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Pharmacology, Biochemistry and Behavior 74 (2003) 587–593
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Behavioural and microdialysis study after neurotoxic lesion of the dorsal raphe nucleus in rats

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Received 7 August 2002; received in revised form 16 October 2002; accepted 30 October 2002

Abstract

The study investigated the effects of a 5,7-dihydroxytryptamine (5,7-DHT) lesion of the dorsal raphe nucleus (DRN) on anxiety-related behaviour and neurochemical correlates in rats. Behaviour was assessed in the elevated plus maze test (X-maze). Lesion of the DRN reduced markedly 5-HT levels in projection areas by at least 60%. Destruction of the serotonergic neurons in the DRN changed neither anxiety-related behaviour on the elevated plus maze, nor aversion-induced 5-HT release in the brain. Exposure of the lesioned rats to the elevated plus maze increased extracellular 5-HT (148%) in the ventral hippocampus similar as in sham-lesioned (162%) and non-lesioned (160%) controls. The results demonstrate that lesioning of 5-HT neurons in the DRN does not abolish totally the control of anxiety-related behaviour.

Keywords: 5,7-dihydroxytryptamine; X-maze; Hippocampus; Dorsal raphe nucleus; Microdialysis; Rat; Serotonin

1. Introduction

The midbrain median and dorsal raphe nuclei are the main origin for ascending and partially overlapping serotonergic projections to brain structures, e.g., hippocampus, striatum, amygdala, and frontal cortex, which are involved in the regulation of anxiety-related behaviour (Azmitia and Segal, 1978). At current knowledge, both raphe nuclei seem to be equally important in this task.

Local administration of 5-HT-1A agonists into the dorsal raphe nucleus (DRN) decreases the firing rate of 5-HT neurons (Verbanac et al., 1996) and causes an anxiolytic-like behaviour in different animal models (File and Gonzalez, 1996; Hogg et al., 1994; Cervo et al., 2000). Application of 5-HT-1A agonists into the median raphe nucleus leads also to an anxiolytic-like behaviour, e.g., in the social interaction test (Andrews et al., 1994) and the conditioned suppression of drinking (Carli and Samanin, 1988).

Local lesions in the CNS are a common tool to interfere with brain structures and neurotransmitter systems, respectively, to assess their relevance for the behaviour and basal brain functions (e.g., File et al., 1979). Lesion of the serotonergic neurons in the origins of the serotonergic system reduces the availability of serotonin in the projection areas. The neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) is used to destroy serotonergic neurons selectively.

Previous studies on the effects of a neurotoxic lesion of the median raphe nucleus did not show a change in anxiety-related behaviour in the social interaction test (File et al., 1979) and in the X-maze (Thomas et al., 2000; Andrade and Graeff, 2001).

Lesioning of the DRN has resulted in remarkably varied findings in the literature, i.e., no changes in the elevated plus maze test (X-maze) (Critchley et al., 1992), but anxiolysis in the social interaction test (File et al., 1979).

The purpose of the present study was to investigate both the behavioural and neurochemical consequences of a 5,7-DHT lesion of the DRN compared to the lesion of the median raphe nucleus (Thomas et al., 2000).

For behavioural assessment, the X-maze introduced by Handley and Mithani (1984) and validated by Pellow et al. (1985) was used. The X-maze test is a reliable method to measure 'anxiety' in rodents (Hogg, 1996; Rodgers and Cole, 1994). The anxiety-related behaviour in the X-maze test was assessed 7–10 days after lesioning.

It has been shown that exposure of rodents to an aversive environment is accompanied by an increase of extracellular 5-HT levels in different brain regions related

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0091-3057/02/$ – see front matter © 2002 Elsevier Science Inc. All rights reserved.
PII: S0091-3057(02)01043-2

to anxiety (Cadogan et al., 1994; Rex et al., 1993a; Voigt et al., 1999).

Therefore, the behavioural testing was combined with neurochemical analysis of 5-HT release using the in vivo microdialysis technique. DRN-lesioned and sham-lesioned rats were placed on the X-maze and extracellular 5-HT levels in the ventral hippocampus were assessed and compared with those of untreated controls. The ventral hippocampus was chosen, since this region is not only involved in the regulation of anxiety-related behaviour, but also receives serotonergic input from both the dorsal raphe and the median raphe, hence allowing the detection of possible compensating mechanisms by the raphe nuclei.

2. Methods

2.1. Animals

Male Sprague–Dawley rats (Schönwalde, Germany) 170 ± 15 g, group housed under a 12-h light–dark schedule with free access to food (Altromin 1326) and tap water were used. All animal experiments were carried out following the 'Principles of laboratory animal care' and the German Law on Protection of Animals. The experimental protocol was approved by the Landesamt für Arbeitsschutz, Gesundheitsschutz und technische Sicherheit Berlin.

