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Electrical stimulation of the dorsal and median raphe nuclei increases extracellular noradrenaline in rat hippocampus: Evidence for a 5-HT-independent mechanism

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Abstract

Recent studies have used raphe stimulation combined with in vivo measurements of extracellular dopamine to investigate interactions between the 5-hydroxytryptamine (5-HT) and dopamine systems. Here we have tested whether the same approach can be used to investigate interactions between the 5-HT and noradrenaline systems. Electrical stimulation of the dorsal raphe nucleus (DRN) or median raphe nucleus (MRN) was performed in anaesthetised rats implanted with microdialysis probes in the hippocampus and locus coeruleus (LC). DRN stimulation (3, 5 and 10 Hz) evoked a frequency-dependent increase in extracellular noradrenaline in the hippocampus. MRN stimulation had a similar effect. Both DRN and MRN stimulations enhanced extracellular 5-HT levels in the LC and previous studies have demonstrated that extracellular 5-HT also increases in the hippocampus. However, the increase in hippocampal noradrenaline evoked by DRN stimulation was not altered by 5-HT neuronal lesions, which reduced 5-HT metabolite levels by 90%. In conclusion, electrical stimulation of the midbrain raphe increases extracellular noradrenaline in the hippocampus, however, experiments in 5-HT-lesioned animals suggest that this response is not mediated by 5-HT. Although raphe stimulation may be useful to investigate interactions between 5-HT and dopamine, our data indicate that the same approach may not be feasible for 5-HT and noradrenaline.

Keywords: Dorsal raphe nucleus; Median raphe nucleus; Electrical stimulation; 5-HT; Locus coeruleus; Noradrenaline

1. Introduction

It is established that the brain 5-hydroxytryptamine (5-HT) system interacts with noradrenergic pathways at the neuroanatomical level. Moreover, there is clear evidence that these anatomical interactions are functional. For example, in vivo neurochemical studies indicate that 5-HT receptor selective drugs have marked effects on the noradrenergic system. Thus, a series of recent microdialysis studies have demonstrated that agonists selective for the 5-HT_{1A} receptor increase extracellular noradrenaline in various regions of the rat forebrain (Done and Sharp, 1994; Chen and Reith, 1995; Suzuki et al., 1995; Hajós-Korcsok and Sharp, 1996; Hajós-Korcsok et al., 1999), and there is evidence to suggest that this excitatory effect is mediated through postsynaptic 5-HT_{1A} receptors (Chen and Reith, 1995; Suzuki et al., 1995; Hajós-Korcsok et al., 1999). In addition, 5-HT_2 receptor antagonists also increase extracellular noradrenaline in microdialysis experiments (Done and Sharp, 1994; Gobert et al., 2000). The latter data are in keeping with the idea that within some systems 5-HT may be inhibitory on noradrenergic activity (Kostowski et al., 1974; Crespi et al., 1980; McRae-Degueurce et al., 1982, 1985; Reader et al., 1986).

An interesting approach to determine how endogenous 5-HT alters other neurotransmitter systems involves studies of the effects of 5-HT pathway stimulation. In recent microdialysis experiments raphe stimulation has been applied to great effect to investigate interactions between 5-HT and dopamine. Thus, raphe stimulation has been demonstrated to evoke an increase in extracellular dopamine in the nucleus accumbens and pharmacological experiments, including the use of 5-HT neurotoxic lesions, established

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the role of 5-HT in this effect (De Deurwaerdere and Spampinato, 1999; De Deurwaerdere et al., 1998).

Earlier biochemical studies reported that electrical stimulation of the dorsal raphe nucleus (DRN) enhanced the noradrenaline content of the locus coeruleus (LC) and also increased tissue levels of a noradrenaline metabolite (DOPEG) in terminal areas (McRae-Degueurce et al., 1985). Furthermore, an increase was found in the levels of the main 5-HT metabolite, 5-HIAA, in LC tissue samples after DRN stimulation, an effect abolished by 5-HT neurotoxin pretreatment (McRae-Degueurce et al., 1985). These studies are limited by the fact that it is now widely recognised that measurements of tissue levels of the monoamine transmitters and their metabolites offer an indirect and sometimes inaccurate measure of monoamine release (e.g., Abercrombie and Zigmond, 1989; Castaneda et al., 1990; Adell et al., 1991; Kirby et al., 1995).

