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Differential regulation of 5-hydroxytryptamine release by $GABA_A$ and $GABA_B$ receptors in midbrain raphe nuclei and forebrain of rats

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1 Extracellular 5-hydroxytryptamine (5-HT) was determined in dorsal raphe nucleus (DRN), median raphe nucleus (MRN) and nucleus accumbens by use of microdialysis in unanaesthetized rats.

2 Infusion of the γ-aminobutyric acid $(GABA)_A$ receptor agonist muscimol into DRN and MRN resulted in decreased 5-HT in DRN and MRN, respectively. Muscimol infusion into nucleus accumbens had no effect on 5-HT.

3 Infusion of the $GABA_A$ receptor antgonist bicuculline into DRN resulted in increased DRN and nucleus accumbens 5-HT. Bicuculline infusion into MRN had no effect on 5-HT. This suggests that endogenous GABA had a tonic, $GABA_A$ receptor-mediated inhibitory effect on 5-HT in DRN, but not in MRN.

4 Infusion of the $GABA_B$ receptor agonist baclofen into DRN produced a decrease in DRN 5-HT. Baclofen infusion into nucleus accumbens resulted in decreased nucleus accumbens 5-HT. This suggests that $GABA_B$ receptors are present in the area of cell bodies and terminals of 5-hydroxytryptaminergic neurones.

5 Infusion of the $GABA_B$ receptor antagonists phaclofen and 2-hydroxysaclofen had no effect on midbrain raphe and forebrain 5-HT. This suggests that $GABA_B$ receptors did not contribute to tonic inhibition of 5-HT release.

6 In conclusion, 5-HT release is physiologically regulated by distinct subtypes of GABA receptors in presynaptic and postsynaptic sites.

Keywords: 5-Hydroxytryptamine (5-HT) release; GABA receptor; dorsal raphe nucleus; median raphe nucleus; microdialysis

Introduction

The role of γ-aminobutyric acid (GABA) as an inhibitory neurotransmitter in the midbrain raphe is well established. GABAergic terminals are present (Belin *et al.*, 1979), and make synaptic connection with 5-hydroxytryptaminergic neurones in the dorsal raphe nucleus (DRN) (Wang *et al.*, 1992). Local application of GABA receptor agonists in the DRN inhibits 5-hydroxytryptaminergic neuronal activity (Gallager & Aghajanian, 1976) and 5-hydroxytryptamine (5-HT) release and metabolism in the forebrain (Hery & Ternaux, 1981; Nishikawa & Scatton, 1983; Romandini & Samanin, 1984; Tao & Auerbach, 1994). These effects are blocked by specific GABA receptor antagonists (Gallager & Aghajanian, 1976; Romandini & Samanin, 1984). Furthermore, GABA receptor agonists inhibit 5-HT release from cortex and striatal slices (Bowery *et al.*, 1980; Schlicker *et al.*, 1984) consistent with extensive evidence that GABA is a presynaptic modulator substance.

$GABA_A$ and $GABA_B$ receptor subtypes have been characterized based on pharmacological criteria and differences in coupling to ion channels. In the DRN compounds with selective affinity for $GABA_A$ or $GABA_B$ receptors mediate inhibition of 5-HT activity by distinct transduction mechanisms (Innis & Aghajanian, 1987). Thus, the inhibition produced by the $GABA_A$ selective agonist muscimol results from activation of a receptor-chloride channel complex (Mathers, 1987). In contrast, $GABA_B$ receptors, selectively stimulated by baclofen, are coupled via G-proteins to potassium channel activation (Hill & Bowery, 1981; Dutar & Nicoll, 1988). Compounds with selective affinity for $GABA_B$ receptors are also effective as presynaptic modulators (Bowery *et al.*, 1980) and thus act in the forebrain to inhibit 5-HT release from nerve terminals (Schlicker *et al.*, 1984).

The present study addressed several unresolved issues in GABAergic regulation of 5-HT release. First, because of possible differences between the raphe nuclei (Hjorth, 1992; Tork, 1990), we have directly compared the effects of GABA receptor compounds on release of 5-HT from DRN and median raphe nucleus (MRN) neurones. Second, the relative importance of $GABA_A$ and $GABA_B$ receptors, and possible role in tonic regulation of 5-HT neuronal activity *in vivo* has not been established. The increase in hippocampal 5-HT metabolism produced after bicuculline injection into the MRN suggests that $GABA_A$ receptors may be tonically activated in this midbrain site (Forchetti & Meek, 1981). In contrast, bicuculline infusion into the DRN had no influence on striatal 5-HT metabolism (Nishikawa & Scatton, 1985b). Thus, we have directly compared the effects of $GABA_A$ and $GABA_B$ agonists and antagonists to investigate the relative importance of these receptor subtypes in the physiological regulation of 5-HT release. Extracellular 5-HT was examined by *in vivo* microdialysis in the DRN, MRN and nucleus accumbens of unanaesthetized, freely-moving rats. Drugs were administered by reverse dialysis infusion into the raphe nuclei or nucleus accumbens. By use of microdialysis, extracellular 5-HT in the raphe and forebrain sites has been shown to be decreased in response to tetrodotoxin, autoreceptor agonists and infusion of low calcium dialysis solution (Auerbach *et al.*, 1989; Bosker *et al.*, 1994 Rutter *et al.*, 1995; Sharp *et al.*, 1989b; Tao & Hjorth, 1992). Furthermore, lesioning the raphe with the selective 5-HT neurotoxin 5,7-dihydroxytryptamine led to very low or undetectable 5-HT in dialysis samples (Kalen *et al.*, 1988; Sharp *et al.*, 1989a). This evidence suggests that changes in extracellular levels in the raphe and forebrain sites reflect changes in 5-HT neuronal activity.

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Methods

Animal preparation

Male Sprague-Dawley rats (Harlan Sprague Dawley Inc., Indianapolis, IN) were individually housed in cages with food and water available *ad libitum*. 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. Before the experiments, the animals were kept at least two weeks on a reversed light-dark cycle (lights off from 9 h 30 min to 21 h 30 min) and were briefly handled three to four times a week. Rats weighing 300–350 g were anaesthetized with a combination of xylazine (4 mg kg^{-1}, 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 the dura (0.9 mm ventral to the skull surface) according to a rat brain atlas (Paxinos & Watson, 1982). The coordinates for the guide cannulae relative to interaural zero were: 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; nucleus accumbens, AP 10.7, ML 1.4. Dialysis measurements were carried out no sooner than one week after surgery.

Microdialysis and analytical techniques

Concentric style (l-shaped) microdialysis probes were constructed from 26 gauge stainless steel tubing and glass silica. 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). For perfusion into the area of the raphe, 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 sampling, rats were briefly anaesthetized with methoxyflurane and dialysis probes were inserted through the guide cannulae and secured with dental cement. Animals were attached to fluid swivels, allowing relatively unrestricted behaviour within the testing chamber. Before the collection of samples, dialysis probes were perfused overnight with artificial cerebrospinal fluid (aCSF) containing (mM): NaCl 140, KCl 3, $CaCl_2$ 1.5, $MgCl_2$ 1.0, NaH_2PO_4 0.27 and Na_2HPO_4 1.2, pH 7.4. To enhance detection of extracellular 5-HT a low concentration of the selective reuptake inhibitor citalopram (1.0 μM) was added to the aCSF. The aCSF was pumped at a rate of 1.0 $\mu l\ min^{-1}$. Sample collection began at the onset of the lights-off period under dim red light conditions. Samples were collected every 30 min and analysed within 30 min by high performance liquid chromatography with electrochemical detection as described previously in detail (Auerbach *et al.*, 1989). Experiments were performed during the dark phase because rats are most active at night, and previous studies suggest that 5-HT neuronal activity is greatest when animals are awake (Jacobs & Fornal, 1991; Wilkinson *et al.*, 1991).

