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A microdialysis study of the *in vivo* release of 5-HT in the median raphe nucleus of the rat

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1 The present study has examined several characteristics of the release of 5-HT in the median raphe nucleus in terms of its dependence of nerve impulse, provenance of a vesicular storage fraction as well as the regulatory role played by 5-HT_{1A} receptors.

2 Tetrodotoxin (1 μM) and reserpine (5 mg kg^{-1}, i.p.) virtually suppressed the output of 5-HT.

3 The administration of EEDQ (10 mg kg^{-1}, i.p.) did not alter the basal release of 5-HT but abolished the reduction of 5-HT release induced by 8-OH-DPAT (0.1 mg kg^{-1}, s.c.).

4 The perfusion of 1 – 100 μM of 8-OH-DPAT or the novel 5-HT_{1A} agonist BAY × 3702 decreased the efflux of 5-HT, whereas the perfusion of the 5-HT_{1A} antagonist WAY-100635 failed to alter 5-HT release.

5 The decrease in dialysate 5-HT induced by 100 μM 8-OH-DPAT was reversed by the concurrent perfusion of 100 μM WAY-100635. Also, the perfusion of 100 μM WAY-100635 for 2 h inhibited partly the reduction of 5-HT release evoked by the systemic administration of 8-OH-DPAT (0.1 mg kg^{-1}).

6 These results indicate that extracellular 5-HT in the median raphe nucleus is stored in vesicles and released in an impulse-dependent manner. Also, the basal release of 5-HT in the median raphe nucleus does not appear to be under the tonic control of somatodendritic 5-HT_{1A} receptors by endogenous 5-HT. Instead, this feedback mechanism seems to be triggered when an excess of the transmitter or a 5-HT_{1A} agonist is present in the extracellular space of the median raphe nucleus.

Keywords: 5-HT; 5-HT_{1A} receptors; reserpine; TTX; EEDQ; 8-OH-DPAT; BAY × 3702; WAY-100635; median raphe nucleus; microdialysis

Introduction

The dorsal and median raphe nuclei (DRN and MRN, respectively) provide the vast majority of axonal processes of serotonin (5-hydroxytryptamine, 5-HT) cells that innervate the forebrain regions of the central nervous system (CNS) of the rat (Azmitia & Segal, 1978; Imai *et al*., 1986; Jacobs & Azmitia, 1992). However, the pattern of distribution of 5-HT fibres to the forebrain differs between the DRN and the MRN. Thus, whereas 5-HT cells within the DRN innervate mainly brain structures related to motor activity such as the basal ganglia, those in the MRN project preferentially, though not exclusively, to limbic regions such as the medial septum and hippocampus (Bobillier *et al*., 1975; Azmitia & Segal, 1978; Köhler *et al*., 1982; Imai *et al*., 1986; Jacobs & Azmitia, 1992; McQuade & Sharp, 1997).

The MRN of the rat contains about 1100 serotonergic neurons, which represents less than one tenth of those observed in the DRN (Jacobs & Azmitia, 1992). In addition, the axons of 5-HT cells of the MRN are thicker and have larger varicosities compared to serotonergic cells in the DRN (Kosofsky & Molliver, 1987; Mamounas *et al*., 1991). Both nuclei also show differences in the sensitivity to neurotoxins (Mamounas & Molliver, 1988; Blier *et al*., 1990), agonists at somatodendritic 5-HT_{1A} autoreceptors (Sinton & Fallon, 1988; Hillegaart *et al*., 1990; Invernizzi *et al*., 1991; Casanovas *et al*., 1997) and in the response to aversive stimuli (Dilts & Boadle-Biber, 1995; Adell *et al*., 1997). However, other studies have shown that the response to neurotoxins (Hensler *et al*., 1994; Gartside *et al*., 1996) and the sensitivity to 5-HT_{1A} agonists or selective 5-HT reuptake inhibitors (Hajós *et al*., 1995) is virtually identical for both nuclei.

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Although several studies have described the existence of a somatodendritic release of 5-HT within the boundaries and in the vicinity of the MRN, the precise origin of this extracellular pool of the transmitter is not well known. On the one hand, a release from cell bodies and/or dendrites has been suggested (Adell *et al*., 1993; Bosker *et al*., 1994). Alternatively, 5-HT can also be released from the proximal portion of axonal processes, recurrent collaterals and/or projections from other 5-HT neurones. Regardless of its origin, the extracellular level of 5-HT in the raphe region is of crucial importance because it can control the activity of serotonergic neurones and the terminal release of 5-HT through its action on somatodendritic 5-HT_{1A} autoreceptors (Adell & Artigas, 1991; Romero *et al*., 1994).