In previous microdialysis studies we have established raphe stimulation protocols that evoke robust and region-specific increases of extracellular 5-HT in terminal areas (McQuade and Sharp, 1995, 1997). Here we have investigated whether the combined raphe stimulation/microdialysis approach can be used to investigate interactions between endogenous 5-HT and the noradrenaline system. Extracellular noradrenaline was measured in the ventral hippocampus, an area that receives a 5-HT innervation from both the DRN and median raphe nucleus (MRN) (Azmitia and Segal, 1978; Vertes and Martin, 1988; Vertes, 1991), as well as a strong noradrenergic innervation from the LC (Lindvall and Björklund, 1974, Moudy et al., 1993). Moreover, there is evidence that both the DRN and MRN send 5-HT projections to the LC although some evidence suggests that this may be limited to the MRN (Morgane and Jacobs, 1979; Aston-Jones et al., 1986; Vertes and Kocsis, 1994; Luppi et al., 1995).

2. Methods

2.1. Animals

Male Sprague–Dawley rats (270–290 g, Harlan-Olac, Bicester, UK) were housed in groups of up to six under conditions of controlled temperature (21 ± 1 °C) and lighting (lights on 08.00–20.00 h); food pellets and water were freely available. The experimental procedures carried out in this study were in compliance with the UK Animal Scientific Procedures Act 1986.

2.2. Microdialysis

Rats were anaesthetised with chloral hydrate (400 mg/kg ip) with supplementary doses (60 mg/kg ip) administered as necessary. The body temperature of the animals was maintained at 35–37 °C throughout the course of the experiment by means of a homeothermic heating pad and rectal probe. Microdialysis probes (4 mm tip length) were implanted in the hippocampus (AP − 5.0 mm and ML − 4.6 mm from bregma, DV − 8.5 mm from dura; Paxinos and Watson, 1982) for measurement of noradrenaline. In some experiments, probes (2 mm tip length) were also implanted into the contralateral LC region (AP − 9.8 mm and ML + 1.2 mm from bregma, DV − 8.8 mm from dura) to allow monitoring of extracellular levels of 5-HT. A further burr hole (1 mm diameter) was drilled for implantation of the stimulating electrode into the raphe nuclei.

Probes were perfused continuously (2 μl/min) with aCSF. For noradrenaline measurements the aCSF contained 5 μM desipramine. For measurement of 5-HT, the aCSF contained 1 μM citalopram. The presence of the uptake blockers was necessary to detect reliable stimulation-evoked increases in noradrenaline/5-HT (basal levels were readily detectable). Perfusates were collected every 20 min and analysed for noradrenaline or 5-HT by HPLC-EC. Stimulation of the DRN or MRN commenced once baseline levels of noradrenaline and 5-HT had stabilised (2–3 h post probe implantation).

2.3. Electrical stimulation

A coaxial bipolar stainless steel electrode (Clark Electromedical Instruments, Pangbourne, UK) was stereotaxically positioned in either the DRN (AP − 7.5 mm, ML 0.0 mm, DV − 6.0 mm) or MRN (AP − 7.5 mm, ML 0.0 mm, DV − 8.0 mm) and left in position for the duration of the experiment. The electrode was connected via a constant-current unit (Grass PS1U6) to a stimulator (Grass S48). The stimulation parameters were: 1 ms pulse width square wave, 300 μA current, 3–10 Hz frequency, 20 min stimulus duration. Stimulations were applied on three occasions with 60-min intervals between stimulations. The stimulation frequency was increased successively (3, 5, 10 Hz) with each stimulation period.

