations of 30 μM and 100 μM produced increases above baseline 5-HT in the nucleus accumbens of about 50% and 100%, respectively.

Kynurenate (10 μM) or DNQX (10 μM), were infused into the DRN beginning 30 min before and during local infusion of kainate (10 μM). As shown in Figure 2, neither kynurenate nor DNQX alone had a significant effect on basal DRN 5-HT. However, kynurenate completely blocked the effect of kainate (Figure 2a). Similarly, DNQX abolished the effect of kainate in the DRN (Figure 2b).

To determine if the effect of kainate was dependent on neuronal discharge, TTX was infused into the DRN before kainate. TTX (10 μM) produced about a 75% reduction from basal 5-HT in the DRN with a return to baseline after TTX was removed from perfusion medium (Figure 3). As shown in Figure 3a, infusion of TTX greatly attenuated the increase in DRN 5-HT produced by kainate (100 μM). Thus, in the absence of TTX (Figure 1a), mean baseline extracellular 5-HT was 3.2 ± 0.5 pg/sample and the area under the curve (AUC) value for the total increase as a result of infusing kainate was 6.7 pg. After TTX infusion, baseline 5-HT was reduced to 1.3 ± 0.2 pg/sample and the AUC value for the increase produced during co-infusion of kainate (100 μM) was 2.3 pg, significantly less than the effect of kainate in the absence of TTX ($F(1,8)=12.21$, $P<0.0081$). In another study, infusion of TTX (10 μM) into the DRN induced a reduction in nucleus accumbens 5-HT from 3.4 ± 0.7 pg/sample to 1.1 ± 0.2 pg/sample. The infusion of TTX into the DRN completely blocked the increase in nucleus accumbens 5-HT in response to kainate (100 μM) infusion into the DRN (Figure 3b).

A two-day experiment was carried out to investigate the possible toxicity of EAA receptor stimulation. Infusing kainate (100 μM) into the DRN on the first day for 1 h produced an increase from baseline levels of 5.3 ± 0.8 pg/sample to a mean maximum value of 10.6 ± 2.0 pg/sample (Figure 4a). On the second day, baseline 5-HT was 4.0 ± 0.8 pg/sample, and infusion of kainate, (100 μM) for 1 h, evoked an increase to a similar mean maximum value of 10.7 ± 2.9 pg/sample (Figure

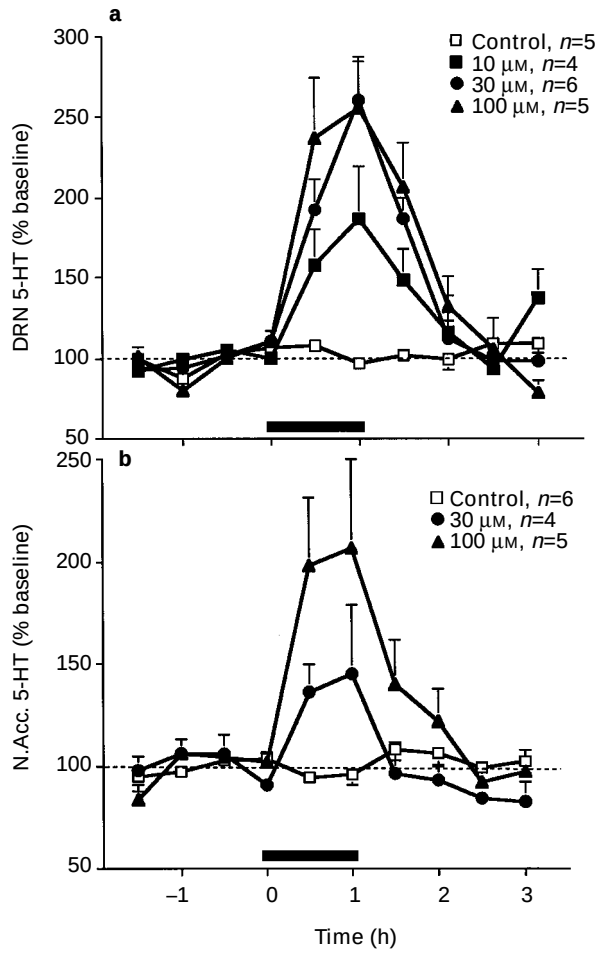

Figure 1 Effect of kainate in DRN on extracellular 5-HT in DRN (a) and nucleus accumbens (b). The data are mean values expressed as a percentage of baseline; vertical lines show s.e.mean. The solid horizontal bar indicates the period of kainate infusion by reverse microdialysis in DRN. (a) Basal extracellular 5-HT in DRN was 4.4 ± 0.9 pg for the control group, 3.5 ± 0.4 pg for kainate 10 μM, 3.6 ± 0.6 pg for kainate 30 μM, and 3.2 ± 0.5 pg for kainate 100 μM. Increases in DRN 5-HT were significant at all doses of kainate: 10 μM; $F(1,7)=13.321$, $P=0.0082$, 30 μM; $F(1,9)=53.372$, $P=0.0001$ and 100 μM; $F(1,8)=20.434$, $P=0.0019$. (b) Basal extracellular 5-HT in nucleus accumbens was 1.9 ± 0.5 pg for the control group, 2.0 ± 0.3 pg for kainate 30 μM, and 2.1 ± 0.4 pg for kainate 100 μM. Kainate (100 μM) infusion into DRN produced significant increases in extracellular 5-HT in nucleus accumbens (N. Acc.); $F(1,9)=8.221$, $P=0.0186$. At a lower dose, kainate (30 μM) had no significant effect; $F(1,8)=2.361$, $P=0.1629$.