Drugs were administered to rats after 5-HT levels in 4 successive samples were stable (less than $\pm$10% fluctuation of baseline). Drugs were dissolved in the aCSF and then infused into target sites via the dialysis probes ('reverse dialysis'). In general, agonists and antagonists were administered by reverse dialysis for a period of 60 min. In receptor blocking experiments, antagonists were infused beginning 30 min before and during the 60 min period of agonist infusion. In most experiments, the dialysis probe used to infuse drugs was also used to measure extracellular 5-HT. However, in some experiments, drugs were infused through a probe in the raphe while extracellular 5-HT was measured via a second probe in the nucleus accumbens. Infusion of citalopram (1 μM) into the DRN did not significantly affect extracellular 5-HT in the nucleus accumbens (see Figure 3b). Thus, during these dual probe experiments, because 5-HT was only measured in the forebrain, citalopram was omitted from the aCSF perfused through the raphe probe. Nevertheless, one experiment investigated the possibility that the reuptake inhibitor might interact with the effect of $GABA_A$ ligands on 5-HT. Thus, muscimol (100–

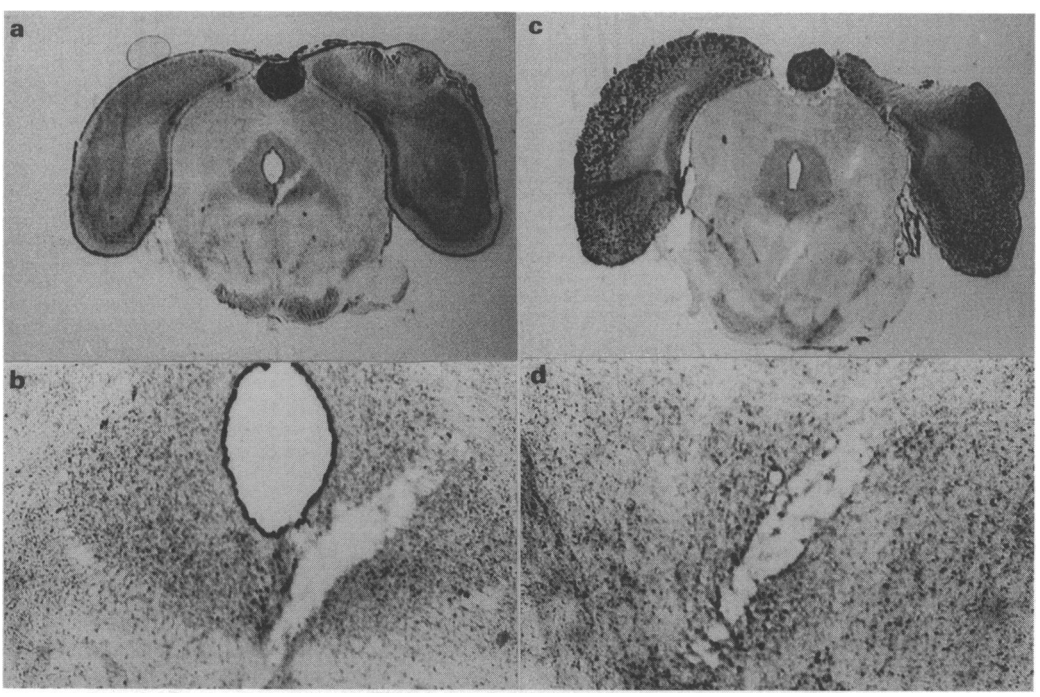

Figure 1 Examples of microdialysis probe tracks in the DRN and MRN. Coronal sections through the area of the DRN and MRN were stained with crystal violet and photographed at low and higher magnification: (a) low magnification overview of a probe track ending in the DRN, (b) higher magnification showing the periaqueductal gray with the probe track ending in the DRN, (c) low magnification overview showing a probe track ending in the MRN, (d) higher magnification showing the microdialysis site in the MRN.

300 μM) in aCSF containing citalopram (1 μM) was infused into the DRN while extracellular 5-HT was measured with a second dialysis probe in the nucleus accumbens.

Histology

At the end of an experiment, the rat was deeply anaesthetized and a 2% fast green solution was perfused through the dialysis probe for several minutes to stain the surrounding tissue. The brain was removed, frozen, and sliced free hand with a razor blade to confirm the location of the probe track. The data were omitted when the probe was located outside of the target site. For making photomicrographs, heavily anaesthetized rats were perfused intracardially with 0.9% saline followed by 4% formalin solution (Fisher Scientific, Springfield, NJ). The brains were removed, frozen and 30 μm sections prepared with a cryostat microtome (Bright Instruments, Huntington, U.K.). The slices were stained with filtered 0.5% crystal violet for 10 min, rinsed in tap water for 2 min, followed by acid alcohol for 30 s, 100% ethanol for 4 min and histoclear (Fisher Scientific, Springfield, NJ) for 3 min. Figure 1 shows examples of typical microdialysis probe tracks in the DRN and MRN (low magnification, Figure 1a and c; high magnification, Figure 1b and d).

Statistics

The data were calculated and presented in the figures as means of the % change from the average of 4 sequential baseline measurements. The significance of changes in 5-HT ($P<0.05$) was determined by repeated measures ANOVA or MANOVA followed by Scheffe's F test.

Materials

All chemicals were reagent grade or better. Muscimol (3-hydroxy-5-aminoethylisoxazole hydrobomide), (−)-bicuculline ([**R**-9**R***,**S***])-5-(6,8-dihydro-8-oxofuro[3,4-e]-1,3-benzodioxol-6yl)-5,6,7,8-tetrahydro-6,6-dimethyl-1,3-dioxolo[4,5-g]isoquindinium chloride), picrotoxin, phaclofen (3-amino-2-(4-chlorophenyl)propylphosphonic acid) and 2-hydroxysaclofen (3-amino-2-(4-chlorophenyl)-2-hydroxypropane sulphonic acid) were purchased from RBI (Natick, MA), (±)-baclofen (4-amino-3-[4-chlorophenyl]-butanoic acid) from Sigma (St. Louis, MO). Citalopram was provided courtesy of H. Lundbeck A/S (Copenhagen-Valby).

Results

Baseline samples were obtained from freely moving rats on the day after implantation of dialysis probes. To enhance detection of 5-HT, the selective reuptake blocker citalopram (1 μM) was present in the dialysis solution. Mean extracellular 5-HT in the four sequential samples just before drug administration was 4.8±0.4 pg per sample for DRN ($n=65$; not corrected for probe recovery), 4.8±0.6 pg per sample for MRN ($n=26$) and 3.5±0.3 pg per 30 μl for nucleus accumbens ($n=80$).