2.4. Localisation of dialysis probes and stimulating electrodes

At the end of the experiment brains were removed and postfixed in 4% paraformaldehyde and the final positions of the electrode tip and dialysis probes were determined histologically. Data were included in the final analysis only if the dialysis probes and stimulation electrode were correctly located.

2.5. HPLC measurement of noradrenaline and 5-HT

Immediately following collection, perfusate samples were analysed for noradrenaline or 5-HT on separate HPLC-EC assays according to the method described elsewhere (see Sharp and Zetterstrom, 1992). In brief, noradrenaline was separated using a Rainin Dynamax HPLC column (4.6 × 150 mm, Microsorb C_{18} 5 μm particles) and a mobile phase comprising 0.1 M NaH_2PO_4, 1.8 mM

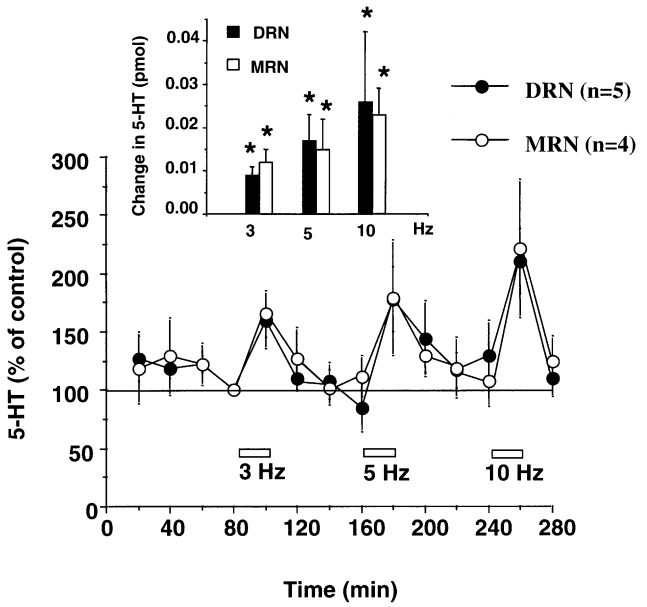

Fig. 1. Effect of electrical stimulation of the DRN and MRN on extracellular 5-HT levels in the LC. The raphe nuclei were stimulated (1 ms, 300 μA, 20 min) with increasing pulse frequency (3, 5 and 10 Hz) at the time points indicated by the bar. Each point is a mean ± S.E.M. Inset shows the effect of stimulation on the absolute amount of 5-HT in the LC (peak minus baseline). Basal dialysate levels of 5-HT in the LC of DRN ($n=5$) and MRN ($n=4$) stimulated rats were 0.029 ± 0.005, and 0.024 ± 0.003 pmol/40 μl sample, respectively. * $P<.05$ versus prestimulation values (Dunnett's t test).

sodium octane sulphonate, 0.5 mM EDTA and 12% (vol/vol) methanol (final pH 4, flow rate 1.2 ml/min). For measurement of 5-HT the mobile phase comprised of 0.12 M NaH_2PO_4, 0.01 mM sodium octane sulphonate, 0.1 mM EDTA and 12.5% (vol/vol) methanol (final pH 3.8, flow rate 1.1 ml/min). Detection was achieved using a BAS LC-4 electrochemical detector connected to a glassy carbon electrode (+0.7 V vs. Ag/AgCl reference).

2.6. Lesioning of 5-HT neurones

5-HT neurones were lesioned with 5,7-dihydroxytryptamine (5,7-DHT) according to Baumgarten et al. (1982). Rats were anaesthetised with halothane and 5,7-DHT (200 μg in 10 μl saline vehicle containing 1% ascorbate) was injected over 5 min into the right lateral ventricle (coordinates: AP −0.9, ML −1.4, DV −4.0 mm). Control animals received intracerebroventricular injection of vehicle. Rats were pretreated with 25 mg/kg ip desipramine 30 min prior to 5,7-DHT or vehicle infusion, to protect noradrenergic neurones from lesion. Microdialysis experiments were carried out 14 days after administration of 5,7-DHT or its vehicle.

