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Involvement of 5-HT_{1A}- and α_2-receptors in the decreased 5-hydroxytryptamine release and metabolism in rat suprachiasmatic nucleus after intravenous 8-hydroxy-2- (*n*-dipropylamino) tetralin

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1 The 5-hydroxytryptamine$_{1A}$-receptor agonist 8-hydroxy-2-(*n*-dipropylamino) tetralin (8-OH-DPAT, 0.1 mg kg^{-1} i.v.) decreased the height of the extracellular 5-hydroxyindoleacetic acid (5-HIAA) oxidation peak recorded in the suprachiasmatic nucleus (SCN) of the anaesthetized rat by use of differential pulse voltammetry.

2 The decrease in extracellular 5-HIAA produced by 8-OH-DPAT could be partially attenuated by prior administration of the non-selective 5-HT receptor antagonist methiothepin (1 mg kg^{-1} i.v.). The 5-HT_2-receptor antagonist ritanserin (0.2 mg kg^{-1} i.v.) did not appear to block the effects of 8-OH-DPAT.

3 The selective ligand for 5-HT_{1A} recognition sites TVX Q 7821 (isapirone, 1 mg kg^{-1} i.v.) decreased the extracellular level of 5-HIAA in the SCN but to a lesser extent than 8-OH-DPAT. The response to 8-OH-DPAT was attenuated by prior administration of TVX Q 7821 to a level suggesting that TVX Q 7821 had blocked the effect of intravenous 8-OH-DPAT.

4 Idazoxan (0.2 mg kg^{-1} i.v.) an α_2-adrenoceptor antagonist, completely blocked the effect of 8-OH-DPAT on the 5-HIAA oxidation peak recorded in the SCN, whilst having no effect on the 5-HIAA oxidation peak when given alone.

5 At a dose of 0.5 mg kg^{-1} i.v. idazoxan induced a 120% increase in the height of the indole oxidation peak, suggesting that 5-HT release and metabolism in the rat SCN may be influenced by tonic adrenergic inputs.

6 The data in this paper suggest that 5-HT_{1A}- and α_2-receptors are involved in the effects of i.v. administered 8-OH-DPAT on 5-HT release and metabolism in the SCN *in vivo*.

Introduction

It is generally accepted that there are several different recognition sites located on neurones within the mammalian central nervous system for 5-hydroxytryptamine (5-HT). Based upon the initial binding studies of Peroutka & Snyder (1979) these sites have been classified as 5-HT_1, which [^{3}H]-5-HT binds to with high affinity, and 5-HT_2, labelled by high affinity [^{3}H]-spiperone binding.

Recent evidence suggests that the 5-HT_1 binding sites can be further sub-divided. These 5-HT_1 subtypes have been identified by high (5-HT_{1A}) and low (5-HT_{1B}) affinity components of the displacement of [^{3}H]-5-HT binding by the neuroleptic spiperone (Pedigo *et al.*, 1981; Schnellman *et al.*, 1984). In addition certain 5-HT-receptor agonists have been found to bind selectively to either the 5-HT_{1A} or 5-HT_{1B} site. One such compound is 8-hydroxy-2-(*n*-dipropylamino) tetralin (8-OH-DPAT) which binds specifically to the 5-HT_{1A} site (Middlemiss & Fozard, 1983). The piperidinyl indole derivative 5-methoxy-3-[1,2, 3,6-terahydro-4-pyridinyl]-1H indole (RU24969) has been proposed as a selective ligand for the 5-HT_{1B} site (Sills *et al.*, 1984; Doods *et al.*, 1985).

We have recently demonstrated that the release and metabolism of 5-HT in the suprachiasmatic nucleus (SCN) of the rat is decreased following peripheral administration of RU 24969 (Martin & Marsden, 1986) by an agonist action on 5-HT_{1B}-receptors. Since

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the evidence in the literature strongly suggests that the prejunctional 5-HT autoreceptor is of the 5-HT_{1B} subtype (Martin & Sanders-Bush, 1982; Middlemiss, 1985b; Engel *et al.*, 1986) it seems likely that RU24969 decreases 5-HT release *in vivo* in the SCN by acting on the 5-HT autoreceptor.

Hjorth and co-workers (1982) have shown that tissue levels of 5-hydroxyindoleacetic acid (5-HIAA) decrease following intravenous administration of 8-OH-DPAT. The *in vitro* release studies of Hamon *et al.* (1984) suggest that 8-OH-DPAT acts on a 5-HT autoreceptor in the cortex and striatum to decrease 5-HT release. Work in our laboratory has also suggested that release and metabolism of 5-HT in the frontal cortex *in vivo* is decreased by peripheral administration of the 5-HT_{1A} agonist 8-OH-DPAT (Routledge & Marsden, 1985; Maidment *et al.*, 1986). However, Middlemiss (1984) has reported that 8-OH-DPAT does not decrease [^{3}H]-5-HT release from superfused rat frontal cortex slices *in vitro*. In addition, we have demonstrated that 8-OH-DPAT injected into the SCN or dorsal raphé nucleus does not affect *in vivo* 5-HT release and metabolism in the SCN (Marsden & Martin, 1985b).