Effect of the $GABA_A$ receptor agonist muscimol

When muscimol was infused by reverse dialysis into the DRN for 60 min, extracellular 5-HT was significantly decreased in a dose-dependent manner. As shown in Figure 2, the highest dose of muscimol (100 μM) produced a maximum reduction of about 35% of baseline 5-HT in the DRN. In another experiment, muscimol was administered by reverse dialysis into the DRN while a second probe in the nucleus accumbens was used to measure extracellular 5-HT in this forebrain projection site of the DRN. In dual probe experiments, citalopram was present in the aCSF perfused through the probe in the nucleus accumbens, and was either absent (Figure 3a) or present

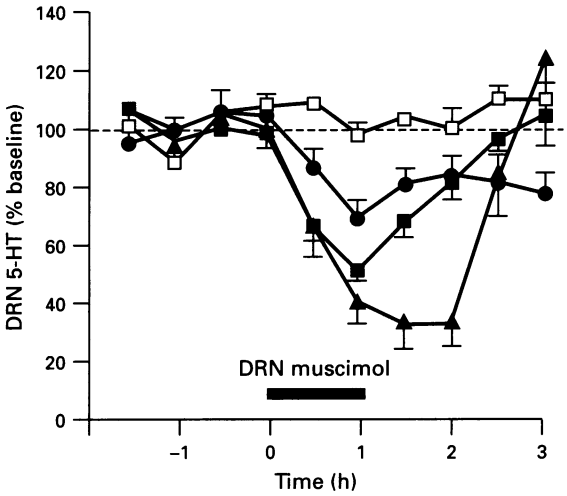

Figure 2 Muscimol infusion into the DRN reduced extracellular 5-HT in the DRN. The solid horizontal bar indicates the period of muscimol infusion by reverse microdialysis in the DRN. Results are extracellular 5-HT in the DRN expressed as mean±s.e.mean (vertical lines) % change from pre-drug baseline levels. aCSF (□, $n=5$); muscimol 10 μM (●, $n=4$); muscimol 30 μM (■, $n=6$); muscimol 100 μM (▲, $n=5$). As compared to aCSF control, DRN 5-HT was significantly decreased at times between 0–2 h after the start of drug infusion: 10 μM ($F(1,7)=48.8$, $P=0.0002$; 30 μM, $F(1,9)=181.2$, $P=0.0001$; 100 μM, $F(1,8)=70.0$, $P=0.0001$.

(Figure 3b) in the probe used to perfuse muscimol into the DRN. As shown in Figure 3a, the maximum decrease in the absence of citalopram in the DRN was to about 40% of basal 5-HT in the nucleus accumbens. Infusion of a low concentration of citalopram (1 μM) alone had no significant effect on nucleus accumbens 5-HT. Furthermore, perfusion of the DRN with aCSF containing citalopram and muscimol produced a maximal decrease in nucleus accumbens 5-HT that was the same as the effect of muscimol alone. However, as compared to changes in DRN 5-HT, a higher dose of muscimol (300 μM) infusion into the DRN was needed to produce the maximum effect on nucleus accumbens 5-HT. To determine if the effect on 5-HT was regionally selective, muscimol (100–300 μM) was infused into the MRN while 5-HT was measured in the nucleus accumbens. As shown in Figure 4, extracellular 5-HT in the nucleus accumbens was not significantly affected by muscimol infusion into the MRN.

Infusion of muscimol into the MRN produced decreases in MRN 5-HT. As compared to DRN, higher doses were required to produce significant effects. Muscimol (300 μM) elicited a reduction to about 50% of baseline extracellular 5-HT (Figure 5a). In contrast to DRN and MRN infusion, muscimol administration by reverse dialysis in the nucleus accumbens, had no significant effect on 5-HT in the nucleus accumbens (Figure 5b).

Effect of $GABA_A$ receptor antagonists

Infusion of the $GABA_A$ receptor antagonist bicuculline into the DRN produced a dose-dependent increase in 5-HT in DRN (Figure 6a) and in the nucleus accumbens (Figure 6b). During DRN perfusion with bicuculline (100 μM) the maximal increase in nucleus accumbens and DRN 5-HT was about 100% and 200%, respectively. In contrast, infusion of bicuculline into the MRN had no significant effect on 5-HT in the MRN (Figure 7). Similarly, infusion of bicuculline (100 μM) into nucleus accumbens had no significant effect on 5-HT in the nucleus accumbens ($n=6$; data not shown).

Reverse dialysis treatment with another GABA receptor antagonist picrotoxin (100 μM) had similar effects (Figure 8) to bicuculline. Perfusion of picrotoxin into the DRN produced an

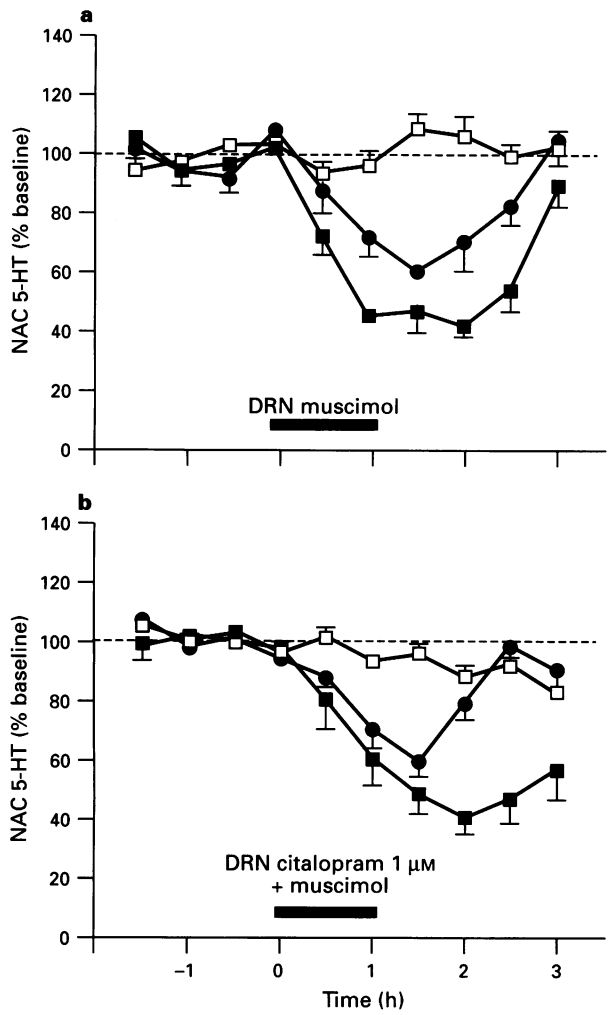

Figure 3 Muscimol infusion into the DRN reduced extracellular 5-HT in the nucleus accumbens. The solid horizontal bar indicates the period of muscimol infusion by reverse microdialysis in the DRN. Results are extracellular 5-HT in nucleus accumbens (NAC) expressed as mean ± s.e.mean (vertical lines) % change from pre-drug baseline levels. (a) aCSF (□, $n=6$); muscimol 100 μM (●, $n=6$); muscimol 300 μM (■, $n=6$). As compared to aCSF control, 5-HT in nucleus accumbens was significantly decreased at times between 0–2 h after the start of drug infusion into the DRN: 100 μM, $F(1,10)=21.3$, $P=0.001$; 300 μM, $F(1,10)=92.4$, $P=0.0001$. (b) aCSF + 1 μM citalopram (□, $n=5$); 1 μM citalopram + muscimol 100 μM (●, $n=6$); 1 μM citalopram + muscimol 300 μM (■, $n=7$). As compared to control, 5-HT in nucleus accumbens was significantly decreased at times between 0–2 h after the start of drug infusion into the DRN: 100 μM, $F(1,9)=15.8$, $P=0.0032$; 300 μM, $F(1,10)=17.8$, $P=0.0018$.