In the present work we have used the technique of *in vivo* microdialysis in unanaesthetized freely moving rats to study some intrinsic characteristics of the release of 5-HT in the MRN such as its dependence on nerve impulse, storage of the transmitter in synaptic vesicles as well as the regulatory role of 5-HT_{1A} receptors.

Methods

Animals

Male Wistar rats (Iffa Credo, Lyon, France) weighing 280 – 360 g at the beginning of the experiments were used. The animals were housed in groups of four per cage until the onset of the experiments and kept under a controlled temperature of 22 ± 2°C and a 12 h lighting cycle (lights on at 07.00 h). After surgery, rats were housed individually. Food and water were always freely available throughout the experiments. All

experimental procedures were in strict compliance with the Spanish legislation and the European Communities Council Directive on 'Protection of Animals Used in Experimental and Other Scientific Purposes' of 24 November 1986 (86/609/EEC).

In vivo *microdialysis*

Concentric microdialysis probes were constructed with 20 mm long 25-gauge stainless-steel tubing (A-M Systems, Everett, WA, U.S.A.). The inflow and outflow lines inserted into the 25-gauge tubing consisted of fused silica capillary tubing of 110 μm OD and 40 μm ID (Polymicro Technologies, Phoenix, AZ, U.S.A.). Dialysis membranes were made from hollow cuprophan fibres with 252 μm OD, 220 μm ID and 5000 daltons molecular weight cutoff (GFE09, Gambro, Lund, Sweden). The total length of the dialysis membrane exposed to the tissue was 1.5 mm. To reduce the void volume, narrow bore (0.15 mm ID) FEP-tubing (Metalant AB, Stockholm, Sweden) was used in the microdialysis system. After an injection of 60 mg kg^{-1} of sodium pentobarbitone, the rats were placed in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, U.S.A.) and a dialysis probe was implanted in the MRN and secured to the skull with anchor screws and dental cement. Stereotaxic coordinates (in mm) from bregma and skull surface were according to the atlas of Paxinos & Watson (1986): AP -7.8, L -2.0 and DV -9.3 with a lateral angle of 13° to avoid obstruction of the cerebral aqueduct. After a recovery period of 20–24 h, rats were placed in a plastic cage with a mounted liquid swivel system (Instech Laboratories, Plymouth Meeting, PA, U.S.A.). The probes were perfused at a constant rate of 0.25 μl min^{-1} with an artificial cerebrospinal fluid (NaCl 125 mM, KCl 2.5 mM, $MgCl_2$ 1.18 mM and $CaCl_2$ 1.26 mM), containing 1 μM of citalopram (H. Lundbeck A/S, Copenhagen-Valby, Denmark). The dialysate level of 5-HT represents a balance between release and reuptake of the transmitter. With the partial blockade of the reuptake process by the addition of citalopram to the perfusion fluid (Chen & Reith, 1994), the concentration of 5-HT in dialysate samples reflects mainly the release of the transmitter. Dialysate samples of 5 μl were collected at 20 min intervals into polypropylene microcentrifuge vials. After an initial 1 h sample of dialysate was discarded, three to four samples were collected to establish stable baseline levels of 5-HT.

Biochemical measurements

Dialysate samples were assayed for 5-HT by h.p.l.c. using an Ultrasphere 3 μm column (7.5 cm × 0.46 cm; Beckman, San Ramon, CA, U.S.A.) and determined with an amperometric detector Hewlett-Packard 1049 (Palo Alto, CA, U.S.A.), set at a potential of +0.6 V. The mobile phase consisted of 0.15 M NaH_2PO_4, 1.3 mM octyl sodium sulphate, 0.2 mM EDTA (pH 2.8 adjusted with phosphoric acid) and 27% methanol and was pumped at 0.7 ml min^{-1}.