The aims of the work presented in this paper were to determine whether peripherally administered 8-OH-DPAT affected 5-HT release and metabolism in the SCN *in vivo* using voltammetry and, in addition, to provide an insight into the receptor subtypes involved.

Methods

Electrode preparation

The working electrodes used in this study were similar to those used in previous studies (Ponchon *et al.*, 1979; Maidment & Marsden, 1985). They were made by inserting three pyrolytic carbon fibres (Le Carbone, Lorraine, 8 o.d., ref AGF/F) into a glass capillary (Clark Electromedical Instruments AG10) pulled to fine tip (25 μm i.d.).

Standard plastic coated silver wire coated with electrical conductive paint (Radio Spares, ref 555–156) was used to make electrical contact with the carbon fibre. This joint was strengthened with resin (Sody 33, ESCIL) and the electrode tip was sealed by the application of a drop of low viscosity resin (Loctite Glassbond), taking care not to coat the fibres with the resin. The fibres were then cut such that approximately 300 μm protruded from the tip. Silver wire (Clark Electromedical Instruments AG–10T) placed in contact with the dura surface was used as an auxiliary electrode. The reference electrode was a silver/silver chloride wire also placed in contact with the dura surface.

Before implantation the working electrode was electrically pretreated in order to distinguish the oxidation of 5-HIAA from that of ascorbic acid (AA) and 3, 4-dihydroxyphenyl acetic acid (DOPAC) (Cespuglio *et al.*, 1981). This pretreatment involved applying the following potentials as a triangular wave form to the working electrode immersed in phosphate buffered saline (pH 7.4, 0.1 M) using a waveform generator and a polarograph (Princeton Applied Research 174A):(a) 0–3V, 70 Hz, 20 s;(b) 0–2V, 70 Hz, 20 s;(c) 0–1V, 70 Hz, 20 s.

Experimental procedure

Throughout this study male Wistar rats weighing 290–310 g were used. They were anaesthetized with chloral hydrate (600 mg kg^{-1} i.p.) and anaesthesia maintained with supplementary doses of 30 mg (i.p.) when necessary. The jugular vein was cannulated with polypropylene tubing (Portex Ltd, PP25) for the administration of drugs. The animals were then placed in a David Kopf stereotaxic frame with the incisor bar set 5 mm above the interaural line. Reference and auxiliary electrodes were placed in contact with the dura surface through small burr holes and held in place with chronoplastic dental cement. The working electrode was implanted into the left SCN at an angle of 10° to the vertical through a 2 mm burr hole using the following co-ordinates obtained from the atlas of Pellegrino *et al.*, (1981):rostro-caudal + 1.7 mm, medial lateral + 1.81 mm from bregma and − 9.2 mm from the dura surface.

Differential pulse voltammetry (DPV) was performed using a Metrohm E506 polarograph with an E608 controller with the following scan parameters: potential range − 0.02 to + 0.4 V; scan rate 5 mV s^{-1}; modulation amplitude 50 mV; pulse frequency 2.5 s^{-1}. Scans lasting 120 s were initiated every 5 min and the height of the oxidation peak at + 0.3 V in nanoamps was taken as a measure of extracellular 5-HIAA (Crespi *et al.*, 1983; Faradji *et al.*, 1983; Sharp *et al.*, 1984). In common with other studies using similar carbon fibre electrodes, we have termed this oxidation peak, 'peak 3'. A stabilization period of one hour was allowed before any interventions were made. Drugs were then administered via the jugular cannula dissolved in 0.9% w/v saline in a volume of 1 ml kg^{-1} and flushed through with 0.25 ml saline. Peak heights following the first injection in antagonist vs. agonist studies or a single injection in agonist or antagonist alone studies were expressed as a percentage of the mean of the peak heights of the six voltammograms obtained before the injection. Where appropriate Student's *t* test was used to compare groups.