2.2. Drugs

Desipramine (Research Biochemicals International, MA, USA) and 5,7-DHT (Research Biochemicals International) were freshly dissolved in 0.9% saline. All other chemicals were obtained from Merck, Darmstadt, Germany. Artificial CSF consisted of 125 mM NaCl, 2.5 mM KCl, 27 mM $NaHCO_3$, 0.5 mM $NaH_2PO_4 \cdot H_2O$, 2.4 mM $Na_2HPO_4 \cdot 2H_2O$, 0.5 mM Na_2SO_4, 1.0 mM $MgCl_2 \cdot 6H_2O$, and 1 mM $CaCl_2 \cdot 2H_2O$ adjusted to pH 7.4.

2.3. 5,7-DHT lesion

Rats were pretreated with desipramine (25 mg/kg ip, 30 min prior to lesion) to prevent destruction of catecholaminergic terminals and anaesthetized with sodium-pentobarbitone (50 mg/kg ip). 5,7-DHT (10 μg in 0.5 μl over 4 min) was injected stereotaxically into the DRN. The coordinates were $A = 0.7$, $L = 4.65$ from interaural zero, $V = 6.05$ with an angle of 35° (König and Klippel, 1963). Sham-lesioned rats received equal volume of saline. According to the experimental procedure, rats were observed after surgery for 7–10 days.

2.4. Behavioural testing

All tests were performed in a sound-proofed chamber. A video camera was suspended above the apparatus and connected with a computer-automated tracking system (VideoTrack, CPL, UK) to record and analyse the behaviour.

One hour before the test, the animals were transferred in their home cages from the animal unit to the observation chamber. The behavioural experiments were performed using an X-maze illuminated with 210 lx on the surface of the open arms, 190 lx in the centre, and 160 lx in the closed arms. The X-maze was 60 cm high with four arms (43 × 17 cm), with a wall on two opposite arms (height: 15 cm). The rats were placed in the centre of the X-maze facing a corner, therefore allowing the animals to choose between the open and the closed arms for the first entry. The experiments were performed for 10 or 20 (combination with the microdialysis) min (Pellow et al., 1985). The behavioural parameters measured were the number of entries into the closed arms (CE) and the open arms, the time spent in the open arms. The percentage of entries into open arms of all entries and the percentage of time spent in the open arms of the total test time was calculated. The number of entries into all four arms (TE) and the entries into the closed arms (CE) were determined as measures of locomotor activity.

2.5. Microdialysis procedure

For the microdialysis experiments, a microdialysis guide cannula (CMA 12, CMA-medicine, Sweden) was implanted stereotaxically in the ventral hippocampus (AP = 5.8 mm, $L = 4.6$ mm from bregma and 4.5 mm from the skull surface, Paxinos and Watson, 1997) immediately after lesioning and fixed to the skull with stainless steel screws and cold curing resin (Technovit, Kulzer, Germany). Rats were allowed to recover from surgery for 8–10 days. During this time, the rats were kept individually in cylindrical cages (35 cm diameter, 40 cm side), which served as home-cages during the microdialysis experiments.

The microdialysis probe was inserted through the guide cannula at 1400 h the day before the experiment. The microdialysis membranes (CMA 12) were 3 mm long, with an outer diameter of 0.5 mm and a 20,000 molecular weight cutoff. According to our in vitro calibration test, the relative recovery was about 25% for 5-HT. Overnight perfusion with artificial CSF was performed with a flow rate of 0.7 μl/min. On the day of the experiment, the flow rate was increased to 1 μl/min allowing stable levels for 5-HT to be reached approximately within 2 h thereafter. This flow rate allowed the collection of 20-μl samples every 20 min into microvials. To each sample, a preload of 5 μl 0.1 M perchloric acid was added, resulting in a total sampling volume of 25 μl.

Dialysates were analysed by reverse-phase high-performance liquid chromatography with electrochemical detection (HPLC-ECD). The detection limit was 0.25 fmol/5 μl. Samples were injected directly into a valve with a 5-μl loop (Rheodyne, USA). The sample was separated by a 100-mm column (SepStik microbore column MF-8494, 3 μm, i.d. 1

mm; BAS, USA). The mobile phase contained 0.15 mM NaH_2PO_4, 1 mM EDTA, 0.23 mM OSA, and 4% isopropanol at pH 3.7. Mobile phase was delivered by a LC-10AD pump (Shimadzu Europe, Germany) with external pulse dampener at flow rate of approximately 50 μl/min. Serotonin was oxidized at 0.650 V (UniJet CC5 electrochemical cell, BAS; and Decade electrochemical detector, Antec, Netherlands).