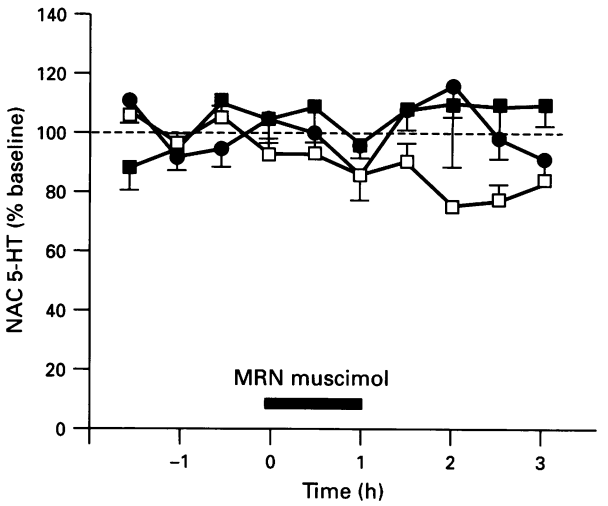

Figure 4 Effect of muscimol infusion into the MRN on extracellular 5-HT in the nucleus accumbens. The solid horizontal bar indicates the period of muscimol infusion by reverse microdialysis in the MRN. Results are extracellular 5-HT in nucleus accumbens (NAC) expressed as mean ± s.e.mean (vertical lines) % change from pre-drug baseline levels. aCSF (□, $n=6$); muscimol 100 μM (●, $n=6$); muscimol 300 μM (■, $n=5$). As compared to aCSF control, 5-HT in nucleus accumbens was not significantly changed after the start of drug infusion into the MRN: 100 μM, $F(1,10)=1.2$, $P=0.2914$; 300 μM, $F(1,9)=4.5$, $P=0.0637$.

increase in 5-HT in DRN and nucleus accumbens to about 150% and 60% above baseline, respectively. Local application of picrotoxin into the MRN or nucleus accumbens had no significant effect on 5-HT in these sites.

Effect of $GABA_B$ receptor ligands

Reverse dialysis administration of baclofen into the DRN induced a small, but significant reduction of extracellular 5-HT. Baclofen (300 μM) produced a decrease to about 80% of baseline 5-HT in the DRN (Figure 9a). Previous studies suggest the possibility that baclofen might have both a direct inhibitory effect on 5-HT neuronal activity (Innis & Aghajanian, 1987) and an indirect effect via stimulation of $GABA_B$ autoreceptors on GABA interneurones (Waldmeier *et al.*, 1988). By stimulating autoreceptors, baclofen might cause reductions in GABA release in the DRN, and thus have an indirect excitatory effect on 5-HT neuronal activity. In order to eliminate the possible indirect excitatory influence of decreased release of endogenous GABA, the $GABA_A$ receptor blocker bicuculline was infused before baclofen. During infusion of bicuculline (30 μM), baseline 5-HT levels in DRN were 10.0 ± 1.9 pg per sample ($n=11$). This was about twice baseline levels in the absence of $GABA_A$ receptor blockade. During bicuculline infusion, the reduction in 5-HT produced by baclofen (100 μM and 300 μM) was greatly augmented. As shown in Figure 9b, 5-HT was reduced to about 50% of baseline. Furthermore, when the concentration of bicuculline was increased to 100 μM, baseline 5-HT levels were 22.9 ± 5.6 pg per sample and baclofen (300 μM) produced a decrease in 5-HT to about 30% of baseline (Figure 9b).

Reverse dialysis infusion of baclofen into the DRN produced a reduction of 5-HT in the nucleus accumbens to about 65% of baseline (Figure 10a). Infusion of baclofen into the nucleus accumbens had a larger effect on 5-HT in the nucleus accumbens with extracellular levels decreased to about 30% of baseline (Figure 10b). Pretreatment with the $GABA_B$ receptor antagonist phaclofen (100 μM) blocked the decrease in nucleus accumbens 5-HT produced by infusing baclofen into the nucleus accumbens (Figure 10b).

By itself, the $GABA_B$ receptor antagonist phaclofen (100 μM) had no significant effect on 5-HT when infused into the DRN or nucleus accumbens (Figure 11a). Similarly, as shown in Figure 11b, another $GABA_B$ receptor antagonist saclofen (100 μM) had no significant effect on 5-HT in the DRN or MRN.

Discussion

The activity of midbrain 5-HT neurones is inhibited by GABA receptor stimulation (Gallager & Aghajanian, 1976). However, the relative importance of different GABA receptor subtypes, and whether these receptors are tonically activated under physiological conditions has not been fully established. We have used microdialysis in freely moving rats to investigate these issues. GABA receptor agonists and antagonists were infused by reverse microdialysis while extracellular 5-HT was simultaneously measured in the DRN, MRN and a forebrain

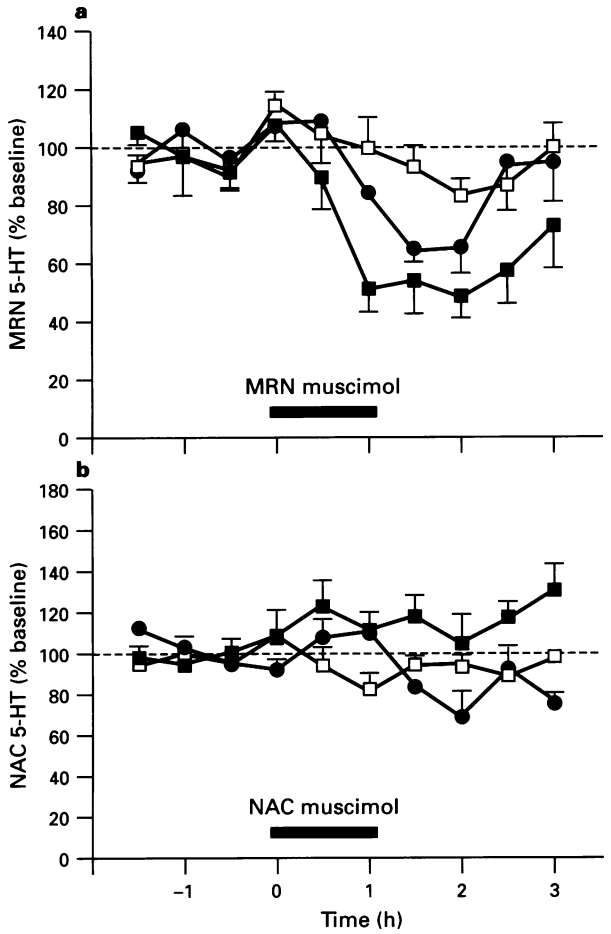

Figure 5 Muscimol infusion into the MRN reduced extracellular 5-HT in the MRN. Muscimol infusion into the nucleus accumbens had no effect on nucleus accumbens 5-HT. The solid horizontal bar indicates the period of muscimol infusion by reverse microdialysis in the MRN and nucleus accumbens (NAC). Results are extracellular 5-HT in (a) MRN and (b) nucleus accumbens expressed as mean ± s.e.mean (vertical lines) % change from pre-drug baseline levels. (a) aCSF (□, $n=6$); muscimol 100 μM (●, $n=4$); muscimol 300 μM (■, $n=5$). As compared to aCSF control, there was a dose-related decrease in MRN 5-HT at times between 0–2 h after the start of drug infusion: 100 μM, $F(1,8)=2.39$, $P=0.161$; 300 μM, $F(1,9)=12.8$, $P=0.006$. (b) aCSF (□, $n=6$); muscimol 300 μM (●, $n=4$); muscimol 1000 μM (■, $n=5$). As compared to aCSF control, there were no significant changes in nucleus accumbens 5-HT during infusion of muscimol into the nucleus accumbens.