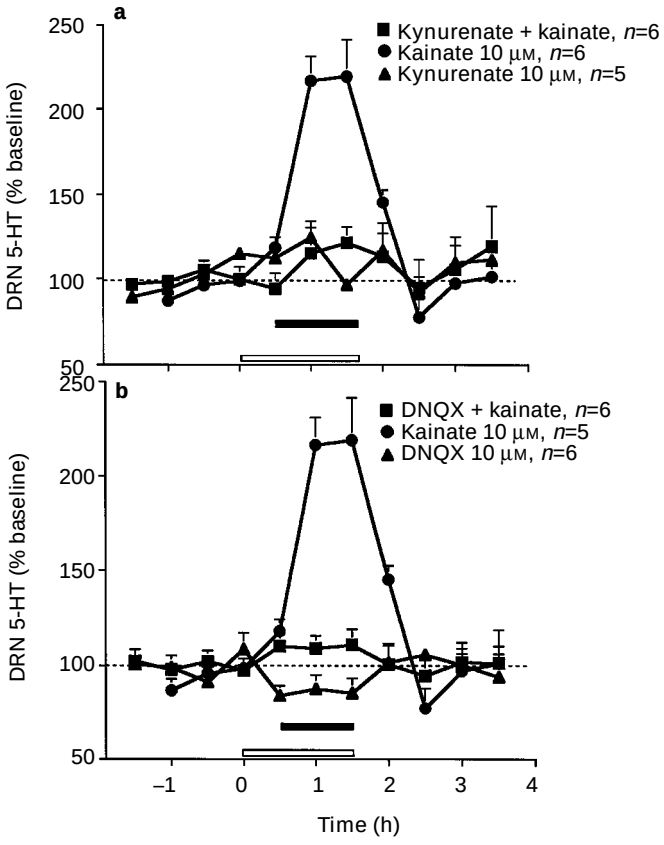

Figure 2 Effect of EAA receptor antagonists on kainate-induced changes in 5-HT in DRN. The data are mean values expressed as a percentage of baseline; vertical lines show s.e.mean. The open and solid horizontal bars indicate the period of DRN perfusion with antagonist and kainate, respectively. Infusing kainate alone as a control (basal extracellular 5-HT 3.5 ± 0.5 pg) induced an increase in DRN 5-HT. (a) Kynurenate alone (basal extracellular 5-HT 5.5 ± 0.3 pg) failed to alter the levels of DRN 5-HT. However, the effect of kainate on extracellular 5-HT (basal extracellular 5-HT 2.5 ± 03 pg) was blocked by pretreatment with kynurenate; $F(1,10)=25.192$, $P=0.0005$. (b) DNQX alone (basal extracellular 5-HT 4.3 ± 1.0 pg) did not affect DRN 5-HT. DNQX pretreatment (basal extracellular 5-HT 4.6 ± 0.5 pg) blocked the effect of kainate; $F(1,10)=33.393$, $P=0.0002$.

4b). Although the % increase above baseline tended to be greater on the second day, the difference from the first day was not significant.

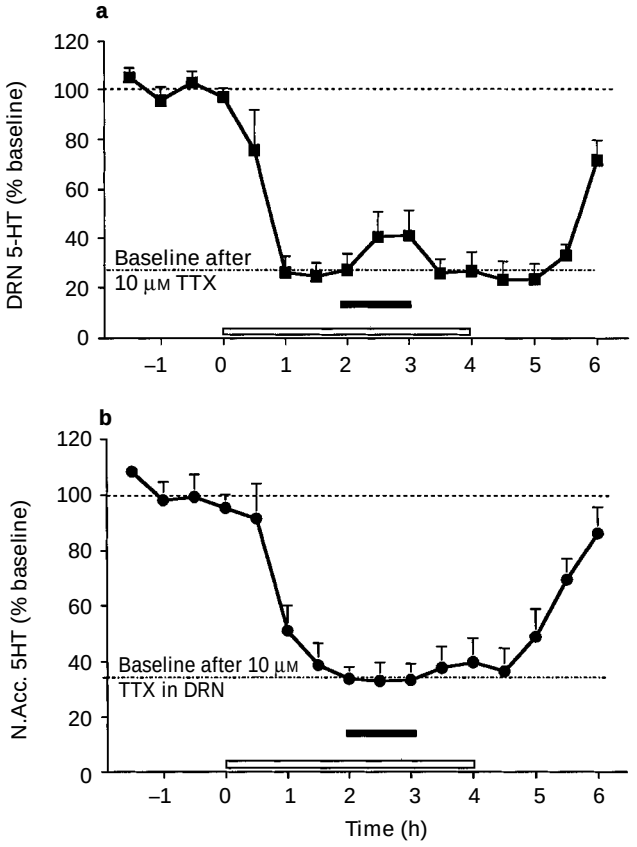

Figure 3 Effect of TTX in DRN on kainate-induced increases in 5-HT in DRN (a) and nucleus accumbens (b). The data are mean values expressed as a percentage of baseline; vertical lines show s.e.mean ($n=6$). The open and solid horizontal bars indicate the period of DRN perfusion with TTX (10 μM) and kainate (100 μM), respectively. (a) Basal extracellular 5-HT in DRN was 6.1 ± 1.3 pg. TTX in the DRN induced a significant decrease in DRN 5-HT ($F(1,8)=11.198$, $P=0.0101$) and attenuated the effect of kainate. (b) Basal extracellular 5-HT in nucleus accumbens was 3.4 ± 0.7 pg. TTX in the DRN induced a significant decrease in nucleus accumbens (N. Acc.) 5-HT ($F(1,10)=11.249$, $P=0.0073$), and blocked the effect of infusing kainate into the DRN on 5-HT in the nucleus accumbens.