At the conclusion of the reserpine experiment, rats were killed and their brains were processed according to a method described elsewhere (Adell *et al.*, 1989) to examine the magnitude of 5-HT depletion in whole brain tissue caused by the drug. Briefly, each brain was quickly removed and placed over a cold plate and a coronal cut was made with a blade at the level of the optic chiasm. Each portion of the forebrain rostral to this cut was then homogenized in 700 μl of iced 0.4 M perchloric acid containing 5.0 mM sodium metabisulfite, 8.3 mM cysteine and 0.3 mM EDTA. After centrifugation at 50,000 × *g* (30 min, 4°C) aliquots of the supernatants were analysed for 5-HT.

Reagents and drugs

All the h.p.l.c. reagents were of analytical grade and obtained from Merck (Darmstadt, Germany). Reserpine, 5-HT, the irreversible receptor inactivator *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) and tetrodotoxin (TTX) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). The 5-HT_{1A} agonist (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT) was provided by RBI (Natick, MA, U.S.A.). The novel 5-HT_{1A} agonist *R*-(−)-2-{4-[(chroman-2-ylmethyl)-amino]-butyl}-1, 1-dioxo-benzo[d]isothiazolone hydrochloride (BAY x 3702), the selective 5-HT_{1A} antagonist *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclo-hexanecarboxamide trihydrochloride (WAY-100635) and the selective 5-HT reuptake inhibitor citalopram hydrobromide were generously supplied by Bayer A.G. (Cologne, Germany), Wyeth-Ayerst Research (Princeton, NJ, U.S.A.) and H. Lundbeck A/S (Copenhagen-Valby, Denmark), respectively. Reserpine was dissolved in a small amount of glacial acetic acid and diluted in distilled water. EEDQ was dissolved in 50% ethanol (v/v). 8-OH-DPAT and WAY-100635 were dissolved in 0.9% NaCl and distilled water, respectively.

When administered through the dialysis probe, the drugs were dissolved in the perfusion fluid. Concentrated solutions of TTX, 8-OH-DPAT, BAY x 3702 and WAY-100635 were stored at −80°C and working solutions (1 μM for TTX and 1, 10 and 100 μM for 8-OH-DPAT, BAY x 3702 and WAY-100635) were prepared immediately before the experiments.

Experimental procedures

EEDQ was injected 24 h before the onset of microdialysis. The 5-HT_{1A} agonists and antagonist were perfused in cumulative concentrations as described in the figures. A separate experiment was carried out to study whether the local perfusion of 100 μM of WAY-100635 in the MRN could attenuate the reduction of the release of 5-HT in this nucleus evoked by the injection of 0.1 mg kg^{-1} of 8-OH-DPAT. For this purpose, 100 μM of WAY-100635 was continuously perfused through the dialysis probe. After 2 h of perfusion, 0.1 mg kg^{-1} of 8-OH-DPAT was injected s.c. and dialysate samples were collected for a further 1 h and 40 min. The animals of the control group were injected with 8-OH-DPAT also, but the MRN was perfused with artificial CSF in absence of WAY-100635. All drugs were injected in volumes of 1 ml kg^{-1} body weight. All doses given refer to the free base. The rats of the control groups received the vehicle used to dissolve the corresponding drug.

At the conclusion of the experiments, rats were given an overdose of sodium pentobarbitone and the placement of the dialysis probes was examined by perfusing fast green dye and visual inspection of the probe track after cutting the brain at the level of the MRN. The brains of several rats were removed immediately from the skulls, immersed in 0.9% NaCl at 4°C for 1 min approximately and then stored at −20°C. Each of these brains was cut in a cryostat in the coronal plane at 14 μm. Midbrain sections were then stained with cresyl violet, according to standard procedures, for localization of the probe track.

Statistics

All data are expressed as means ± s.e.mean. Baseline levels of 5-HT were determined averaging the three or four pre-drug samples and, unless otherwise specified, all samples were

expressed as a percentage of the mean baseline. Statistical analysis of the overall response in the different treatment groups was carried out by two-way analysis of variance (ANOVA) for repeated measures with treatment and time as main factors, followed by *post hoc* multiple comparison tests. The effects of reserpine on the tissue levels of 5-HT in the forebrain were analysed by Student's *t*-test. All statistical procedures were performed using the Statistica software for Windows (StatSoft, Inc., Tulsa, OK, U.S.A.). Values of P less than 0.05 were considered statistically significant.