At the end of each experiment an electrolytic lesion was made to identify the position of the electrode by applying a d.c. potential of 2 V for 20 s to the working electrode. The animal was then killed, the brain

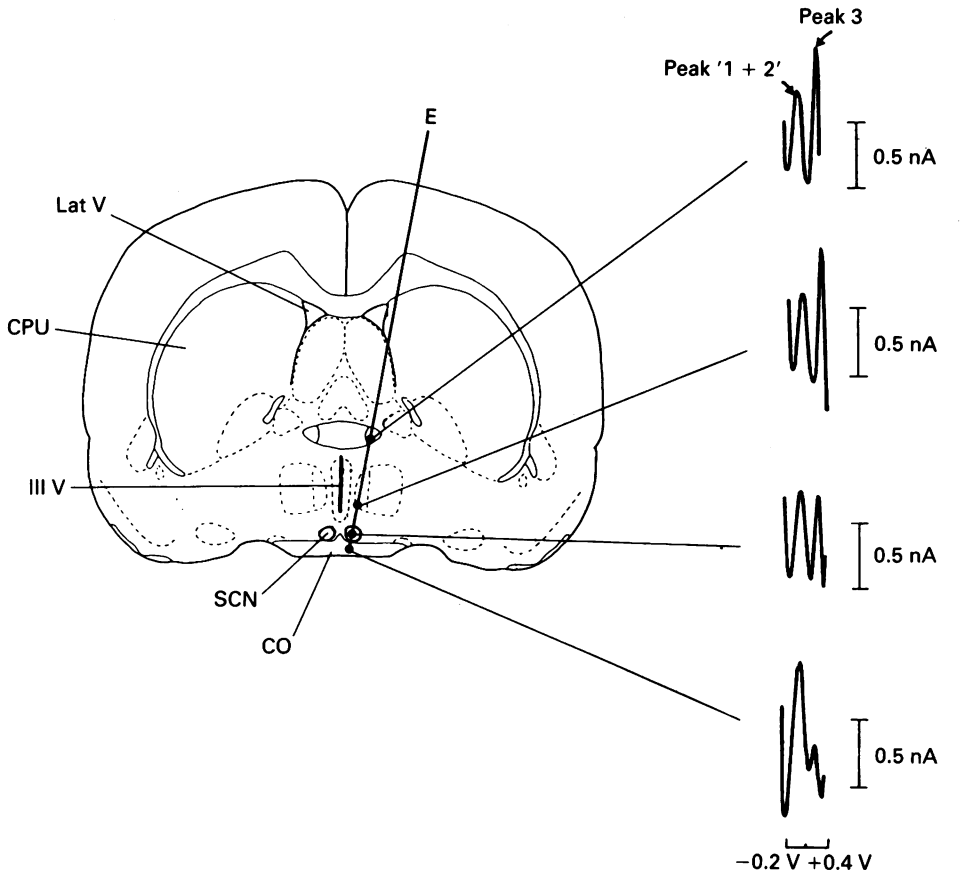

Figure 1 Coronal section of the rat brain taken from the atlas of Pellegrino *et al.* (1981) illustrating the path of implantation of the working electrode (E) to the suprachiasmatic nucleus (SCN). Representative voltamograms from a single animal are shown on the right. Each voltamogram was obtained when the electrode tip was in the approximate region of the black dot on the line. The position of the electrode in the SCN was verified histologically. Note the decrease in the size of the oxidation peak at + 300 mV (peak 3) when the electrode tip was in the SCN. Abbreviations used: Lat V, lateral ventricle; CPU, caudate putamen; IIIV, IIIrd ventricle; CO, optic chiasm.

removed and fixed in 10% phosphate buffered formalin pH 7.4 for 2 days. Paraffin wax blocks were then prepared from which 7 μm thick sections were cut. They were stained with Mayers Haematoxylin, counterstained with Eosin and examined under a microscope for electrode position. Only data obtained from animals where the working electrode was in the SCN are included here.

Drugs

The following drugs were used in this study: 8-OH-DPAT (I.C.I. plc), methiothepin, R-55667 (ritanserin, Janssen), idazoxan (Reckitt & Colman), TVX Q 7821 (isapirone, Troponwerke, Cologne, F.R.G.).

Results

As shown previously by ourselves (Martin & Marsden, 1986) and other workers (Faradji *et al.*, 1983) an increase in the height of peak 3 occurred as the carbon fibre working electrode was lowered towards the SCN. However, once the electrode entered the nucleus the magnitude of peak 3 decreased (Figure 1). This phenomenon provided preliminary evidence of electrode position prior to the histological verification (Martin & Marsden, 1986).