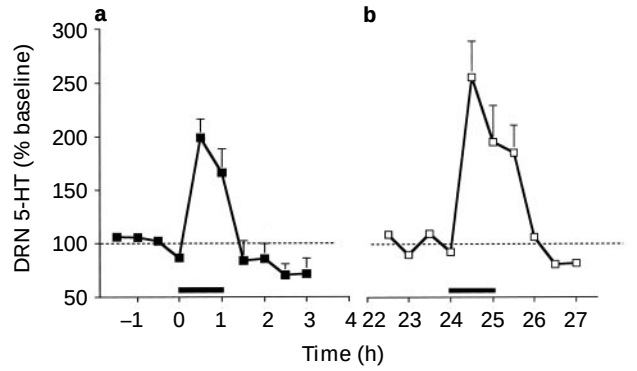

Figure 4 Effect of repeated kainate infusion on extracellular 5-HT in the DRN. The data are mean values expressed as a percentage of baseline; vertical lines show s.e.mean ($n=6$). The solid horizontal bar indicates the period of kainate (100 μM) infusion Basal extracellular 5-HT was 5.3 ± 0.8 pg on day 1 (a) and 4.0 ± 0.8 pg on day 2 (b). Kainate induced a significant increase in extracellular 5-HT on both day 1 (a) and day 2 (b). There was no significant difference between the effect on day 1 and day 2 ($F(1,10)=1.614$, $P=0.2327$).

Infusion of AMPA into the DRN had a weak effect on DRN 5-HT (Figure 5a). At a concentration of 300 μM, there was no effect, and at 1000 μM, the increase was transient and non-significant. Electrophysiological studies indicate that diazoxide and cyclothiazide block the desensitization of AMPA receptors (reviewed by Bettler and Mulle, 1995). To investigate the possible role of receptor desensitization in the small and transient increase in DRN 5-HT, diazoxide (300 μM) or cyclothiazide (300 μM) was infused into the DRN 30 min before AMPA. As shown in Figure 5b, diazoxide or cyclothiazide alone had no effect, but enhanced the effect of AMPA (300 μM), resulting in a significant increase to about 100–150% above baseline 5-HT in the DRN.

Effects in the MRN Reverse dialysis infusion of kainate into the MRN produced a dose-dependent increase in MRN 5-HT (Figure 6a). Thus, at a low dose, kainate (10 μM) induced a 30% increase above baseline 5-HT in the MRN. The effect of

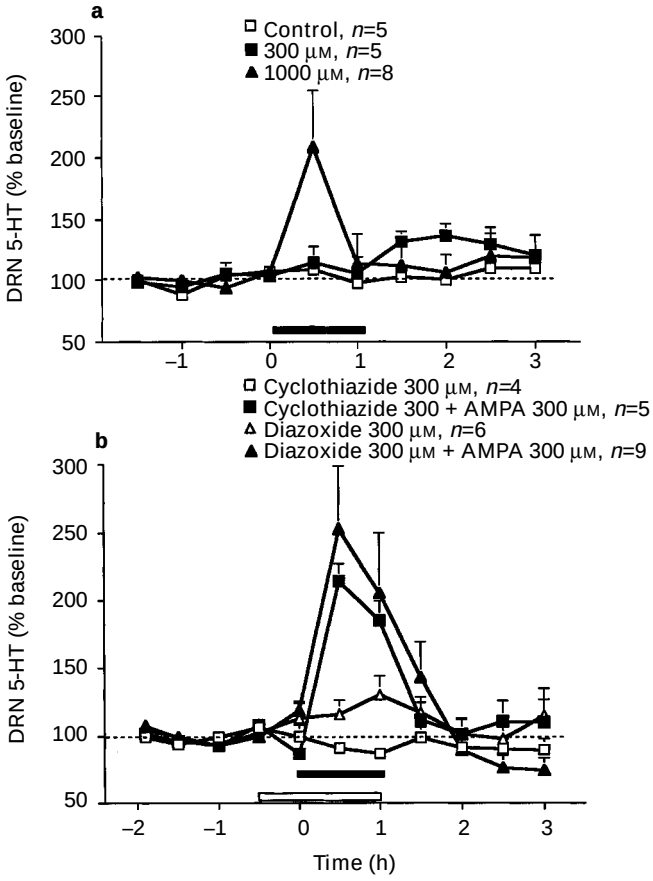

Figure 5 Effect of AMPA in DRN on extracellular 5-HT in DRN. The data are mean values expressed as a percentage of baseline; vertical lines show s.e.mean. (a) Basal extracellular 5-HT was 4.4 ± 0.9 pg in the control group, 4.2 ± 0.9 pg in AMPA 300 μM and 3.2 ± 0.6 pg in AMPA 1000 μM. The solid horizontal bar indicates the period of AMPA infusion by reverse microdialysis in DRN. AMPA infusion had no significant effect on extracellular 5-HT in DRN; 300 μM, $F(1,8)=0.26$, $P=0.6242$; 1000 μM, $F(1,11)=2.797$, $P=0.1226$. (b) The open horizontal bar indicates the period of diazoxide (300 μM) or cyclothiazide (300 μM) infusion into the DRN. The solid horizontal bar indicates the period that AMPA (300 μM) was present in the perfusion medium. Diazoxide alone (basal extracellular 5-HT 5.8 ± 1.3 pg) did not influence 5-HT level in the DRN. However, pretreatment with this drug (basal extracellular 5-HT 6.5 ± 0.9 pg) significantly enhanced the effect of AMPA on release of DRN 5-HT ($F(1.13)=5.013$, $P=0.0433$). Similarly, cyclothiazide alone (basal extracellular 5-HT 5.9 ± 1.9 pg) did not produce a change in DRN 5-HT. Pretreatment with cyclothiazide (basal extracellular 5-HT 5.6 ± 1.4 pg) significantly enhanced the effect of AMPA on the release of DRN 5-HT ($F(1,7)=44.089$, $P=0.0003$).

30 μM kainate was maximal, an increase to about 70% above baseline. Also, extracellular 5-HT was measured in an MRN projection site, the dorsal hippocampus, during kainate infusion into the MRN. As shown in Figure 6b, infusing kainate at concentrations of 100 or 300 μM into the MRN evoked significant increases in dorsal hippocampus 5-HT, about 75% and 130%, respectively.