Results

The basal value of 5-HT in dialysate samples of the MRN in the presence of 1 μM of citalopram ($N=52$) was 45.8 ± 3.7 fmol 5 μl^{-1}.

Effect of TTX

The effects of the perfusion of 1 μM TTX through the dialysis probe on dialysate 5-HT in the MRN are shown in Figure 1. This neurotoxin induced a rapid reduction ($P<0.001$) of 5-HT to 7–15% of basal values. When TTX was removed from the perfusion fluid, the concentration of 5-HT in dialysate samples returned shortly to basal values.

Effects of reserpine

At 24 h after the administration of 5 mg kg^{-1} of reserpine, the tissue concentration of 5-HT was significantly reduced ($P<0.001$) to 8% of vehicle-injected rats (13.7 ± 1.0 ng g^{-1} vs 171.3 ± 5.9 ng g^{-1}, respectively). Reserpinized rats also displayed the characteristic closure of eyelids (ptosis) and hunched-back posture (Shore & Giachetti, 1978). With regard to the effect of reserpine on dialysate 5-HT, two-way ANOVA revealed a significant effect of treatment ($P<0.001$), time ($P<0.001$) and a significant treatment × time interaction ($P<0.001$). Reserpine produced a significant long-lasting reduction of dialysate levels of 5-HT shortly after its administration as shown in Figure 2. A transient increase of the release of 5-HT was observed at 20 min after the injection of the vehicle solution and this effect was attenuated by reserpine (Figure 2).

Effects of EEDQ

The administration of 10 mg kg^{-1} of EEDQ failed to alter basal dialysate levels of 5-HT (Figure 3). However, EEDQ blocked the reduction of the levels of 5-HT induced by the injection of 0.1 mg kg^{-1} of 8-OH-DPAT (Figure 3) as revealed

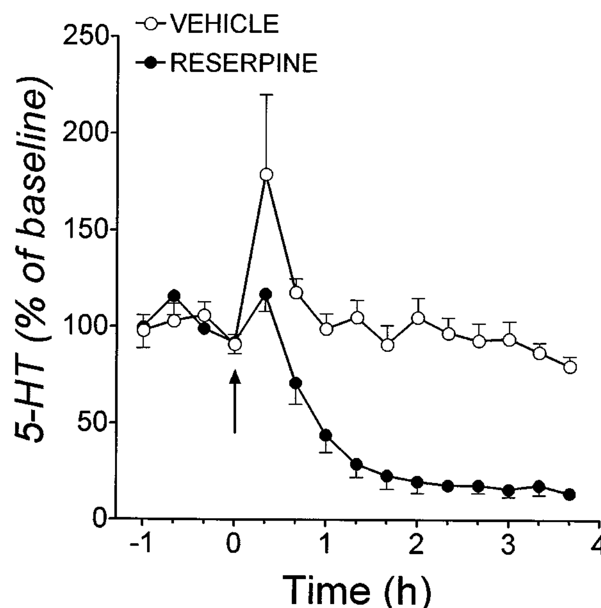

Figure 2 Effect of the injection (arrow) of 5 mg kg^{-1} reserpine on the output of 5-HT. Each point is the mean ± s.e.mean of six rats. See text for statistical details.

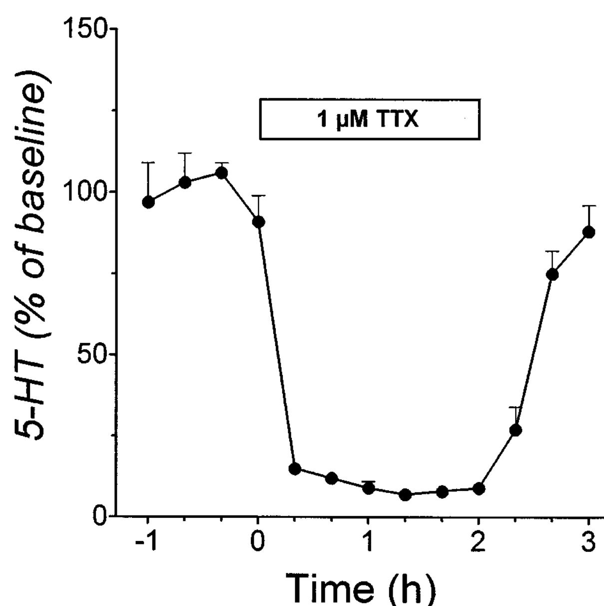

Figure 1 Effect of the perfusion of 1 μM tetrodotoxin on the output of 5-HT. Each point is the mean ± s.e.mean of five rats. See text for statistical details.