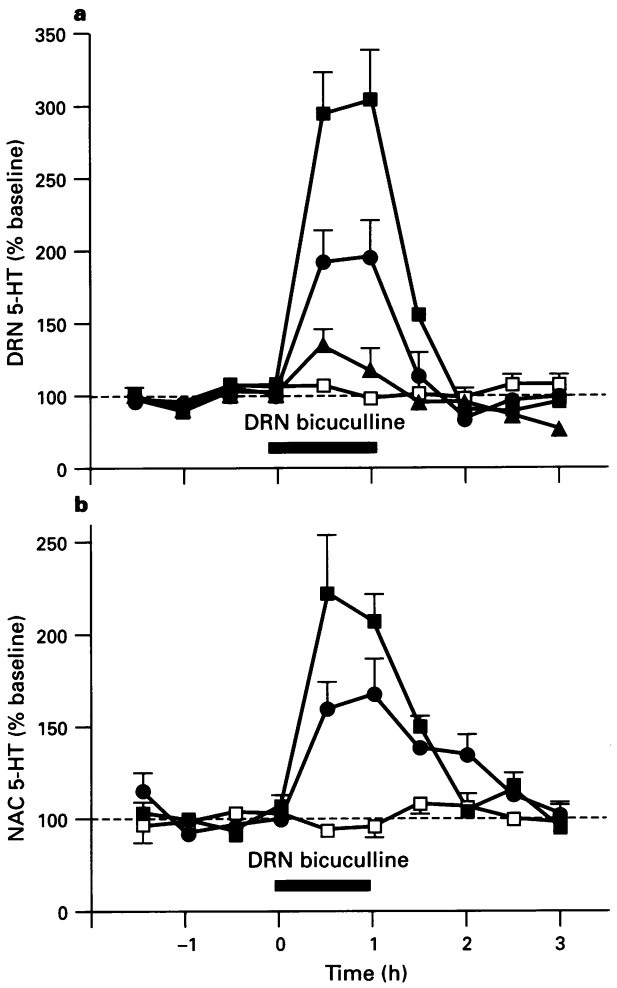

Figure 6 Bicuculline infusion into the DRN increased extracellular 5-HT in the (a) DRN and (b) nucleus accumbens (NAC). The solid horizontal bar indicates the period of bicuculline infusion by reverse microdialysis in the DRN. Results are extracellular 5-HT in (a) DRN and (b) nucleus accumbens expressed as mean ± s.e.mean (vertical lines) % change from pre-drug baseline levels. (a) aCSF (□, $n=5$); bicuculline 10 μM (▲, $n=5$); bicuculline 30 μM (●, $n=5$); bicuculline 100 μM (■, $n=5$). As compared to aCSF control, there was a dose-dependent increase in DRN 5-HT at times between 0–1 h after the start of drug infusion: 10 μM, $F(1,8)=4.8$, $P=0.0646$; 30 μM, $F(1,8)=19.3$, $P=0.0023$; 100 μM, $F(1,8)=41.9$, $P=0.0002$. (b) aCSF (□, $n=6$); bicuculline 30 μM (●, $n=6$); bicuculline 100 μM (■, $n=4$). As compared to aCSF control, nucleus accumbens 5-HT was significantly increased at times between 0–1 h after the start of drug infusion into the DRN: 30 μM, $F(1,10)=16.6$, $P=0.0022$; 100 μM, $F(1,8)=40.5$, $P=0.0002$.

projection site, nucleus accumbens. Our data substantiate the view that $GABA_A$ and $GABA_B$ receptor subtypes play a differential role in regulation of 5-HT release within the midbrain raphe nuclei and forebrain. Infusion of $GABA_A$ receptor agonists into the DRN and MRN produced larger decreases in extracellular 5-HT than $GABA_B$ agonists. Conversely, infusion of $GABA_B$ receptor agonists into a forebrain site potently decreased extracellular 5-HT while $GABA_A$ agonists had no effect. The results also suggest that $GABA_A$ receptors in the DRN have an important tonic inhibitory influence on 5-HT neuronal activity under our experimental conditions.

Methodological issues

Detection of extracellular 5-HT was facilitated by infusion of the reuptake blocker citalopram. Reuptake blockade enhances levels of extracellular 5-HT around the dialysis probe, and this presumably resulted in somatodendritic autoreceptor stimulation. This inference is based on evidence that the spontaneous activity of DRN 5-HT neurones (Chaput *et al.*, 1986) and 5-HT release in the forebrain (Auerbach *et al.*, 1995) were dose-dependently inhibited after systemic administration of citalopram. However, local infusion of a reuptake blocker apparently results in only a partial inhibition of 5-HT release as the autoreceptor agonist 8-OH-DPAT decreased DRN 5-HT even with citalopram in the DRN perfusate (Tao & Auerbach, 1996). In further support of this conclusion, infusion of the $GABA_A$ receptor agonist muscimol produced a decrease in extracellular 5-HT in the DRN and MRN. Conversely, $GABA_A$ receptor antagonists produced large increases in extracellular 5-HT in the DRN. Together these data suggest that extracellular 5-HT in the raphe was neuronal in origin and dependent on depolarization-induced release. The conclusion that 5-HT release in the raphe is dependent on neuronal discharge during local perfusion with a reuptake inhibitor is in agreement with data from other studies (Bosker *et al.*, 1994; Rutter *et al.*, 1995).

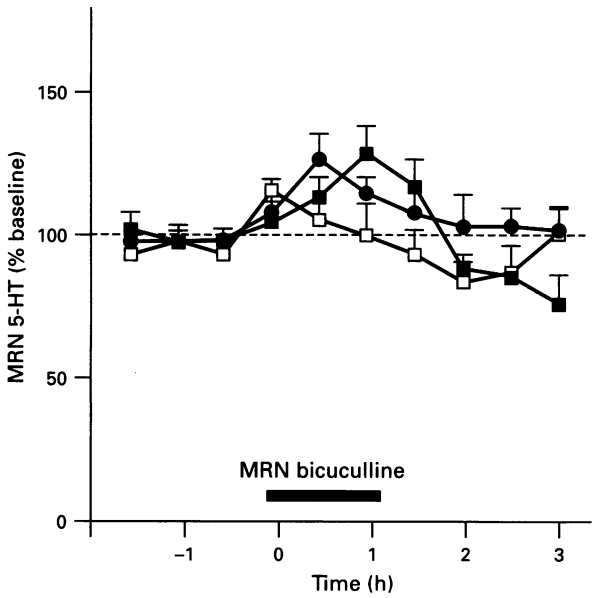

Figure 7 Effect of bicuculline on 5-HT release in the MRN. Results are means ± s.e.mean (vertical lines) expressed as a percentage of pre-drug baseline 5-HT levels. The solid horizontal bar indicates the period of bicuculline infusion into the MRN. aCSF (□, $n=6$); bicuculline 30 μM (●, $n=8$); bicuculline 100 μM (■, $n=9$). Bicuculline infusion into the MRN between 0–1 h had no significant effect on MRN 5-HT as compared to the group treated with aCSF: 30 μM, $F(1, 12)=3.28$, $P=0.095$; 100 μM, $F(1, 13)=2.44$, $P=0.1425$.