Effect of infusing kainate into forebrain sites

Kainate was dissolved in Ringer solution and locally administered by reverse dialysis into the nucleus accumbens, ventral hypothalamus, ventral hippocampus or frontal cortex. In the nucleus accumbens (Figure 7a), and ventral hippocampus (Figure 7b), kainate produced dose-dependent increases in extracellular 5-HT. Significant increases in 5-HT were obtained at kainate concentrations between 30–300 μM. The maximal increase in nucleus accumbens 5-HT induced by 100 μM and 300 μM kainate was similar, ~60% above baseline, but the higher dose produced a more sustained increase. Pretreatment with cyclothiazide (300 μM) did not significantly enhance the effect of kainate (30 μM) on extracellular 5-HT in the nucleus accumbens (data not shown). In ventral hypothalamus (Figure 7c), kainate had a significant but much less potent effect. In frontal cortex (Figure 7d), kainate had no significant effect on 5-HT.

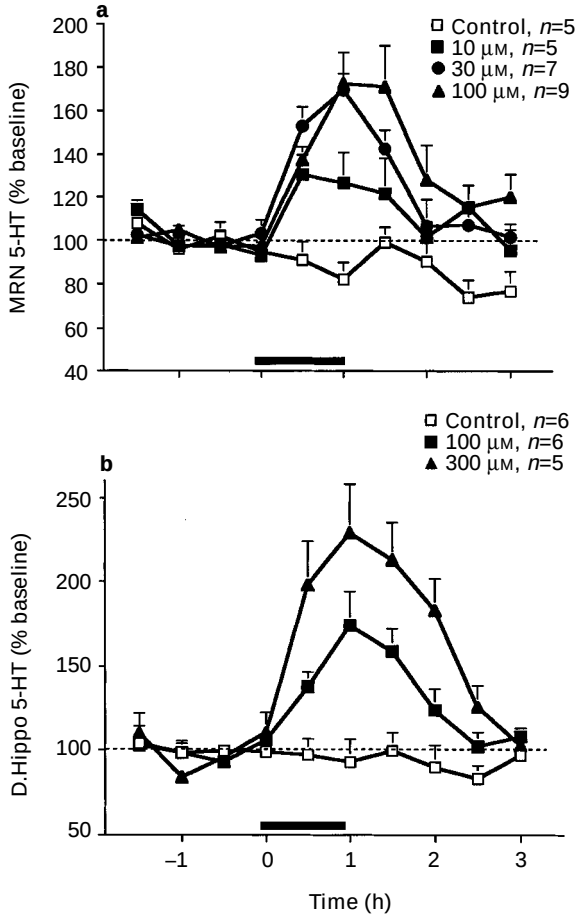

Figure 6 Effect of kainate in MRN on 5-HT in the MRN (a) and dorsal hippocampus (b). The data are mean values expressed as a percentage of baseline; vertical lines show s.e.mean. The solid horizontal bar indicates the period of kainate infusion by reverse microdialysis in MRN. (a) Basal extracellular 5-HT in MRN was 6.9±0.4 pg in the control group, 5.9±0.6 pg in kainate 10 μM, 5.9±1.3 pg in kainate 30 μM, 5.3±1.1 pg in kainate 100 μM. Increases in DRN 5-HT were significant at all doses of kainate: 10 μM, $F(1, 8)=7.323$, $P=0.0268$; 30 μM, $F(1,10)=42.976$, $P=0.0001$; 100 μM, $F(1,12)=15.179$, $P=0.0021$. (b) Basal extracellular 5-HT in dorsal hippocampus was 2.5±0.3 pg in the control group, 2.2±0.3 pg in kainate 100 μM, 2.6±0.3 pg in kainate 300 μM. Kainate infusion into MRN produced significant increases in extracellular 5-HT in dorsal hippocampus (D. Hippo): 100 μM, $F(1,10)=10.181$, $P=0.0096$; 300 μM, $F(1,9)=22.763$, $P=0.001$.

As shown in Figure 8a, kynurenate (100 μM) infusion into the nucleus accumbens blocked the effect of kainate (100 μM) on 5-HT in nucleus accumbens. As shown in Figure 8b, infusing TTX (1 μM) induced a decrease in extracellular 5-HT from 3.5±0.5 pg/30 μl to 1.2±0.1 pg/sample, or about a 65% reduction in nucleus accumbens. This reduction was significant ($F(1,10)=18.456$, $P=0.0016$). The effect of kainate (300 μM) in the nucleus accumbens was completely blocked by pretreatment with TTX. To determine if the kainate-induced increase in 5-HT was dependent on intrinsic neurones, ibotenic acid (10 μg in a volume of 1 μl) was micro-injected into the nucleus accumbens 10 days before the start of microdialysis experiments. In the nucleus accumbens, as determined by silver staining for degenerating neurones (Figure 9), ibotenate had neurotoxic effects on soma, while basal 5-HT from ibotenic acid-treated rats, 2.4±0.4 pg/sample ($n=9$), was not significantly different from controls, 2.3±0.5 pg/sample ($n=7$). Infusion of kainate (300 μM) into the nucleus accumbens of the ibotenate-treated rats produced about a 150% increase in 5-HT (Figure 8c). This was significantly larger than the effect of kainate in the intact nucleus accumbens. AMPA (300 μM) infusion into the nucleus accumbens had no effect on 5-HT in the nucleus accumbens (data not shown). Furthermore, in contrast with the DRN, AMPA (300 μM) infusion beginning 30 min after diazoxide (300 μM) had no significant effect on nucleus accumbens 5-HT (data not shown).

Discussion

EAA receptors in the midbrain raphe

These experiments used *in vivo* microdialysis to investigate the role of non-NMDA ionotropic EAA receptors in the regulation of 5-HT release in the CNS of freely-behaving rats. Local infusion of kainate elevated extracellular 5-HT in the midbrain raphe, an effect that was blocked by kynurenate and the more selective AMPA/kainate antagonist DNQX. However, neither kynurenate nor DNQX alone produced a decrease in basal levels of 5-HT. Similarly, NMDA infusion into the midbrain raphe elicited increases in extracellular 5-HT that could be blocked by NMDA receptor antagonists, but the antagonists alone had no effect on basal 5-HT release (Tao & Auerbach, 1996). Thus, EAA receptors do not appear to have a tonic excitatory influence under our experimental conditions. This is consistent with electrophysiological evidence that kynurenate had no effect on spontaneous discharge of 5-HT neurones recorded in the DRN of head-restrained, awake cats (Levine & Jacobs, 1992). Kynurenate did block the effect of repeated auditory stimuli on single unit discharge, suggesting that EAA inputs to the DRN are involved in mediating the response to phasic sensory stimuli, but are not involved in tonic regulation of 5-HT neuronal activity (Levine & Jacobs, 1992).