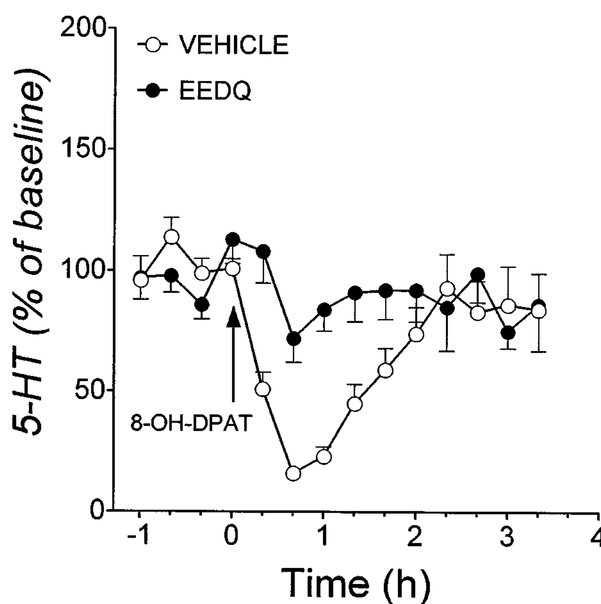

Figure 3 Effect of pretreatment with 10 mg kg^{-1} EEDQ on the suppression of the release of 5-HT induced by the injection (arrow) of 0.1 mg kg^{-1} 8-OH-DPAT. See text for statistical details. Each point and each bar represent the mean ± s.e.mean of five rats.

by a significant effect of treatment ($P<0.05$), time ($P<0.001$) and a significant treatment × time interaction ($P<0.01$).

Effects of 5-HT_{1A} agonists and antagonist

The local perfusion of the 5-HT_{1A} agonists 8-OH-DPAT and BAY × 3702 through the dialysis probe placed in the MRN decreased the level of 5-HT ($P<0.001$) at the concentrations of 10 μM and 100 μM (Figure 4). In contrast, the local perfusion of the 5-HT_{1A} antagonist WAY-100635 at the same range of concentrations did not alter significantly the levels of 5-HT (Figure 4). The perfusion of 100 μM WAY-100635 into the MRN reversed partly ($P<0.01$) the decrease of dialysate 5-HT evoked by 100 μM 8-OH-DPAT (Figure 5). In addition, the local perfusion of 100 μM of WAY-100635 was able to block partly the reduction of the efflux of 5-HT ($P<0.01$) induced by the systemic administration of 0.1 mg kg^{-1} of 8-OH-DPAT (Figure 6).

The injection of 0.3 mg kg^{-1} of WAY-100635 increased significantly ($P<0.01$) dialysate 5-HT in the MRN for 40 min after its administration (Figure 7). However, this effect was not

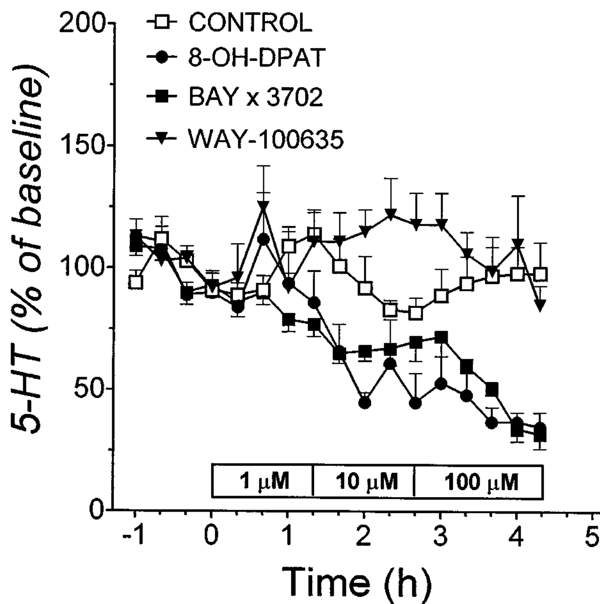

Figure 4 Effect of the local perfusion of artificial CSF (control) and cumulative concentrations of 8-OH-DPAT, BAY × 3702 and WAY-100635 on the output of 5-HT. Each point is the mean ± s.e.mean of seven (control), four (8-OH-DPAT), six (BAY × 3702) and five (WAY-100635) rats. See text for statistical details.