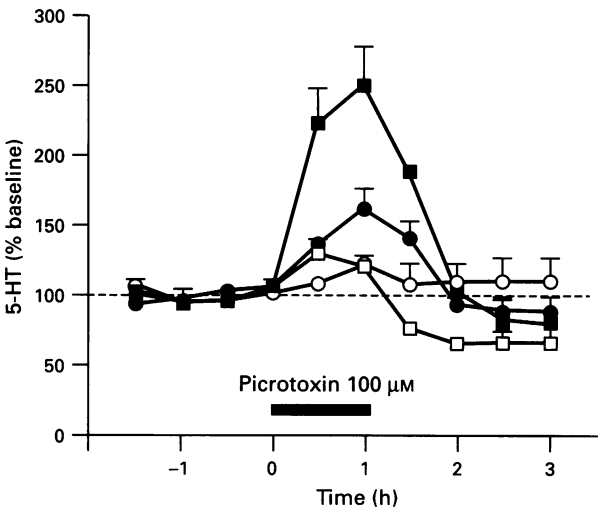

Figure 8 Effect of picrotoxin infusion on 5-HT in the midbrain raphe and forebrain. The solid horizontal bar indicates the period of picrotoxin infusion by reverse microdialysis. Results are extracellular 5-HT expressed as mean ± s.e.mean (vertical lines) % change from pre-drug baseline levels. As compared to aCSF controls (data shown in Figure 2 for DRN aCSF control and in Figure 3a for nucleus accumbens aCSF control), 5-HT was significantly increased in the DRN (■, $n=6$; $F(1,9)=21.3$, $P=0.0013$) and nucleus accumbens (●, $n=4$; $F(1,8)=12.3$, $P=0.0056$) during picrotoxin infusion into the DRN. Infusion of picrotoxin into the MRN or nucleus accumbens had no significant effect on 5-HT in the MRN (□, $n=5$; $F(1,9)=3.94$, $P=0.0753$) or nucleus accumbens (○, $n=6$; $F(1,10)=4.42$, $P=0.0619$), as compared to aCSF controls (control data shown in Figure 5a for MRN and in Figure 5b for nucleus accumbens).

We used a dual probe protocol to validate the significance of changes in raphe extracellular 5-HT. Measurements of 5-HT in the nucleus accumbens were made during infusion of citalopram alone or GABA receptor ligands into the DRN. Infusion of citalopram alone into the DRN produced no significant decrease in 5-HT in the nucleus accumbens. However, similar to the changes in the raphe, 5-HT in the nucleus accumbens was decreased during infusion of a GABA receptor agonist into the DRN, and increased by GABA receptor antagonist treatment. The magnitude of these changes was not affected by the absence or presence of 1 μM citalopram in the DRN perfusate. This suggests that muscimol at high doses in the DRN produced a widespread inhibition of 5-HT neuronal activity and thus, decreased release in forebrain projection sites. In contrast, at the low dose used, citalopram presumably blocked 5-HT reuptake around the dialysis probe without fully activating 5-HT somatodendritic autoreceptors in widespread areas of the DRN. Thus, microdialysis in the raphe can be used to examine the localized effects of receptor ligands in order to test hypotheses concerning the role of GABA in regulation of 5-HT neuronal activity. In comparison to electrophysiological techniques, microdialysis has poor time resolution but is more readily applied to localized drug application and measurements in awake, unrestrained animals. Although the concentration range of GABA receptor ligands used in these infusion studies appears high, it is important to realize that there is a steep drop in concentration across the dialysis probe membrane. Also the probe is relatively small and is perfused at a slow rate. Thus, actual drug concentrations at the receptor site will be substantially lower than in the perfusion medium (Dykstra *et al.*, 1992).

Effect of $GABA_A$ receptor stimulation

In contrast to the DRN and MRN, muscimol infusion into the nucleus accumbens had no effect on 5-HT. This agrees with *in vitro* evidence that presynaptic inhibition of 5-HT release in forebrain is not mediated by $GABA_A$ receptors (Schlicker *et al.*, 1984). The decrease in nucleus accumbens 5-HT during infusion of muscimol into the DRN can be explained by activation of $GABA_A$ receptors on 5-HT cell bodies. However, in comparison with the effect on DRN 5-HT, it was necessary to infuse a higher concentration of muscimol into the DRN to produce an equivalent effect on nucleus accumbens 5-HT. Presumably, localized infusion into the raphe did not activate all raphe 5-HT neurones with projections to the nucleus accumbens. At higher muscimol concentrations, diffusion from the site of the dialysis probe probably activated a larger proportion of DRN 5-HT neurones with projections to the nucleus accumbens. In contrast, muscimol infusion into the MRN had no effect on nucleus accumbens 5-HT. These data suggest that the spread of the drug from the site of infusion was limited, and are consistent with anatomical evidence that the DRN is the predominant source of 5-HT projections to the basal ganglia (Tork, 1990).

Previous studies of 5-HT turnover have provided evidence that $GABA_A$ receptor stimulation in the MRN inhibits 5-HT neuronal activity (Forchetti & Meek, 1981; Nishikawa & Scatton, 1985b; Wirtshafter *et al.*, 1987), and GABA-mediated inhibition of MRN 5-HT neurones has been associated with the appearance of hippocampal theta rhythm (Kinney *et al.*, 1995). Nevertheless, in comparison to the DRN, our results indicate that MRN 5-HT is less sensitive to muscimol. This is of interest in the context of other differences between DRN and MRN 5-HT neurones (Hjorth, 1992; Tork, 1990). In particular, MRN 5-HT neurones were less sensitive to morphine (Tao & Auerbach, 1995), the effects of some amphetamine derivatives (Blier *et al.*, 1990; Mamounas & Molliver, 1988) and N-methyl-D-aspartate (NMDA) (Tao & Auerbach, 1996). Thus, it appears that, in comparison to the DRN, drugs have less influence on MRN 5-HT neuronal activity. It is possible that this could be due to the lower density of 5-HT neurones in the MRN as compared to the DRN. During infusion of drugs into the MRN a smaller proportion of cell bodies may be affected. However, this cannot readily account