In contrast to the effects of EAA receptor antagonists, local infusion of the γ-aminobutyric acid$_A$ ($GABA_A$) receptor antagonist bicuculline into the DRN of freely-behaving rats produced large increases in extracellular 5-HT (Tao *et al.*, 1996). In head-restrained cats, bicuculline application produced significant increases in DRN 5-HT neuronal activity only during slow wave sleep (Levine & Jacobs, 1992). Thus, the apparent predominance of tonic GABA over EAA receptor stimulation during our experiments might be an indication that rats were frequently asleep. These experiments were carried out during the dark period when rats tend to be more active. However, the rats were housed singly in the experimental chamber with minimal environmental disturbances and appeared inactive for much of the time. Because samples were

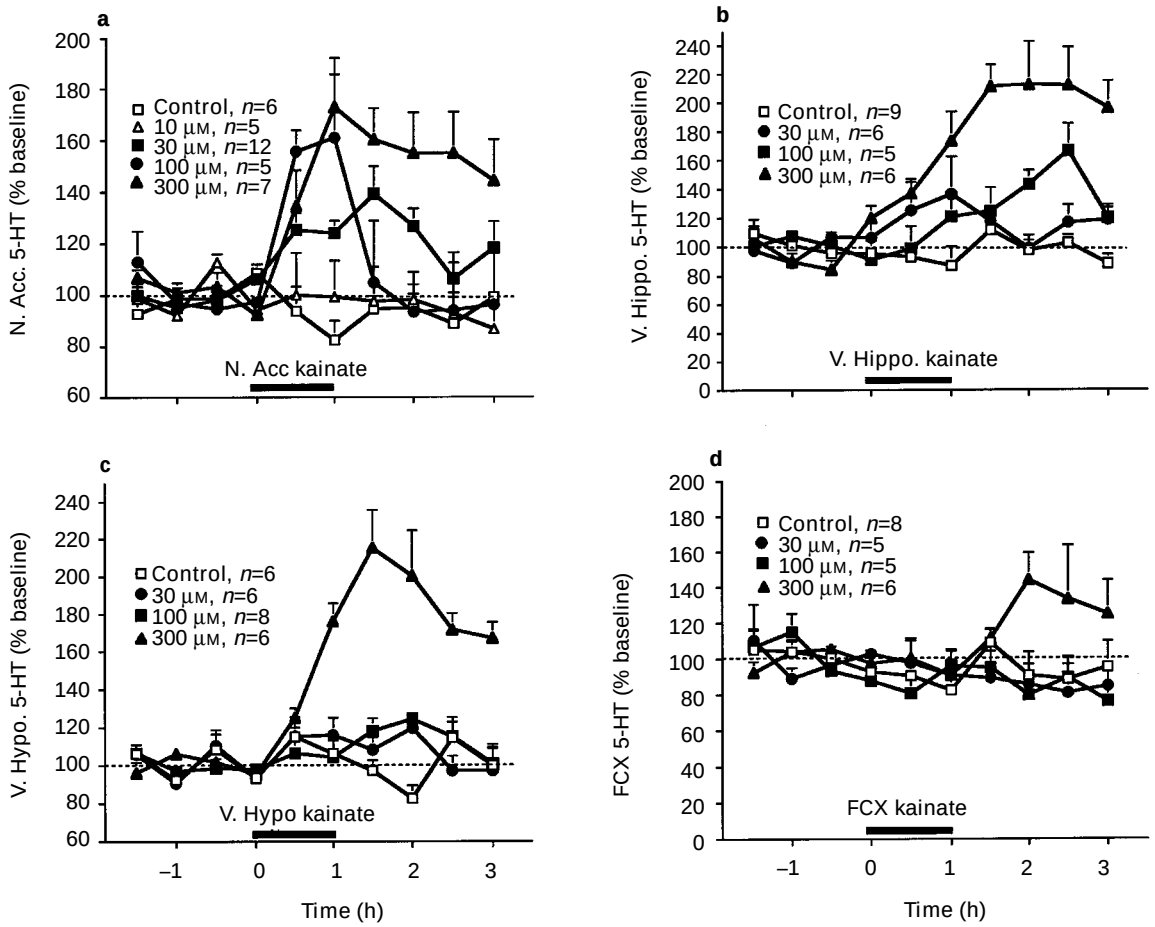

Figure 7 Effect of kainate on the extracellular 5-HT in the nucleus accumbens. (a), ventral hippocampus (b), ventral hypothalamus (c) and frontal cortex (d). The data are mean values expressed as a percentage of baseline; vertical lines show s.e.mean. (a) Basal extracellular 5-HT was 2.1 ± 0.2 pg in nucleus accumbens (N. Acc.). Extracellular 5-HT was significantly increased after infusion of kainate 30 μM ($F(1,10) = 15.568$, $P = 0.0012$); 100 μM ($F(1,9) = 5.475$, $P = 0.044$); 300 μM ($F(1,11) = 51.447$, $P = 0.0001$). (b) Basal extracellular 5-HT was 7.1 ± 0.5 pg in ventral hippocampus (V. Hippo.). Significant increase was found in 30 μM ($F(1,13) = 4.747$, $P = 0.0484$); 100 μM ($F(1,12) = 14.946$, $P = 0.0022$); 300 μM ($F(1,13) = 38.518$, $P = 0.0001$). (c) Basal extracellular 5-HT was 8.4 ± 0.8 pg in ventral hypothalamus (V. Hypo.). A significant increase was observed in response to kainate at the highest dose: 300 μM, ($F(1,10) = 40.783$, $P = 0.001$) but not 30 μM ($F(1,10) = 0.552$, $P = 0.4746$) or 100 μM ($F(1,12) = 1.692$, $P = 0.2171$). (d) Basal extracellular 5-HT was 3.5 ± 0.2 pg in frontal cortex (FCX). Kainate failed to alter extracellular 5-HT even at a high dose: 300 μM, $F(1,12) = 3.889$, $P = 0.0721$.

collected over a 30 min period, more phasic changes in EAA receptor tone could not be assessed.