Administration of 0.9% w/v NaCl solution (saline 1 ml kg^{-1} i.v.) was not associated with any significant change in the height of the indole oxidation peak though the peak increased by about 10% over the two

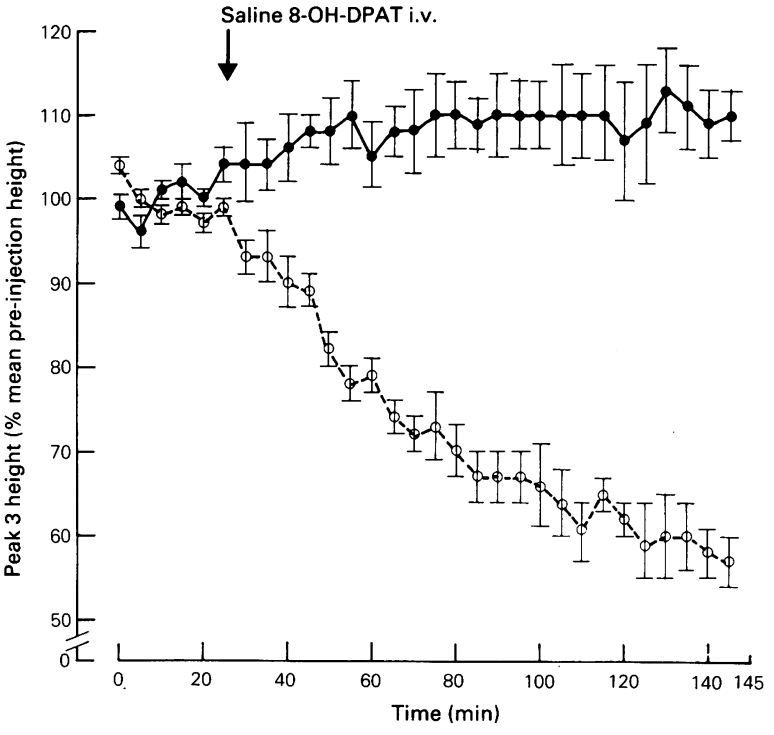

Figure 2 Effect of 0.9% w/v NaCl solution (saline 1 ml kg^{-1} i.v., ●, $n = 4$) 8-hydroxy-2-(*n*-dipropylamino) tetralin (8-OH-DPAT, 0.1 mg kg^{-1} i.v. ○, n = 4) on the height of peak 3 recorded in the suprachiasmatic nucleus. Each point represents the mean and the s.e.mean is illustrated by the vertical lines.

hour recording period (Figure 2). In contrast, 15 min after the administration of 8-OH-DPAT (0.1 mg kg^{-1} i.v.), the height of peak 3 decreased rapidly and significantly; by 22 ± 3 ($n = 4$) 30 min, 33 ± 3 ($n = 4$) 60 min and 43 ± 3% ($n = 4$) 120 min following the injection of 8-OH-DPAT (Figure 2). In order to determine the receptor types involved in this response various 5-HT antagonists were administered 5 min before the administration of 8-OH-DPAT but the effects of the antagonists themselves were studied first. However, as the non-selective 5-HT receptor antagonist methiothepin has previously been found to increase the height of peak 3 recorded in the SCN (Martin & Marsden, 1986) and in the ventromedial hypothalamus (Baumann & Waldmeier, 1984) at comparable doses to those used here, this experiment was not repeated.

The height of peak 3 was not significantly different from that in control animals for the first 50 min following injection of ritanserin (0.2 mg kg^{-1} i.v.) alone. However, after this time peak 3 increased significantly above control values (Figure 3a). Conversely, the purported (Goodwin *et al.*, 1986) 5-HT_{1A}-receptor antagonist TVX Q 7821 (1 mg kg^{-1} i.v.) significantly decreased peak 3 from 30 min following its administration (Figure 3b).

Methiothepin (1 mg kg^{-1} i.v.) given 5 min before 8-OH-DPAT (0.1 mg kg^{-1} i.v.) significantly attenuated the response to 8-OH-DPAT (Figure 4). Similarly, the 5-HT_2-receptor antagonist ritanserin (0.2 mg kg^{-1} i.v.) also significantly attenuated the response 15 and 45 min after 8-OH-DPAT (0.1 mg kg^{-1} i.v.) (Figure 4). However, when the effects of ritanserin alone are taken into account (Figure 3) it is doubtful whether ritanserin genuinely blocked the response to 8-OH-DPAT. The height of peak 3 was significantly lower than control levels 30, 45 and 60 min after TVX Q 7821 (1 mg kg^{-1} i.v.) followed by 8-OH-DPAT (0.1 mg kg^{-1} i.v.). At the latter two times this decrease was significantly less than that obtained when 8-OH-DPAT was given alone (Figure 4).

Idazoxan, an α_2-adrenoceptor antagonist, at doses of 0.1 and 0.2 mg kg^{-1} i.v. had no significant effect on the height of peak 3 recorded in the SCN (Figure 5). However, following a dose of 0.5 mg kg^{-1} i.v. there was a large increase in peak 3 which was maximal at 45 min (119 ± 12%, $n = 4$, Figure 5).