2.6. Experimental design

2.6.1. X-maze test

Seven days after surgery, 5,7-DHT-lesioned, sham-lesioned and untreated rats were placed on the X-maze and observed for 10 min (divided into two time bins).

2.6.2. 5-HT release during exposition to the X-maze

In 5,7-DHT-lesioned and sham-lesioned animals not habituated to the X-maze, four 20-min baseline samples were collected in the home cage 8–10 days after surgery. Thereafter, the rats were placed on the X-maze for 20 min with the dialysis continued. The behaviour was recorded. The rats were then returned to the home cage, and microdialysis sampling was continued for a further 120 min.

2.7. Histology

At the end of the behavioural and microdialysis experiments, the correct placements of the microinjection cannula (Fig. 1) and microdialysis probe were verified histologically by an observer unaware of the experimental data. Animals with a placement of the cannula tip or microdialysis probe out of the target area were excluded from statistical analysis.

Fig. 1. Example of histological verification of the cannula placement for microinjection of 5,7-DHT into the dorsal raphe nucleus. Coordinates were $A = 0.7$, $L = 4.65$ from an interaural zero, $V = 6.05$ with an angle of 35°.

2.7.1. 5-HT content

Analysing the 5-HT content in the lesion area and projection areas controlled the success of the lesion. 5,7-DHT-lesioned and sham-lesioned rats were sacrificed by decapitation 18 days after surgery. The brains were quickly removed, immediately frozen, and stored in fluid nitrogen.

Later, the brains were dissected and the ventral hippocampus, the frontal cortex and the hypothalamus region gently removed.

The tissue samples were homogenized at 4 °C by ultrasonication in 30–40 volumes of N_2-saturated deionised water. Part of the aliquot of the homogenate (200–300 μl) was added to an equal volume of 0.2 M perchloric acid containing 0.8 mM $NaHSO_3$ and centrifuged at $25{,}000 \times g$ at 4 °C for 15 min. The supernatant was used for the measurement of 5-HT (ng/mg tissue).

2.8. Statistics

In the X-maze test number of entries and time spent on the open arms were expressed as percent of total entries or total time, respectively. In the microdialysis experiments, data from three dialysates before exposure to the X-maze were averaged for each animal and the mean regarded as baseline and set as 100%. All other individual data were calculated as percent of this individual baseline.

Based on the results of the Kolmogorov–Smirnov test for normality, the behavioural and microdialysis data were analysed using the nonparametric Kruskal–Wallis ANOVA on ranks followed by Dunn's method. Mann–Whitney rank sum test or *t* test was used where appropriate. Data were presented as box plots showing the medians and means with the 25 and 75 percentile (median, 25:75) or as mean ± S.E.M. where appropriate. A probability level of $P < .05$ was regarded as significant.

3. Results

3.1. X-maze test

In the first experiment, the behaviour of 5,7-DHT-lesioned rats did not differ from sham-lesioned and untreated rats tested in the X-maze. Neither percent time spent on the open arms [$H(2) = 2.454$, $P = .293$] nor percent entries into the open arms [$H(2) = 2.77$, $P = .25$] were changed in 5,7-DHT-lesioned rats when compared with sham-lesioned rats (Fig. 2). There were no differences in locomotor activity indicated by total arm entries [$H(2) = 2.288$, $P = .319$] nor by closed arm entries [$H(2) = 1.683$,