Kainate infusion into the DRN also produced increases in extracellular 5-HT in the nucleus accumbens. However, the potency of kainate was lower in comparison with its effect on DRN 5-HT. At low doses, kainate diffusion is probably limited to a small area within the DRN. Thus, higher doses may be needed to activate a larger proportion of 5-HT neurones with projections to the nucleus accumbens. Also, activation of somatodendritic 5-HT autoreceptors as a consequence of kainate-induced increases in extracellular 5-HT may restrain increases in 5-HT neuronal activity and thus, increases in depolarization-dependent release in forebrain projection sites.

In agreement with another study (Bosker *et al*., 1994), local infusion of TTX into the DRN produced a large decrease in extracellular 5-HT in the DRN. Furthermore, TTX infusion into the DRN also greatly reduced 5-HT in a DRN projection site, the nucleus accumbens. By blocking voltage-dependent sodium channels, TTX inhibits the propagation of action potentials. Thus, these results suggest that changes in extracellular 5-HT in the raphe and in the forebrain both reflect changes in 5-HT neuronal discharge and are dependent mainly on depolarization-dependent release. Furthermore, TTX largely attenuated the ability of kainate in the DRN to elicit increased 5-HT in the DRN and nucleus accumbens. Thus, kainate infusion into the raphe enhanced extracellular 5-HT by a depolarization-dependent mechanism. It is possible that the effect of kainate on 5-HT was indirectly mediated by interneurones in the raphe. Furthermore, as suggested by a moderate increase in locomotor activity (Tao & Auerbach, unpublished observations), arousal was enhanced by kainate infusion into the raphe at higher concentrations. Thus, it is conceivable that long loop mechanisms, possibly involving reductions in GABAergic tone in the raphe (Levine & Jacobs, 1992), might have contributed to the increase in extracellular 5-HT. However, the evidence that iontophoretic application of glutamate stimulated 5-HT neuronal discharge (Levine & Jacobs, 1992; VanderMaelen *et al*., 1986) suggests that the effect of kainate on extracellular 5-HT was due at least in part to direct activation of EAA receptors on 5-HT neurones.

Microinjection of kainate into the brain can have an excitotoxic effect resulting in local neuronal degeneration as early as 6 h later (Pollard *et al*., 1994). Thus, in the present experiments increases in extracellular 5-HT could have resulted at least in part from kainate neurotoxicity. However, kainate was effective when infused into the raphe at concentrations in the range of 10–30 μM. The dialysis membrane serves as a barrier to free diffusion, and the total estimated amount of kainate