When idazoxan (0.2 mg kg^{-1} i.v.) was administered 5 min before 8-OH-DPAT (0.1 mg kg^{-1} i.v.) the height of peak 3 was not significantly different from that in

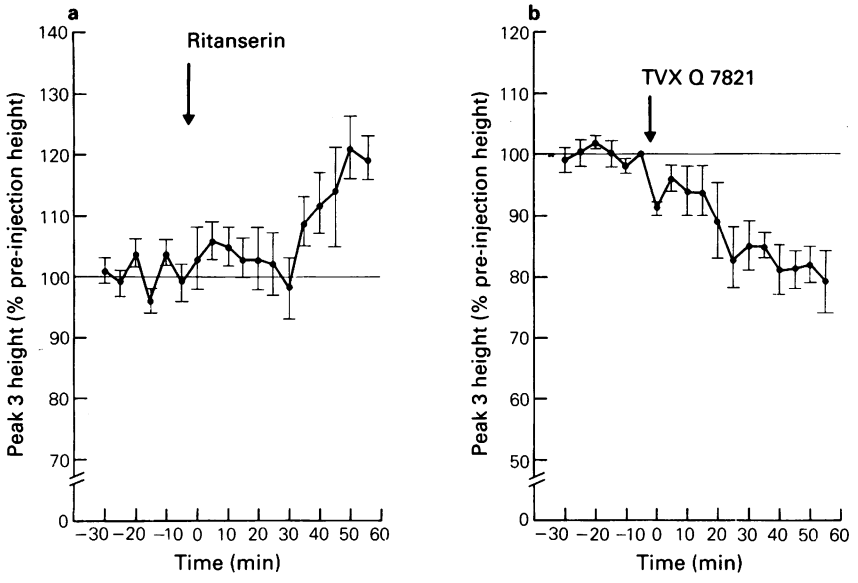

Figure 3 The effect of (a) ritanserin (0.2 mg kg^{-1} i.v., $n = 4$) and (b) TVX Q 7821 (1 mg kg^{-1} i.v., $n = 4$) on the height of peak 3 recorded in the suprachiasmatic nucleus (SCN). Each point represents the mean and s.e.mean is also shown. Note the decrease in peak 3 height following injection of TVX Q 7821.

the control group (Figure 6). This was in marked contrast to the response following 8-OH-DPAT alone (Figures 2 and 6) which was associated with a significant marked decrease in peak 3 height.

Discussion

Previous studies using carbon fibre working electrodes have demonstrated that the height of the oxidation peak recorded at + 300 mV principally represents the oxidation of 5-HIAA (Cespuglio *et al.*, 1981; Sharp *et al.*, 1984; Brazell *et al.*, 1985). However, in the striatum up to 30% of peak 3 was accounted for by the oxidation of uric acid but in the SCN this figure is probably much lower since injection of the 5-HT$_{1B}$-agonist RU 24969 into the SCN results in at least a 90% decrease in peak 3 height (Marsden & Martin, 1985a).

Intracerebral dialysis has allowed measurement of neurotransmitter amines and their metabolites (Ungerstedt, 1984) and studies in which this technique has been combined with voltammetry have shown that drugs such as RU 24969 which decrease extracellular levels of 5-HIAA also decrease extracellular levels of 5-HT (Brazell *et al.*, 1985). Thus, using *in vivo* voltammetry it is possible to monitor 5-HT release and metabolism *in vivo* in small nuclei such as the SCN.

In this paper we present data that intravenously administered, 8-OH-DPAT decreases 5-HT metabolism in the suprachiasmatic nucleus measured by *in vivo* voltammetry. Previous intracerebral dialysis experiments have demonstrated that in the frontal cortex, there is a decrease in extracellular 5-HIAA levels following s.c. administration of 8-OH-DPAT (Maidment *et al.*, 1986). In addition there was an associated decrease in the extracellular level of 5-HT (Routledge & Marsden, 1985) suggesting that this compound decreases release as well as metabolism of 5-HT. It is likely, therefore, that release as well as metabolism of 5-HT in the SCN was also decreased in our present experiments. These results are in general agreement with those of Hjorth *et al.*, (1982) who reported that 8-OH-DPAT produced a dose-dependent decrease in tissue levels of 5-HIAA in the rat brain. They suggested that this may have been due to an agonist action at the 5-HT autoreceptor. A view which has received support from the *in vitro* release studies of Hamon *et al.* (1984) demonstrating that 8-OH-DPAT reduced the K^+-evoked release of previously taken up [^{3}H]-5-HT from cortical or striatal slices.