2.7. Data analysis

Data are presented as a percentage of the absolute amount of 5-HT and noradrenaline in the sample collected immediately before stimulation. In the 5,7-DHT experiment, absolute amounts of noradrenaline (picomoles) collected over a 20-min sample period are also shown. Mean basal levels were calculated as the average of the last three samples before any stimulation. The effect of raphe stimulation on 5-HT and noradrenaline was analysed statistically using one-way ANOVA followed by Dunnett's t test. Comparisons between the amount of 5-HT or NA released by DRN and MRN stimulations were made using Student's unpaired t test. Probability levels of 5% or less were considered statistically significant.

3. Results

3.1. Effect of raphe stimulation on extracellular 5-HT in the LC region

Electrical stimulation of the DRN or MRN for 20 min (3, 5 and 10 Hz) evoked a marked but short-lasting increase of 5-HT in perfusates collected from the region of the LC (Fig. 1). Stimulation of the DRN increased 5-HT above prestimulation control values by 60%, 78% and 110%, $F(13,52)=4.62$, $P<.0001$, which was similar to that observed following MRN stimulation (65%, 80% and 122%) $F(13,39)=3.24$, $P<.002$. In both groups, the effect of stimulation was statistically significant at all frequencies when compared to prestimulation values (post hoc Dunnett's t test), and the absolute amounts of 5-HT released

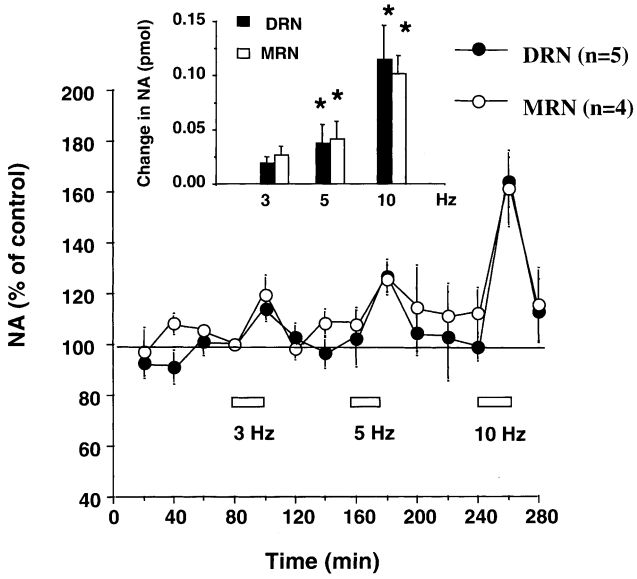

Fig. 2. Effect of electrical stimulation of the DRN and MRN on extracellular noradrenaline in the hippocampus. See Fig. 1 for experimental details. Each point is a mean ± S.E.M. Basal dialysate levels of noradrenaline in the hippocampus of DRN ($n=5$) and MRN ($n=4$) stimulated rats were 0.196 ± 0.044 and 0.219 ± 0.036 pmol/40 μl sample, respectively. Inset shows the effect of stimulation on the absolute amount of noradrenaline in the hippocampus (peak minus baseline). * $P<.05$ versus prestimulation values (Dunnett's t test).

(peak minus baseline) were similar for DRN versus MRN (Student's unpaired *t* test).

3.2. Effect of raphe stimulation on extracellular noradrenaline

Electrical stimulation of DRN evoked a short-lasting, frequency-dependent increase in extracellular noradrenaline in the hippocampus, $F(13,52)=9.18$, $P<.0001$, one-way ANOVA, (Fig. 2). Frequencies of 3, 5 and 10 Hz increased noradrenaline by 13%, 27% and 64%, respectively with effects at 5 and 10 Hz being statistically significant (post hoc Dunnett's *t* test). MRN stimulation also caused a frequency-dependent increase of noradrenaline, $F(13,39)=6.19$, $P<.0001$, (Fig. 2). Moreover, the absolute amount of noradrenaline released by electrical stimulation of the DRN versus MRN was not statistically different at any stimulation frequency (Student's unpaired *t* test).