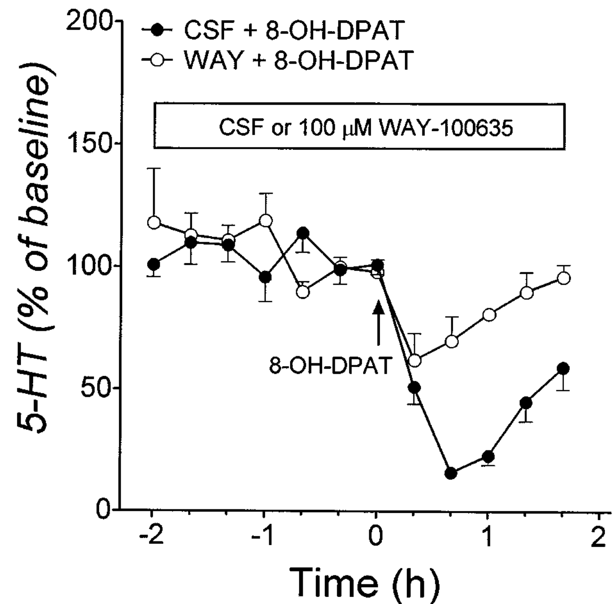

Figure 6 Inhibition by 100 μM WAY-100635 in the median raphe nucleus of the reduction of the output of 5-HT induced by the injection (arrow) of 0.1 mg kg^{-1} 8-OH-DPAT. Each point is the mean ± s.e.mean of five rats. See text for statistical details.

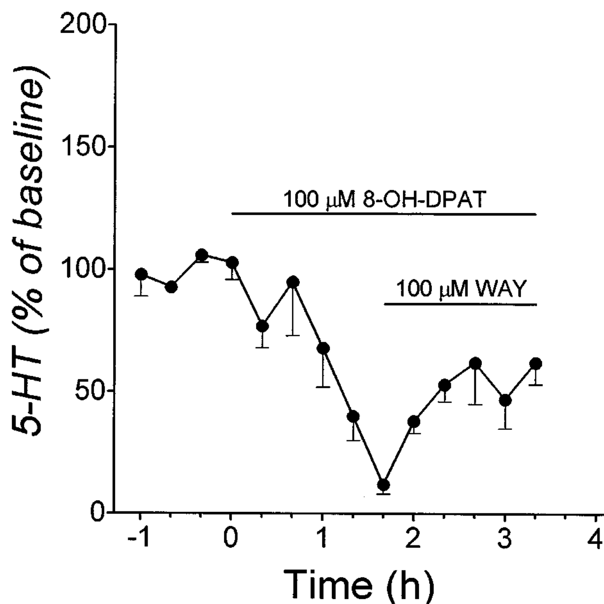

Figure 5 Inhibition by the concurrent perfusion of 100 μM WAY-100635 in the median raphe nucleus of the reduction of the release of 5-HT induced by the perfusion of 100 μM 8-OH-DPAT. Each point is the mean ± s.e.mean of four rats. See text for statistical details.

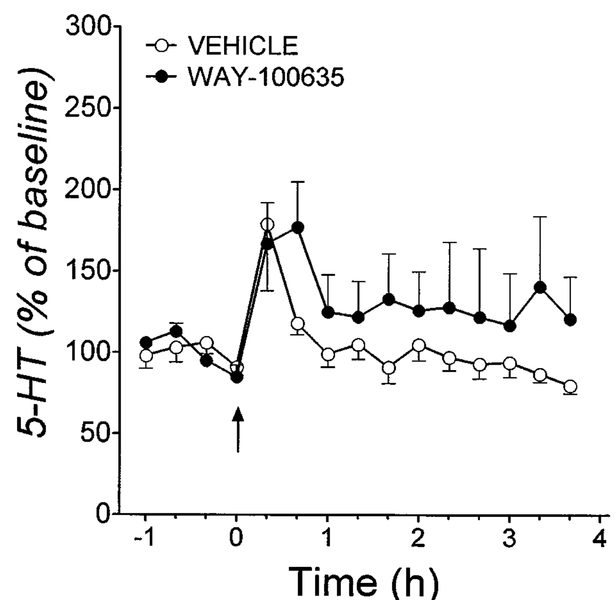

Figure 7 Effect of the systemic administration (arrow) of vehicle or 0.3 mg kg^{-1} WAY-100635 on the output of 5-HT. Each point is the mean ± s.e.mean of six rats. See text for statistical details.

significantly different from that observed after an injection of vehicle.