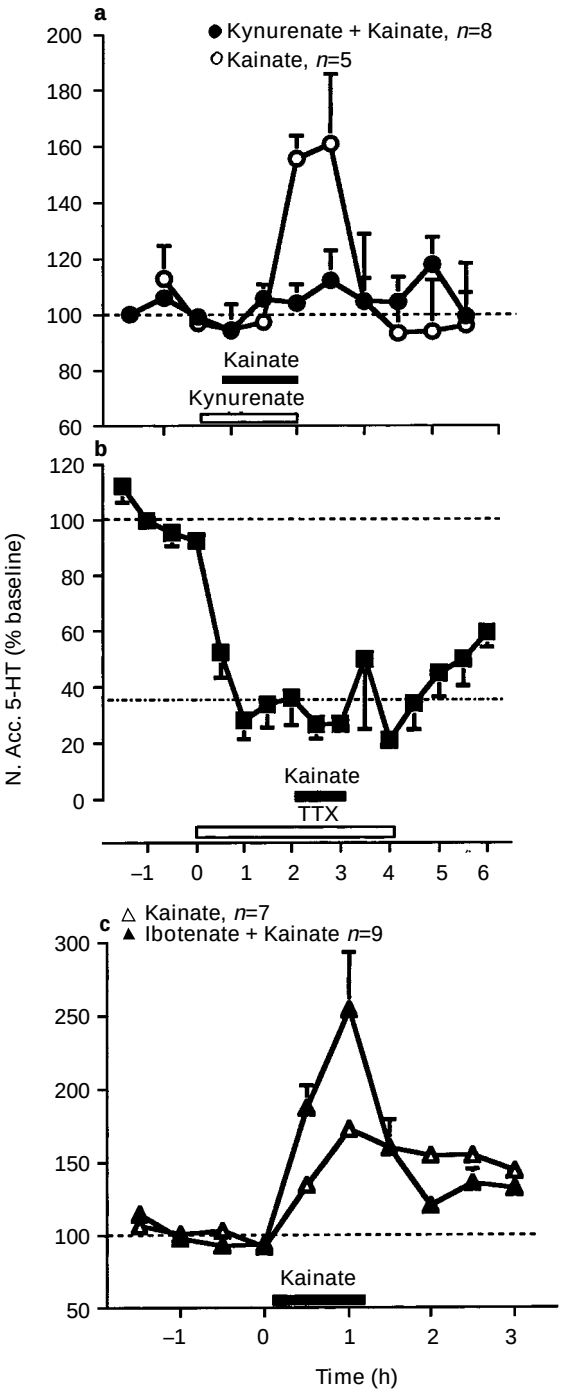

Figure 8 Effect of kynurenate (a), TTX (b) or ibotenate lesioning (c) on kainate-induced increases in 5-HT in nucleus accumbens (N. Acc.). The data are mean values expressed as a percentage of baseline; vertical lines show s.e.mean. The open horizontal bars indicate the period of kynurenate or cyclothiazide infusion by reverse microdialysis in nucleus accumbens. The solid horizontal bars indicate the period of kainate infusion into nucleus accumbens. (a) Kainate (100 μM) alone (basal extracellular 5-HT 1.7 ± 0.3 pg) evoked an increase in 5-HT in the nucleus accumbens. Kynurenate (100 μM) blocked the effect of kainate (100 μM) on extracellular 5-HT in nucleus accumbens (basal extracellular 5-HT 3.6 ± 0.3 pg; $F(1, 11) = 14.946$, $P = 0.0026$). (b) Basal extracellular 5-HT in nucleus accumbens was 3.5 ± 0.5 pg. TTX (1 μM) induced a decrease in extracellular 5-HT in nucleus accumbens and blocked the effect of kainate (300 μM) in the nucleus accumbens ($n = 6$). (c) As compared with kainate (300 μM) alone (basal extracellular 5-HT 2.3 ± 0.5 pg), the kainate (300 μM)-induced increase in nucleus accumbens 5-HT was enhanced by pretreatment with ibotenate 10 μg (basal extracellular 5-HT 2.4 ± 0.4 pg), and this enhancement was significant ($F(1,14) = 5.06$, $P = 0.041$).

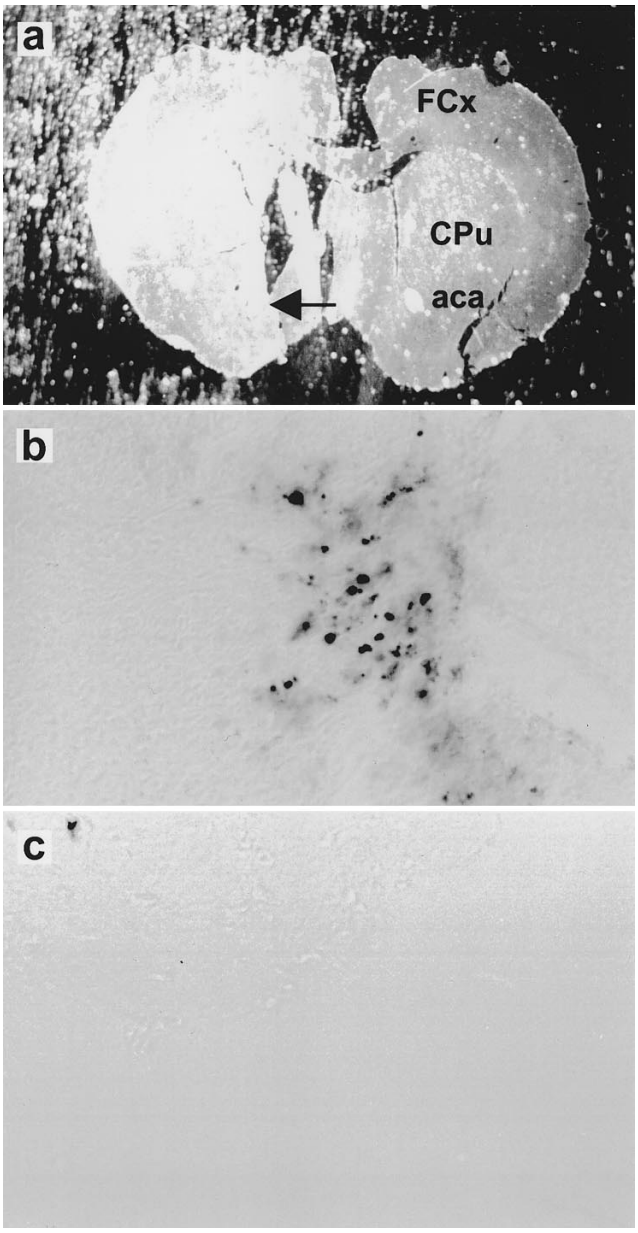

Figure 9 Histological characteristics of ibotenate-induced neuronal degeneration as determined by silver staining. Rats were injected with ibotenate (10 μg) in the nucleus accumbens 10 days before microdialysis experiments. (a) Coronal section through the area of the nucleus accumbens as viewed at low magnification under dark field illumination. The arrow at the bottom end of the probe tract indicates the area viewed at high magnification. (b) Ten days after ibotenate treatment, the strong staining pattern in the nucleus accumbens is evident as viewed at high magnification (400×). (c) In contrast, 3 h after infusing kainate (300 μM) for 60 min via a microdialysis probe in the nucleus accumbens produced no staining. Abbreviations: FCx, frontal cortex; CPu, caudate putamen; aca, anterior commissure.

reaching extracellular space, 0.1 μg, is less than the toxic level found previously (Schwarcz *et al*., 1979). Furthermore, 24 h after infusing kainate into the DRN, basal levels of DRN 5-HT and the ability of kainate to elicit increases in DRN were not significantly altered (Figure 4). Nevertheless, a small component of kainate-elicited increases in 5-HT was insensitive to TTX. Similarly, TTX nearly completely blocked the increase in dopamine in response to infusion of a lower concentration (100 μM) of kainate into the striatum. In contrast, at a higher concentration (300 μM), 50% of dopamine release elicited by kainate was insensitive to TTX (Westerink *et al*., 1992). Thus, kainate-evoked 5-HT and dopamine neurotrans-

mitter release is partly independent of physiological activity of neurones, but this occurs mainly at higher concentrations than used in the present study.

Kainate was about ten fold more potent than NMDA which was effective in the range of 100–300 μM in the DRN (Tao & Auerbach, 1996). However, the maximal effect of NMDA on raphe 5-HT was about two fold greater than kainate. This is similar to the effects of infusing EAA receptor ligands into an area of dopaminergic cell bodies in the substantia nigra. As compared to NMDA, which was effective in the range 100–300 μM, kainate produced smaller increases in nigral dopamine but was effective in the range 10–30 μM (Westerink *et al*., 1992). The nearly equipotent effect of kainate in the DRN and MRN contrasts with regional differences in the effects of NMDA and GABA receptor ligands. The $GABA_A$ receptor agonists muscimol was about five fold more potent in reducing 5-HT in the DRN as compared to the MRN (Tao *et al*., 1996). Also, NMDA was more potent in evoking increased 5-HT in the DRN in comparison with the MRN (Tao & Auerbach, 1996). The equipotent effect of kainate in the DRN and MRN suggests that the differences in sensitivity to NMDA and GABA receptor ligands cannot be ascribed to technical problems with localized drug infusion by reverse dialysis into the midbrain raphe nuclei.