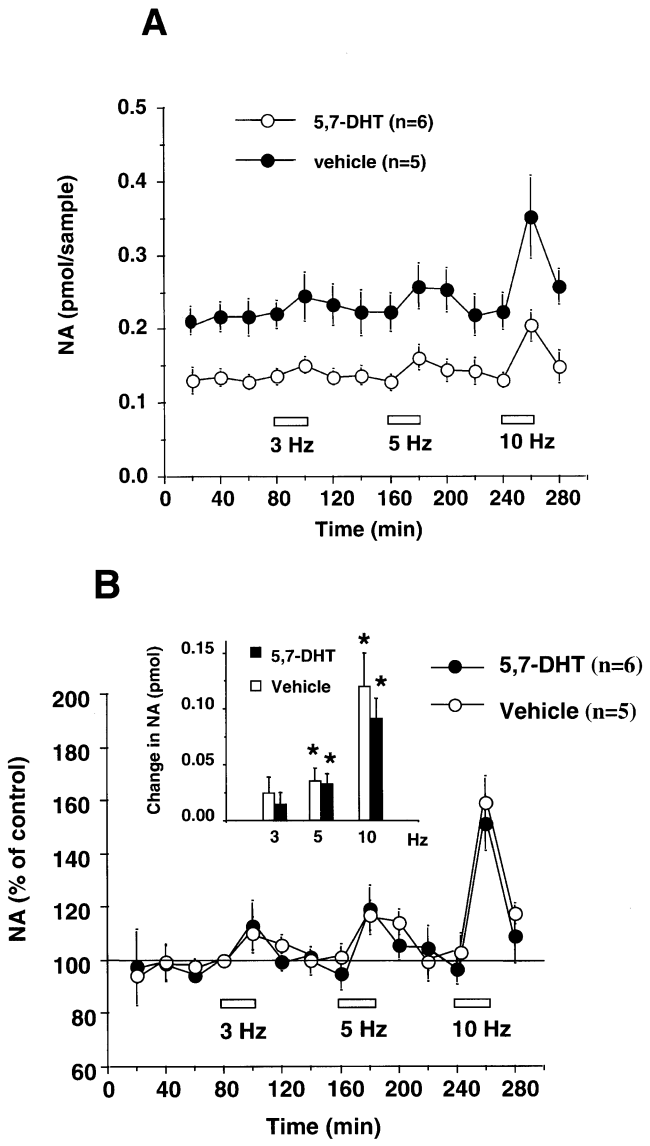

Fig. 3. Effect of electrical stimulation of the DRN on extracellular noradrenaline in the hippocampus of 5-HT-lesioned animals. Data are expressed as (A) absolute noradrenaline (pmol/20 min) and (B) percent of basal noradrenaline. See Fig. 1 for experimental details. Animals received either 5,7-DHT (200 μg in 10 μl vehicle) or vehicle intracerebroventricularly, 14 days prior to the microdialysis experiment. Each point is a mean ± S.E.M. Inset shows the effect of stimulation on the absolute amount of noradrenaline in the hippocampus (peak minus baseline). * $P<.05$ versus prestimulation values (Dunnett's *t* test).

3.3. Effect of raphe stimulation on extracellular noradrenaline after a 5-HT lesion

To test whether the noradrenaline response to raphe stimulation was mediated by 5-HT, the stimulation paradigm (DRN only) was repeated in animals administered the 5-HT toxin, 5,7-DHT versus vehicle-treated controls. Basal 5-HIAA levels were reduced by over 90% in 5,7-DHT-treated rats compared to controls, 0.54 ± 0.18 ($n=6$) vs. 5.68 ± 1.24 ($n=5$) pmol/sample, $P<.001$ Student's unpaired *t* test.