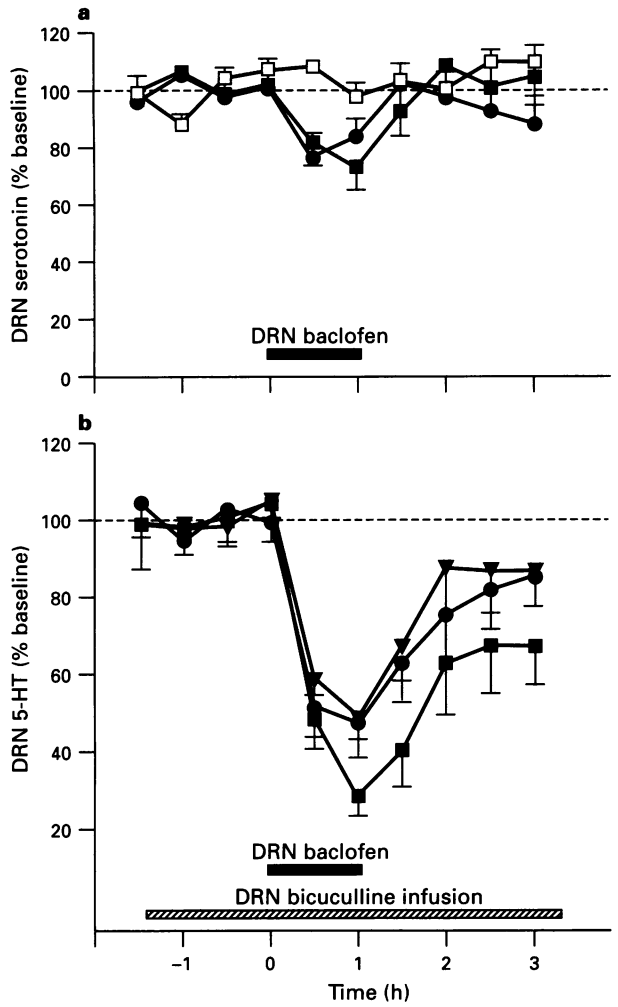

Figure 9 Baclofen infusion into the DRN reduced extracellular 5-HT in the DRN. The solid horizontal bar indicates the period of baclofen infusion by reverse microdialysis in the DRN. Results are extracellular 5-HT in DRN expressed as mean ± s.e.mean (vertical lines) % change from pre-drug baseline levels. (a) aCSF (□, $n=5$); baclofen 100 μM (●, $n=6$); baclofen 300 μM (■, $n=7$). As compared to aCSF control, 5-HT was significantly decreased at times between 0–1 h: 100 μM, $F(1,9)=48.8$, $P=0.023$; 300 μM, $F(1,10)=10.1$, $P=0.0099$. (b) Bicuculline treatment during the period indicated by the hatched bar enhanced the effect of baclofen on DRN 5-HT. Baclofen-induced decreases in extracellular 5-HT between 0–1.5 h were significantly potentiated by bicuculline treatment: baclofen 100 μM (●, $n=6$; from (a)) vs. bicuculline 30 μM + baclofen 100 μM (▼, $n=6$), $F(1, 10)=13.4$, $P=0.0043$; baclofen 300 μM (■, $n=7$; from (a)) vs. bicuculline 30 μM + baclofen 300 μM (●, $n=4$), $F(1, 9)=7.18$, $P=0.0209$; baclofen 300 μM (■, $n=7$; from (a)) vs. bicuculline 100 μM + baclofen 300 μM (■, $n=5$), $F(1,10)=21.7$, $P=0.0009$. Baseline 5-HT levels during infusion of bicuculline at doses of 30 μM and 100 μM were 10.0 ± 1.9 ($n=11$) and 22.9 ± 5.6 ($n=5$) pg per sample, respectively. This is about two and four fold greater than normal baseline 5-HT levels in the DRN.

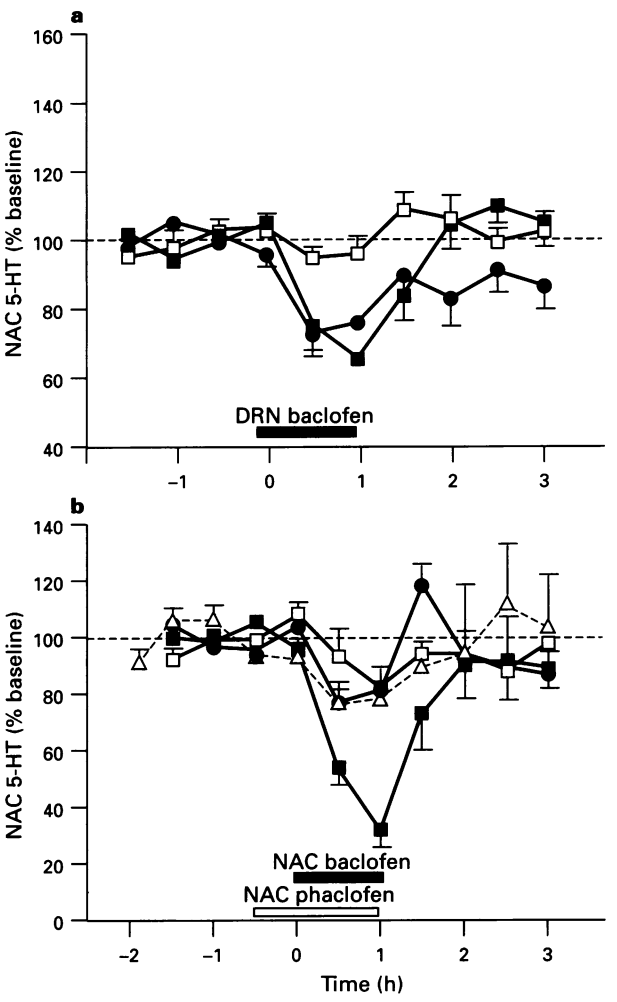

Figure 10 Baclofen infusion into the DRN or nucleus accumbens reduces extracellular 5-HT in the nucleus accumbens. The solid horizontal bar indicates the period of baclofen infusion by reverse microdialysis in (a) DRN and (b) nucleus accumbens (NAC). The open horizontal bar in (b) indicates the period of phaclofen infusion into the nucleus accumbens beginning 30 min before baclofen. Results are extracellular 5-HT in the nucleus accumbens expressed as mean ± s.e.mean (vertical lines) % change from pre-drug baseline levels. (a) aCSF (□, $n=6$); baclofen 100 μM (●, $n=5$); baclofen 300 μM (■, $n=6$). As compared to aCSF control, 5-HT in the nucleus accumbens was significantly decreased at times between 0–1.5 h after the start of drug infusion into the DRN: 100 μM, $F(1,9)=9.59$, $P=0.0128$; 300 μM, $F(1,10)=23.7$, $P=0.0007$. (b) aCSF (□, $n=6$); baclofen 30 μM (●, $n=5$); baclofen 100 μM (■, $n=5$). As compared to aCSF control, there was a dose-related decrease in 5-HT in the nucleus accumbens at times between 0–1 h after the start of drug infusion into the nucleus accumbens: 30 μM, $F(1,9)=1.13$, $P=0.31$; 100 μM, $F(1,9)=24.2$, $P=0.0008$. In addition, 100 μM phaclofen blocked the effect of 100 μM baclofen on inhibition of 5-HT release (△, $n=6$; $F(1,9)=18.9$, $P=0.0019$).

for the greater responsiveness of DRN 5-HT neurones to systemic administration of drugs such as MDMA (Mamounas & Molliver, 1988) and morphine (Tao & Auerbach, 1995). Another possible explanation for the difference in sensitivity could be greater microdialysis-induced damage to MRN 5-HT neurones. However, the MRN and DRN probes have the same dimensions and histological examination showed no apparent difference in the extent of the lesions. Furthermore, basal dialysate levels of 5-HT in the DRN and MRN were similar. This suggests that the lack of response to muscimol in MRN may be due to differences in either intrinsic properties of 5-HT neurones or local circuitry.

Effect of $GABA_B$ receptor stimulation

Consistent with the results of an *in vitro* study (Schlicker *et al.*, 1984), 5-HT in the nucleus accumbens was decreased during local infusion of baclofen. The ability of phaclofen to block this effect supports the conclusion that $GABA_B$ receptors on nerve terminals are involved in presynaptic modulation of 5-HT release. Infusion of baclofen into the DRN also produced decreased 5-HT in the nucleus accumbens as well as the DRN. Compared to infusion into the nucleus accumbens, baclofen alone in the DRN had a smaller, less potent effect on 5-HT. Furthermore, baclofen in the DRN had a smaller effect than