Conversely, Middlemiss (1984) found that 8-OH-DPAT at concentrations of less than 1 μmol l^{-1} did not decrease K^+ stimulated release of tritium from rat frontal cortex slices pre-loaded with [^{3}H]-5-HT and we have shown that 8-OH-DPAT injected into the SCN does not decrease extracellular levels of 5-HIAA (Marsden & Martin, 1985b). In addition, [^{3}H]-8-OH-DPAT does not bind to the 5-HT autoreceptor in rat

striatum (Middlemiss, 1985a). Hjorth *et al.* (1982) suggested that the effects of 8-OH-DPAT reported by them were consistent with a decrease in 5-HT neurone firing although they provided no direct evidence that this was the case. Thus the actual mechanism whereby 8-OH-DPAT decreases release and metabolism of 5-HT in the SCN remains unclear.

In an attempt to determine the 5-HT receptor subtype involved in our response to 8-OH-DPAT we administered methiothepin, a 5-HT_1- and 5-HT_2-receptor antagonist which is claimed to show some selectivity for the 5-HT_1 site (Hibert & Middlemiss, 1985). At a dose which would block the response to RU 24969 (Martin & Marsden, 1986) we could only partially attenuate the response to 8-OH-DPAT. In addition, although the selective 5-HT_2-receptor antagonist ritanserin (Laduron & Janssen, 1984; Goodwin & Green, 1985) also appeared to attenuate the effects of 8-OH-DPAT, when its effects on extracellular levels of 5-HIAA are taken into account it is doubtful whether 5-HT_2-receptors are involved in the response to 8-OH-DPAT.

From these data we postulated that the effects of 8-OH-DPAT involved 5-HT_1-receptors. However, since methiothepin binds to α-adrenoceptors, albeit with a lower affinity than for 5-HT_1-receptors (Leysen *et al.*, 1981), we could not discount the involvement of α_2-adrenoceptors as well as 5-HT_1-receptors at this stage.

Recent findings have suggested that TVX Q 7821 is a selective 5-HT_1-receptor ligand (Dompert *et al.*, 1985) and that this selectivity may be to the 5-HT_{1A} site (Schuuman *et al.*, 1984) where it has antagonist properties (Goodwin *et al.*, 1980; Martin *et al.*, 1986). Further, in binding displacement studies noradrenaline did not displace specifically bound [^{3}H]-TVX Q 7821 (Dompert *et al.*, 1985). We therefore tested this compound in our experimental protocol from which two interesting points emerged. First, administration of TVX Q 7821 was associated with a decrease in extracellular 5-HIAA levels, indicating decreased 5-HT metabolism. Second, in the second half of our recording period it attenuated the response to 8-OH-DPAT to an extent similar to that of methiothepin. The fact that TVX Q 7821 given alone resulted in a 20% decrease in peak 3 makes interpretation of its effects on the 8-OH-DPAT response difficult. However, this finding is in accordance with the data of Goodwin *et al.*, (1986) demonstrating that the hypoth-

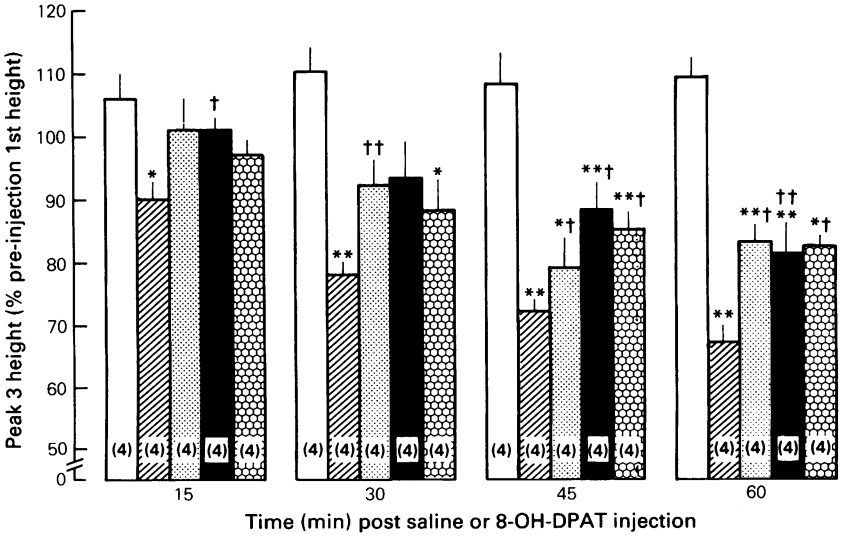

Figure 4 The effect of 0.9% w/v NaCl solution (saline 1 ml kg^{-1} i.v., open columns) 8-hydroxy-2-(*n*-dipropylamino) tetralin (8-OH-DPAT, 0.1 mg kg^{-1} i.v., hatched columns) on the height of peak 3 recorded in this suprachiasmatic nucleus (SCN) 15, 30, 45 and 60 min after their injection. The effect of administration of methiothepin (1 mg kg^{-1} i.v., stippled columns) ritanserin (0.2 mg kg^{-1}, solid columns) TVX Q7821 (1 mg kg^{-1} i.v., honeycombed columns) 5 min before an injection of 8-OH-DPAT (0.1 mg kg^{-1} i.v.,) is also shown. Each column represents the mean of 4 observations and the s.e.mean is shown by the vertical lines. $*P<0.05$, $**P<0.01$ compared to saline. $\dagger P<0.05$, $\dagger\dagger P<0.01$ compared to 8-OH-DPAT.