In 5,7-DHT-treated rats basal dialysate noradrenaline levels in the hippocampus were 40% lower than controls (0.132 ± 0.009 ($n=6$) vs. 0.217 ± 0.018 ($n=5$) pmol/sample; $P<.05$, Student's unpaired *t* test) (Fig. 3A). However, electrical stimulation of the DRN at 3, 5 and 10 Hz in 5,7-DHT-treated rats increased noradrenaline, +13%, +19% and +51%; $F(13,52)=16.42$, $P<.0001$, one-way ANOVA, to the same extent as controls, +10%, +17% and +59%; $F(13,65)=5.79$, $P<.0001$, one-way ANOVA (Fig. 3B). In both treatment groups, the effect of DRN stimulation on noradrenaline was statistically significant at 5 and 10 Hz (post hoc Dunnett's *t* test). The absolute amount of noradrenaline (peak minus baseline) released by stimulation of DRN in 5,7-DHT-treated rats was not statistically significant from controls (Student's unpaired *t* test) at any frequency (Fig. 3B).

4. Discussion

Recent in vivo microdialysis studies of the interaction between 5-HT and noradrenaline have demonstrated marked effects of 5-HT receptor selective ligands on extracellular noradrenaline (see Introduction). Here we used raphe stimulations with microdialysis in an attempt to determine the effect of endogenous 5-HT on the noradrenergic system. The approach used follows that used by De Deurwaerdere and Spampinato (1999) and De Deurwaerdere et al. (1998) to study the effects of endogenous 5-HT on the dopaminergic system.

In the present study we found that electrical stimulation of the DRN and MRN elicited a frequency-dependent increase in extracellular noradrenaline in the ventral hippocampus of the anaesthetised rat. The frequencies at which noradrenaline increased (3–10 Hz) are close to those at which 5-HT neurones normally fire (0.5–3 Hz: Aghajanian et al., 1978; Hajós et al., 1995). In the same animals,

stimulation of the DRN and MRN also evoked the release of 5-HT in the LC. A recent push–pull perfusion study also found that DRN stimulation increased 5-HT release in this nucleus (Kaehler et al., 1999). The LC is rich in noradrenergic cell bodies, and provides the source of noradrenergic terminals for the hippocampus (Lindvall and Björklund, 1974, Moudy et al., 1993). Several previous studies have shown that the raphe stimulation paradigm used here also evokes a release of 5-HT in the ventral hippocampus, as well as other forebrain regions (Sharp et al., 1990; McQuade and Sharp, 1995, 1997). Therefore, activation of the midbrain raphe nuclei at physiologically relevant frequencies evokes a release of 5-HT, which would be able to interact with both the noradrenergic cell bodies in the LC and noradrenergic terminals in the hippocampus.

Such an interaction could explain the raphe-evoked increase in hippocampal noradrenaline. Moreover, this effect was predicted by recent pharmacological experiments showing that certain 5-HT receptor agonists (in particular 5-HT_{1A} agonists) increase extracellular noradrenaline in this region (see Introduction). Surprisingly, however, we found that raphe stimulation also evoked the release of noradrenaline in animals with 5-HT lesions. This finding contrasts with experiments showing that 5-HT lesions induced by 5,7-DHT completely abolish the effects of raphe stimulation on dopamine release (De Deurwaerdere et al., 1998).

We have previously reported that the 5,7-DHT treatment used in the present study decreased tissue concentrations of both 5-HT and 5-HIAA by 80–90% in various forebrain areas of the rat, and also significantly reduced (80%) the occurrence of 5-HT neurones in the DRN as revealed by electrophysiological studies (Hajós and Sharp, 1996). Lesioned animals in the present study had 5-HT metabolite levels that were 90% of levels in non-lesioned controls. Previously we have found that 5-HT neurotoxic lesions, which lower 5-HT metabolite levels by 80%, cause a striking reduction in the amount of hippocampal 5-HT released by stimulation (5 Hz, 20 min) of the DRN and MRN (McQuade and Sharp, 1995). Our experiments do not exclude the possibility that a small residual release of 5-HT remained in the 5-HT-lesioned animals, which then could activate supersensitive postsynaptic 5-HT receptors and facilitate noradrenaline release. However, the two postsynaptic 5-HT receptors, 5-HT_{1A} and 5-HT_3, so far identified to facilitate noradrenaline release when activated (see Introduction), do not up-regulate in animals with 5-HT lesions (Kidd et al., 1992).