Discussion

The existence of an *in vivo* release of 5-HT in the raphe nuclei was first described in the brain of the cat using the push-pull technique (Héry *et al*., 1982) and later in the rat brain using intracerebral microdialysis (Adell & Artigas, 1991; Adell *et al*., 1993; Bosker *et al*., 1994; Casanovas & Artigas, 1996; Matos *et al*., 1996). However, the intrinsic mechanisms that regulate the release of 5-HT in the raphe region are not fully understood. In the present study, we have shown that 5-HT release in the MRN is virtually abolished by TTX, which indicates a dependency of nerve impulse. A similar effect of TTX had been already described in the DRN (Matos *et al*., 1996) and in the MRN (Bosker *et al*., 1994).

In addition, the results obtained with reserpine suggest that 5-HT in the MRN is stored in vesicles similarly to what occurs in forebrain regions (Adell *et al*., 1989; Carboni & Di Chiara, 1989; Martin & Artigas, 1992; Heslop & Curzon, 1994). The presence of vesicles in cell bodies and dendrites of 5-HT neurones has been documented in the DRN of the cat (Chazal & Ralston III, 1987). However, to the best of our knowledge, no such studies have been conducted in the raphe nuclei of the rat. In keeping with our findings, it has been described that tissue 5-HT in the DRN is decreased shortly after reserpine (Long *et al*., 1983), which suggests also a vesicular storage pool of this transmitter in this nucleus.

In a previous study carried out in our lab (Adell *et al*., 1993), we found that the *in vivo* release of 5-HT in the vicinity of the DRN and MRN was not affected by TTX and reserpine. Matos and colleagues also showed that the release of 5-HT was not influenced by TTX when the probe was placed in close apposition to but outside the DRN (Matos *et al*., 1996). Therefore, it appears that only the extracellular pool of 5-HT measured within the boundaries of the raphe nuclei is stored in vesicles and released as a consequence of nerve impulse.

The inactivation of 5-HT_{1A} receptors by the alkylating agent EEDQ (Meller *et al*., 1990; Cox *et al*., 1993) failed to affect basal dialysate 5-HT, which would suggest that, under physiological conditions, the release of 5-HT in this nucleus does not tonically activate such receptors. However, it should be taken into consideration that the systemic administration of EEDQ not only inactivates 5-HT_{1A} and other 5-HT receptor subtypes (Pinto & Battaglia, 1993; Gozlan *et al*., 1994; Raghupathi *et al*., 1996) but also other monoaminergic receptors (Norman & Creese, 1986; Nowak *et al*., 1988; Pilc & Vetulani, 1990). Therefore, the possibility that the lack of effect of systemic EEDQ on the basal release of 5-HT in the MRN may be accounted for by a balance of opposing actions on different brain receptors cannot be ruled out.

The systemic injection of 8-OH-DPAT reduced the output of 5-HT in the MRN similarly to what had been previously observed in raphe nuclei (Adell *et al*., 1993; Bosker *et al*., 1994; Bosker *et al*., 1996; Casanovas *et al*., 1997) and the inhibition of this effect by EEDQ would agree with an involvement of 5-HT_{1A} receptors.

The perfusion of 8-OH-DPAT and the novel 5-HT_{1A} agonist BAY $\times$ 3702 (De Vry *et al*., 1998) in the MRN reduced the dialysate level of 5-HT in a concentration-dependent manner. In contrast, previous results have shown that 100 μM of 8-OH-DPAT increased extracellular 5-HT in the raphe nuclei when a 5-HT uptake blocker was absent from the perfusion fluid (Adell *et al*., 1993; Bosker *et al*., 1994). An explanation of such difference is that 8-OH-DPAT has been shown to block the 5-HT uptake mechanism at high concentrations both *in vitro* (Hamon *et al*., 1984; Cheng *et al*., 1993) and *in vivo* (Assié & Koek, 1996). However, when a 5-HT uptake inhibitor is already present in the dialysis fluid, the local perfusion of 8-OH-DPAT acts preferentially upon 5-HT_{1A} receptors thereby decreasing the release of 5-HT. Obviously, the present study does not allow for a comparison between the alleged differences in the sensitivity to 5-HT_{1A} agonists in DRN and MRN (Sinton & Fallon, 1988; Hillegaart *et al*., 1990; Invernizzi *et al*., 1991; Casanovas *et al*., 1997). Nevertheless, it is evident from our results that 5-HT_{1A} autoreceptors in the MRN are functional and play a regulatory role in the control of local 5-HT release.