AMPA elicited an increase in DRN 5-HT, but its effect was less potent and more transient relative to kainate. Nevertheless, it is possible that the effects of AMPA in the raphe as well as kainate were mediated by AMPA-preferring receptors. AMPA-preferring receptors have a widespread distribution in the CNS. In particular, neurones in the periaqueductal gray (PAG) area encompassing the DRN have abundant expression of mRNA for the AMPA-preferring GluR-1 and GluR-2 subunits but only weakly- or non-detectable levels of mRNA for kainate-preferring subunits (Tolle *et al*., 1993). Kainate has effects in many areas of the CNS but this could be due to its ability to bind to both kainate and AMPA receptors (Hall *et al*., 1994). AMPA subunit responses to kainate show little desensitization (reviewed by Bettler & Mulle, 1995). In contrast, recombinant kainate-preferring subunits rapidly desensitize in response to kainate. Studies of AMPA-preferring receptors indicate that the desensitization in response to AMPA, but not kainate, can be attenuated by cyclothiazide and diazoxide (Partin *et al*., 1993). Thus, in the present study, both the transient response to AMPA (that was enhanced by diazoxide and cyclothiazide), and the sustained response to kainate are consistent with the possibility that AMPA-preferring receptors were responsible for the effects of both drugs on raphe 5-HT.

EAA receptors on 5-HT terminals in forebrain sites

Infusion of kainate into several forebrain sites also elicited increases in extracellular 5-HT. In the hypothalamus and frontal cortex, the increase was delayed, prolonged and only apparent at the highest dose. This suggests that in these two sites the increase was due to excitotoxic effects of kainate. In the nucleus accumbens and ventral hippocampus, the effect of kainate on 5-HT was apparent in an intermediate concentration range, about three times less potent than in the midbrain raphe. This was similar to the potency of kainate in eliciting increased dopamine in the area of terminals in the striatum relative to dopaminergic cell bodies in the substantia nigra (Keefe *et al*., 1992; Westerink *et al*., 1992).

Infusion of TTX into the nucleus accumbens produced a large decrease in baseline levels and completely blocked kainate-induced increases in 5-HT. TTX-sensitivity has been interpreted as indicating that the presynaptic interaction between two neurotransmitters is indirectly mediated by changes in the activity of a third interposed neurone (reviewed by Chesselet, 1984). This is probably valid for *in vitro* studies in which basal neurotransmitter release is evoked by electrical stimulation or high potassium-induced release from terminals and thus, is unaffected by TTX. However, for *in vivo* microdialysis, basal release is dependent on axonal conduction of impulses and is greatly inhibited by TTX. Thus, TTX sensitivity of kainate-evoked increases in extracellular 5-HT only discriminates between effects that are dependent on axonal impulses from effects that are independent of physiological depolarization, for example pathological influx of calcium or non-exocytic release of neurotransmitter (discussed by Westerink *et al*., 1992). Furthermore, in contrast to TTX, ibotenate lesioning of the nucleus accumbens did not block, but instead led to a significant enhancement of the effect of kainate. Ibotenate induces localized destruction of intrinsic neurones without damage to terminals or changes in 5-HT levels (Hastings *et al*., 1985; Markowska *et al*., 1985). By use of a silver impregnation method for detecting degenerating neurones, as shown in Figure 9b, ibotenate infusion induced strong staining in the nucleus accumbens. In contrast kainate infusion did not have a detectable neurotoxic effect under our experiment conditions (Figure 9c). Thus, our results suggest that kainate in the nucleus accumbens acted directly on 5-HT nerve terminals to enhance release in an impulse-dependent manner. The enhanced effect of kainate in the nucleus accumbens after lesioning interneurones suggests that direct excitation of 5-HT nerve terminals was partially offset in unlesioned rats by co-stimulation of presynaptic inhibitory interneurones.

Whitton and co-workers (1994) showed that AMPA in the rat hippocampus was very potent in eliciting increased extracellular 5-HT, an effect that was potentiated by diazoxide. However, we observed that infusion of AMPA (300 μM) into the nucleus accumbens alone or together with diazoxide had no effect on 5-HT. This difference suggests the possibility that the receptors on 5-HT nerve terminals in the nucleus accumbens are kainate-preferring, and in the hippocampus, AMPA-preferring. In contrast, glutamate infusion into cat caudate produced a decrease in extracellular 5-HT (Reisine *et al*., 1982). Glutamate-mediated inhibition of 5-HT release in forebrain sites was blocked by an NMDA receptor antagonist and thus, may involve NMDA receptors (Becquet *et al*., 1990; Whitton *et al*., 1994). Infusion of bicuculline into the caudate also blocked the decrease in 5-HT induced by glutamate (Becquet *et al*., 1990). This suggests that NMDA may stimulate GABA release which in turn acts on 5-HT terminals to inhibit 5-HT release. Thus, changes in release induced by EAAs may differ in specific forebrain sites, depending on which EAA receptor subtypes are present and complex interactions between intrinsic neurones and 5-HT terminals.

In conclusion, microdialysis can provide information concerning which inputs are important in tonic regulation of 5-HT release in the CNS of freely behaving animals. Stimulation of kainate/AMPA receptors in both the midbrain raphe and forebrain projection sites induced increases in extracellular 5-HT. However, EAA receptor antagonists did not produce a decrease in extracellular 5-HT. Together with previous studies (Tao *et al*., 1996), our results suggest that GABA is the predominate tonic influence on DRN neurones under our experimental conditions. In future studies it would be of interest to determine if the relative importance of GABA and EAA inputs varied with spontaneous or evoked changes in behavioural state.

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