Recently, raphe stimulations were found to evoke changes in dopamine release that were prevented by 5-HT lesions as well as 5-HT receptor antagonists (De Deurwaerdere et al., 1998). Interestingly the latter studies used stimulation frequencies of 10–20 Hz, which are in a range higher than those used here. It cannot be excluded that higher stimulation frequencies would evoke a 5-HT dependent release of noradrenaline, however such frequencies would be outside the physiological range.

Although the 5-HT lesion did not alter raphe-evoked release of noradrenaline, basal levels of noradrenaline in the hippocampal dialysates were reduced by about 40%. Whilst it is possible that the 5-HT toxin also lesioned the noradrenergic neurones, desipramine was used to prevent this. In addition, the magnitude of the raphe stimulation-induced noradrenaline release was not altered in lesioned animals. Therefore, this particular observation may reflect a functional interaction between the 5-HT and noradrenergic systems. In support of this, we recently found that the 5-HT synthesis inhibitor *p*-chlorophenylalanine also caused a significant reduction (− 35%) in basal noradrenaline levels (Hajós-Korcsok et al., 1999). It should be pointed out that whilst these in vivo measurements of extracellular noradrenaline suggest that 5-HT is tonically excitatory on the noradrenergic system, previous studies on the effects of 5-HT lesion/synthesis inhibition on whole tissue levels of noradrenaline and its metabolites have concluded that 5-HT is inhibitory (see Introduction and Hajós-Korcsok et al., 1999 for further discussion).

A possible explanation for the raphe stimulation-induced noradrenaline response is direct stimulation of noradrenaline cell bodies or fibres in the raphe region. Neurones immunoreactive for the noradrenaline synthesising enzyme, dopamine β-hydroxylase, have been identified in the mesencephalic raphe nuclei (Grzanna and Molliver, 1980). However, the number of these neurones is small, and it is not clear whether any of these neurones project to the hippocampus. Ascending catecholaminergic fibres pass adjacent to the midline raphe nuclei (Lindvall and Björklund, 1974). In previous studies we have established that electrodes located outside the DRN (where there are few 5-HT cell bodies) do not evoke a release of 5-HT, suggesting that the current spread from our electrodes is very localized (McQuade and Sharp, 1995). In the present study all electrodes were located within the DRN and MRN. Therefore, any current spread to the catecholaminergic fibres is likely to be of low density.

As an alternative to direct activation of the noradrenergic pathways by raphe stimulation, another candidate mechanism is the activation of non-5-HT projections from the raphe nuclei to the hippocampus or LC. Indeed, neuroanatomical studies provide evidence for a sizeable number of non-5-HT neurones in the DRN that contain other putative neurotransmitters, including various peptides, nitric oxide, glutamate, GABA and dopamine (e.g., Kirifides et al., 2001 and references therein). Furthermore, non-5-HT projections from the raphe to the hippocampus have been reported (Kohler and Steinbusch, 1982) although the neurochemical identity of these pathways awaits clarification.

In summary, electrical stimulation of the midbrain raphe nuclei was found to increase the release of noradrenaline in the hippocampus. However, data from 5-HT-lesioned animals suggest that this response is not likely to be mediated by 5-HT. Although recent data indicate that raphe stimulation may be useful to investigate interactions between

5-HT and dopamine, our data indicate that the same approach may not be feasible for 5-HT and noradrenaline.

Acknowledgments

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