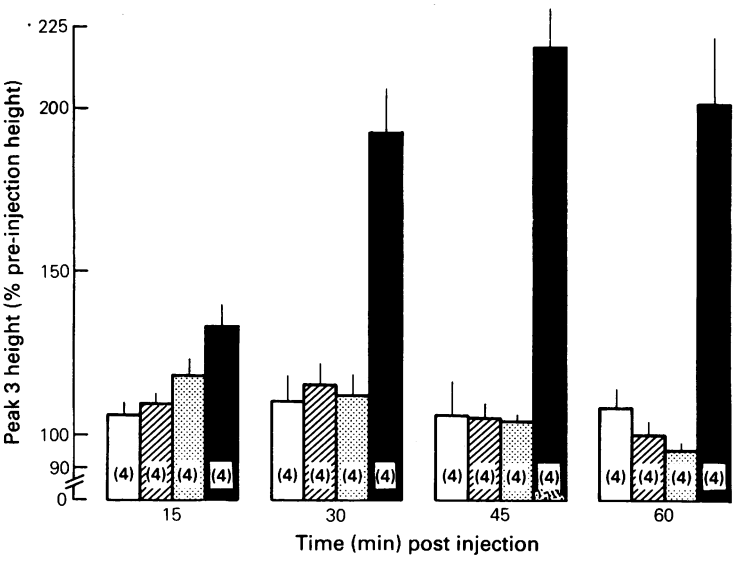

Figure 5 The effect of 0.9% w/v NaCl solution (saline 1 ml kg^{-1}, open columns) and idazoxan (0.1 mg kg^{-1} i.v., hatched columns; 0.2 mg kg^{-1} i.v., stippled columns and 0.5 mg kg^{-1}, i.v., solid columns) on peak 3 height recorded in the suprachiasmatic nucleus. Each column represents the mean and the s.e.mean is shown by the vertical lines. The number of animals in each group is shown in parentheses at the bottom of each column.

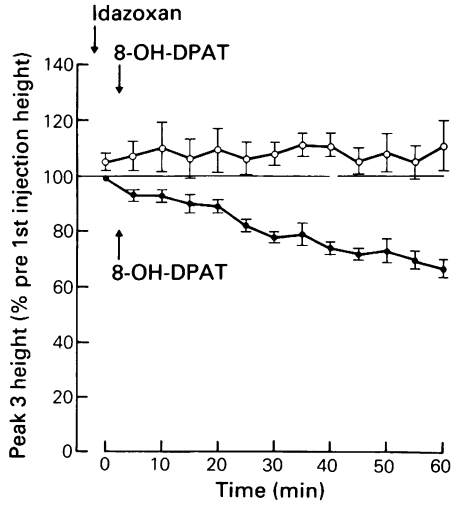

Figure 6 The effect of idazoxan (0.2 mg kg^{-1} i.v., ○, $n = 4$) 5 min before 6-hydroxy-2-(*n*-dipropylamino) tetralin (8-OH-DPAT, 0.1 mg kg^{-1} i.v.) and the effect of 8-OH-DPAT (0.1 mg kg^{-1} i.v., ●, $n = 4$) on peak 3 height recorded in the suprachiasmatic nucleus (SCN) of the chloral hydrate anaesthetized rat. Each point represents the mean and the s.e.mean is shown by the vertical lines.

ermic response to 8-OH-DPAT in the rat was attenuated by TVX Q 7821 even though this compound alone produced a decrease in core temperature. In recent electrophysiological studies in the rat hippocampus TVX Q 7821 had the properties of a partial agonist (Martin *et al.*, 1986). Low ejection currents of TVX Q 7821 antagonized 8-OH-DPAT-induced inhibition of neurone firing whereas higher ejection currents suppressed neurone firing as well as attenuating responses to 8-OH-DPAT. TVX Q 7821 may therefore be a partial agonist at the 5-HT_{1A}-receptor and the data presented here support that view. We do not discount the possibility, however, that TVX Q 7821 may be an agonist at another receptor. In addition, although it appears that TVX Q 7821 only attenuated the response to 8-OH-DPAT, when the effect of TVX Q 7821 alone is taken into account, the effects of 8-OH-DPAT may indeed be completely blocked by TVX Q 7821. Such a result would be in accordance with its proposed 5-HT_{1A}-antagonist properties (Goodwin *et al.*, 1986; Martin *et al.*, 1986) already mentioned. Therefore, our results suggest the involvement of 5-HT_{1A}-receptors in the decreased release and metabolism of 5-HT in the SCN following i.v. administered 8-OH-DPAT.