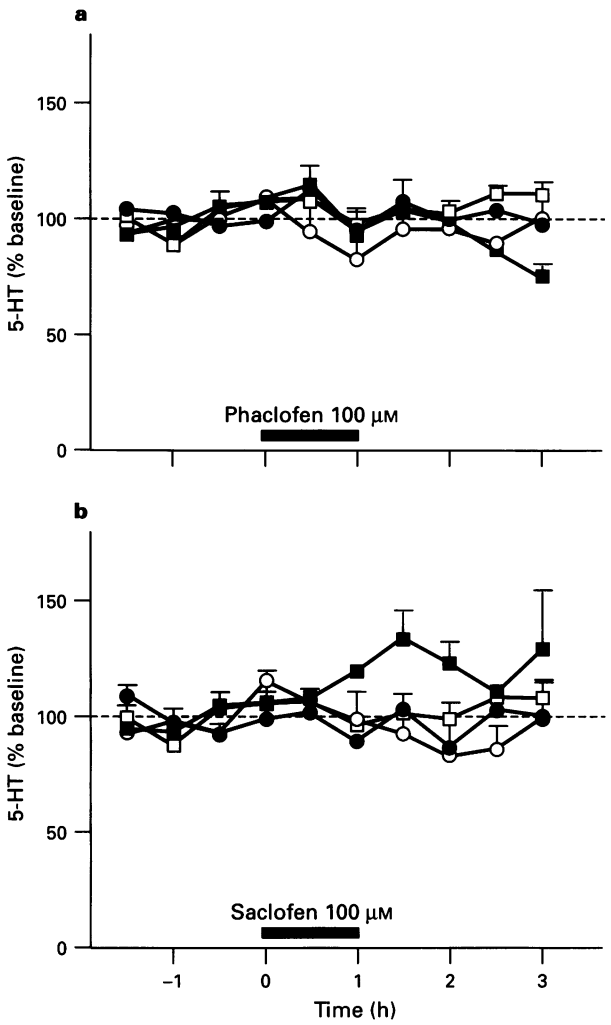

Figure 11 Effect of $GABA_B$ receptor antagonists on extracellular 5-HT in DRN, MRN, or nucleus accumbens. The solid horizontal bar indicates the period of (a) 100 μM phaclofen or (b) 100 μM saclofen infusion by reverse microdialysis. Results are extracellular 5-HT expressed as mean ± s.e.mean (vertical lines) % change from pre-drug baseline levels. (a) As compared to aCSF control (□, $n=5$), infusion of phaclofen into the DRN (■, $n=6$) had no significant effect on DRN 5-HT ($F(1,9)=0.01$, $P=0.9201$). Similarly, phaclofen infusion into the nucleus accumbens (●, $n=6$) had no significant effect on nucleus accumbens 5-HT ($F(1,10)=3.706$, $P=0.0831$), when compared to aCSF control (○, $n=6$). (b) As compared to aCSF control (□, $n=5$), infusion of saclofen into the DRN (■, $n=5$) had no significant effect on DRN 5-HT ($F(1,8)=5.24$, $P=0.0513$). Similarly, saclofen infusion into the MRN (●, $n=5$) had no significant effect on MRN 5-HT ($F(1,9)=0.41$, $P=0.5403$), when compared to aCSF control (○, $n=6$).

the $GABA_A$ agonist muscimol. These results are in agreement with electrophysiological evidence that 5-HT neuronal activity is inhibited by baclofen in the raphe (Innis & Aghajanian, 1987; Smith & Gallager, 1987), but that the influence of $GABA_B$ receptor stimulation in some brain structures is relatively weak (Newberry & Nicoll, 1985).

One possible explanation for the low efficacy of baclofen infusion is based on a model of local circuitry in the raphe. According to this model, baclofen could activate $GABA_B$ autoreceptors on GABAergic interneurones to inhibit the release of GABA (Waldmeier *et al.*, 1988). Reduced stimulation of $GABA_A$ receptors due to decreased release of endogenous GABA in the DRN would thus have an excitatory influence opposing the direct baclofen-induced inhibition of 5-HT neurones. As predicted by this model, pretreatment with bicuculline to block $GABA_A$ receptors on 5-HT cells greatly augmented levels of DRN 5-HT, and subsequent infusion of baclofen produced a much larger decrease in 5-HT. Presumably, with bicuculline present, the excitatory influence of decreased endogenous GABA release was prevented and the full direct inhibitory effect of baclofen was observed. A more speculative explanation for this result is the possibility of a direct negative interaction between $GABA_A$ and $GABA_B$ receptors within the membrane of 5-HT neurones. However, we are not aware of any other experimental evidence that would support this hypothesis. Other unresolved issues concerning local circuitry include the extent to which inhibitory synapses on raphe 5-HT neurones involve GABAergic interneurones (Belin *et al.*, 1979; Wang *et al.*, 1992) or afferents from other sites such as pontine reticular formation and lateral habenula (Wang *et al.*, 1976; Wang & Aghajanian, 1977; Nishikawa & Scatton, 1985a; Kalen *et al.*, 1989).

Tonic inhibitory control of 5-HT neuronal activity

Whether GABA receptors have a tonic physiological role in regulating 5-HT neuronal activity has not been clearly established. Hippocampal 5-hydroxyindole acetic acid (5-HIAA) was increased after microinjection of bicuculline or picrotoxin into the MRN (Forchetti & Meek, 1981). Similarly, striatal 5-HT was increased during microinfusion of bicuculline into the DRN (Kalen *et al.*, 1989), but others found no change in forebrain 5-HT metabolism or release in response to $GABA_A$ receptor blockade in the DRN (Reisine *et al.*, 1982; Nishikawa & Scatton, 1983). The present results suggest that $GABA_A$ receptors on 5-HT neurones in the DRN were tonically activated. Thus, bicuculline and picrotoxin infusion enhanced extracellular 5-HT levels during infusion into the DRN, but local blockade of $GABA_A$ receptors in MRN and nucleus accumbens had no effect. One possible explanation for these diverse findings is evidence that tonic activation of GABA receptors varies with changes in the behavioural state. Thus, as determined by single unit recordings in freely behaving cats, tonic $GABA_A$ receptor-mediated inhibition of 5-HT neurones was apparent only during slow wave sleep (Levine & Jacobs, 1992). In the present study, dialysis measurements were carried out during the dark when rats are most active. However, animals were housed singly in the recording chamber with minimal environmental disturbances and appeared to be inactive much of the time. Thus, it is conceivable that rats were frequently sleeping, during which time, as suggested by the cat data, GABAergic inputs would be most active in regulating 5-HT neuronal discharge. Because microdialysis samples were collected over a 30 min period, with our method it was not possible to assess more phasic changes in GABAergic tone.

The present results provide no evidence of tonic activation of $GABA_B$ receptors on 5-HT cell bodies or terminals. Thus, at doses that blocked the inhibitory effect of baclofen, phaclofen failed to increase 5-HT in the DRN, MRN or nucleus accumbens. Some evidence suggests that stronger activation of GABAergic afferents is necessary to activate $GABA_B$ in comparison with $GABA_A$ receptor-activated currents (Dutar & Nicoll, 1988). Thus, it is possible that *in vivo* activation of $GABA_B$ receptors occurs only when synaptic levels of GABA are unusually high.

In conclusion, the present microdialysis studies of awake rats demonstrate the utility of local drug infusion during measurement of extracellular 5-HT for determining the role of GABA afferents in regulation of 5-HT release. $GABA_A$ receptors appear to inhibit tonically 5-HT release from DRN 5-HT neurones under our experimental conditions. In contrast, although $GABA_A$ receptor agonists in MRN inhibited 5-HT release, these receptors apparently were not tonically activated. Similarly, pharmacological stimulation of $GABA_B$ receptors in the raphe and nucleus accumbens inhibited 5-HT release but these receptors were not tonically activated. Additional microdialysis studies examining the effect of locally infused GABA antagonists during defined behavioural states may yield more information concerning the physiological regula-

tion of 5-HT neurones by endogenous GABA. The results are important in delineating the role of different GABA receptor subtypes and circuitry in the raphe and forebrain in controlling 5-HT release, and thus ultimately the role of GABA-5-HT interactions in physiology and behaviour.

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