The local perfusion of the selective 5-HT_{1A} antagonist WAY-100635 (Fletcher *et al*., 1995; Forster *et al*., 1995) did not alter dialysate 5-HT in the MRN. Such a finding is coincident with previous results (Bosker *et al*., 1996) and confirms that WAY-100635 is a silent 5-HT_{1A} antagonist (Fletcher *et al*., 1995; Gartside *et al*., 1995) at the doses tested. This is also consistent with the lack of effect of the inactivation of 5-HT_{1A} receptors by EEDQ on basal dialysate 5-HT in the MRN (this study). This further supports the idea that, under physiological conditions, the extracellular concentration of 5-HT in the MRN is not sufficient to exert a tonic control on its own release through the activation of 5-HT_{1A} autoreceptors. Instead, only when 5-HT or a 5-HT_{1A} agonist is present in excess, an activation of such autoreceptors can be produced, which would lead to a reduction of 5-HT release in the raphe region. This would be in keeping with the hypothesis proposed by Di Chiara (1996) for dopamine neurones that the basal release of the transmitter contributes to the formation of an extracellular pool of the transmitter in the extracellular space, but it is not sufficient to activate synaptic receptors.

The reduction of extracellular 5-HT in the MRN evoked by the local perfusion or the systemic administration of 8-OH-DPAT was reversed by the concurrent perfusion of WAY-100635. The inability of the local application of WAY-100635 in the MRN to prevent completely the reduction of 5-HT release induced by the systemic injection of 8-OH-DPAT is consistent with an involvement of postsynaptic 5-HT_{1A} receptors in this effect. In support of this idea is evidence that suggests a role of postsynaptic 5-HT_{1A} receptors in the control of the activity of serotonergic neurones of the DRN (Blier & de Montigny, 1987; Ceci *et al*., 1994; Romero *et al*., 1994). Alternatively, it is also possible that the concentration of WAY-100635 that actually can interact with 5-HT_{1A} receptors after perfusion was not sufficient to block completely the effect of systemically administered 8-OH-DPAT.

In line with the lack of effect of locally-applied WAY-100635, the systemic administration of this compound *per se* also failed to alter dialysate 5-HT. Other work has also shown that WAY-100635 does not modify the firing of serotonergic neurones of the DRN (Fletcher *et al*., 1995; Forster *et al*., 1995; Gartside *et al*., 1995) or the release of 5-HT in the MRN (Bosker *et al*., 1996) and frontal cortex (Gartside *et al*., 1995). Furthermore, electrophysiological and neurochemical studies have shown that the systemic administration of (S)-UH-301 (Arborelius *et al*., 1995) or non-selective 5-HT_{1A} antagonists such as spiperone (Lum & Piercey, 1988), (−)pindolol (Sharp *et al*., 1989; Romero *et al*., 1996) and (−)propranolol (Kreiss & Lucki, 1994) also failed to alter the 5-HT neuronal firing and/or 5-HT release. However, an increased firing rate was observed in certain cases (Corradetti *et al*., 1996; Fornal *et al*., 1996).

In summary, the present results show that the release of 5-HT in the MRN comes from a vesicular storage and is dependent on nerve impulse although it does not appear to be tonically inhibited by 5-HT_{1A} autoreceptors. This feedback regulation occurs only when an excess of endogenous 5-HT or of a 5-HT_{1A} agonist is present in the extracellular space. The reduction of the dialysate concentration of 5-HT evoked by a 5-HT_{1A} agonist is blocked, though not completely, by the local perfusion of WAY-100635. This suggests a pivotal role for somatodendritic 5-HT_{1A} autoreceptors. However, postsynaptic 5-HT_{1A} receptors may also participate in this effect although to a much lesser extent.

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