In the final series of experiments we attempted to look for an involvement of other neurotransmitter receptors in the response produced by 8-OH-DPAT.

Evidence in the literature has suggested that α_2-adrenoceptors may be involved in the cardiovascular responses to peripheral administration of 8-OH-DPAT (Fozard & McDermott, 1985). In addition, the presence of inhibitory α_2-adrenoceptors on 5-hydroxytryptaminergic nerve terminals has also been demonstrated in rat brain cortex (Göthert *et al.*, 1981), and hypothalamus (Galzin *et al.*, 1984). Both these groups have shown that α_2-adrenoceptor agonists decrease [^{3}H]-5-HT release from pre-loaded tissues using *in vitro* methodology.

At low doses (0.1 and 0.2 mg kg^{-1} i.v.) idazoxan did not affect the extracellular level of 5-HIAA. However, at the highest dose that we used (0.5 mg kg^{-1} i.v.) there was a dramatic increase in the size of peak 3, which suggested that 5-HT release and metabolism had increased following administration of idazoxan. At these doses (0.1–0.5 mg kg^{-1} i.v.) idazoxan has been found to be a selective α_2-adrenoceptor antagonist (Doxey *et al.*, 1983). Therefore, on the basis of our data, we suggest that blockade of central α_2-adrenoceptors can increase 5-HT release and metabolism, but, we cannot say where these receptors are located from the present experiments. Thus, although our data appear to contradict the *in vitro* findings of Galzin *et al.*, (1984) that idazoxan does not increase 5-HT release in the hypothalamus, they support the earlier data of Göthert & Hüth (1980), who suggested a physiological role for presynaptic α_2-adrenoceptors in the control of 5-HT release. In our *in vivo* experiments the animal is intact, allowing adrenoceptor mediated inhibition of 5-HT release to occur at cell bodies or nerve terminals in contrast to slice preparations where only nerve terminal receptors can be studied.

Idazoxan also completely blocked the effects of 8-OH-DPAT on peak 3 recorded in the SCN at a dose which had no effect on extracellular 5-HIAA levels (0.2 mg kg^{-1} i.v.). We do not know of any data demonstrating that idazoxan inhibits 8-OH-DPAT binding to the 5-HT$_{1A}$ binding site. Data from isolated tissue experiments (guinea-pig ileum, rat vas deferens) have not shown any direct agonist effects of 8-OH-DPAT on α_2-adrenoceptors (Fozard & McDermott, 1985). However, it has been demonstrated that idazoxan does block the effects of i.v. 8-OH-DPAT on rat blood pressure (Fozard & McDermott, 1985).

On the basis of these data, it is tempting to speculate that those effects of 8-OH-DPAT on 5-HT release and metabolism described here involve not only an agonist action of 5-HT$_{1A}$-receptors but also involve α_2-adrenoceptor stimulation. However, it remains to be determined whether this is mediated by a direct effect on α_2-adrenoceptors, though this seems unlikely.

To summarize, our data demonstrate that intravenously administered 8-OH-DPAT decreases 5-HT metabolism in the SCN *in vivo* and that is probably associated with decreased 5-HT release. This effect was blocked by the 5-HT$_{1A}$-receptor ligand TVX Q 7821 and the α_2-adrenoceptor antagonist idazoxan. In addition, methiothepin (5-HT$_1$ and 5-HT$_2$-receptor antagonist) attenuated the response. However, the specific 5-HT$_2$-receptor antagonist ritanserin did not appear to block the effects of 8-OH-DPAT. We conclude, therefore, that the decrease in 5-HT metabolism produced by peripherally administered 8-OH-DPAT involves 5-HT$_{1A}$-receptors and α_2-adrenoceptors. In addition these data are consistent with those of Goodwin *et al.*, (1986) and Martin *et al.*, (1986) who found that TVX Q 7821 is a partial agonist at 5-HT$_{1A}$-receptors. We have previously demonstrated that 8-OH-DPAT injected into the SCN or dorsal raphé has no effect on 5-HT metabolism in the SCN (Marsden & Martin, 1985), therefore the site at which 8-OH-DPAT exerts its inhibitory effect on 5-HT metabolism in the SCN remains unclear.

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