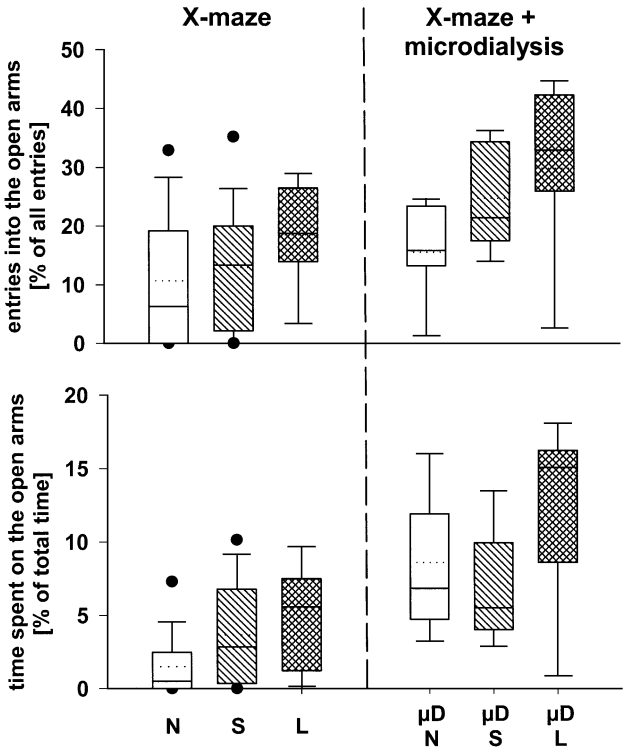

Fig. 2. Percent time spent on the open arms and percent entries into the open arms (median, 25:75 percentile) of the X-maze in 5,7-DHT-lesioned rats (L) compared with sham-lesioned (S) and non-lesioned controls (N) on Day 7 after surgery (10 min) and of lesioned rats (µD L) compared with sham-lesioned (µD S) and non-lesioned controls (µD N) with a microdialysis probe implanted on Days 8-10 post-lesion (20 min) (number of animals, see Table 1).

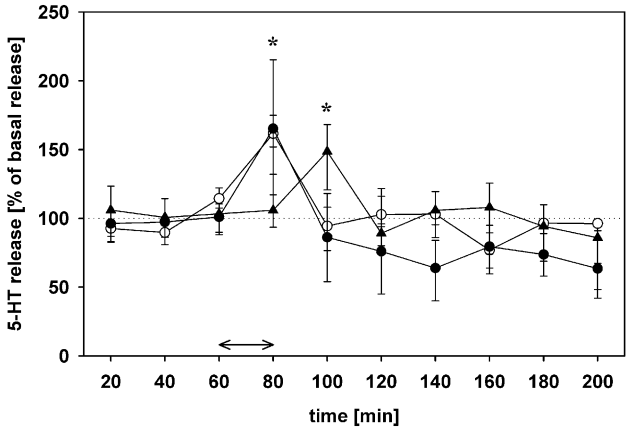

Fig. 3. Effect of exposure to the X-maze (20 min, ↔) on hippocampal dialysate 5-HT (median, 25:75 percentile) in 5,7-DHT-lesioned ($n=6$, ▲), sham-lesioned rats ($n=7$, ○), and non-lesioned rats ($n=7$, ●). Increases in extracellular 5-HT levels were analysed by Kruskal–Wallis ANOVA on ranks followed by Dunn's method, * $P<.05$.

P=.431] (Table 1). Dividing the observation time into two time bins (2 × 5 min) did not reveal differences in the behaviour (data not shown).

3.2. 5-HT release during exposition to the X-maze

Hippocampal extracellular 5-HT levels were increased directly after placing the animal on the X-maze ($H(2)=8.488$, P=.014) to 162% (117:269) ($Q=2.455$, $P<.05$) and 160% (128:241) ($Q=2.584$, $P<.05$) of baseline in sham-lesioned and non-lesioned rats, respectively (Fig. 3). In rats with a 5,7-DHT lesion, the extracellular 5-HT levels were increased in the sample after the exposure (+20 min) of the animal to the X-maze (144, 120:148% of baseline) ($H(2)=8.051$, P=.018; $Q=2.713$, $P<.05$) (Fig. 3). Similar to the first experiment, 5,7-DHT-lesioned, sham-lesioned, and non-lesioned rats exhibited no differences in percentage of time spent on [$H(2)=2.451$, P=.294], percentage of entries into [$H(2)=2.77$, P=.25] the open arms, and number of total entries during the 20 min of exposure to the X-maze test (Fig. 2). However, the lesioned rats showed a tendency to enter the open arms more often [$H(2)=4.711$, P=.095].

3.3. 5-HT content

In 5,7-DHT-lesioned rats ($n=10$), 5-HT tissue content was significantly reduced by 61.9% in the ventral hippocampus ($P<.05$, $t=4.086$, df 18), 80.6% in the frontal cortex ($P<.05$, $t=9.597$, df 18), and 53.5% in the hypothalamus ($P<.05$, $t=3.239$, df 18) compared with sham-lesioned controls ($n=10$) (Table 2).

Table 1
Number of total arm entries and closed arm entries in the X-maze

	Arm entries	Non lesioned	Sham lesioned	5,7-DHT lesioned
X-maze (10 min)	total	14 (3:16)	15 (13:21)	17 (11:21)
	closed	12 (2:16)	14 (12:18)	12 (9:17)
		$n=11$	$n=11$	$n=8$
Microdialysis + X-maze (20 min)	total	27 (17:29)	27 (20:28)	31 (26:37)
	closed	19 (13:23)	21 (15:26)	22 (21:26)
		$n=7$	$n=7$	$n=6$

Data are expressed as median, 25:75 percentile of 5,7-DHT-lesioned rats, sham-lesioned, and non-lesioned controls.

Table 2
5-HT content

	Sham lesioned ($n=10$)	5,7-DHT lesioned ($n=10$)
Ventral hippocampus	307 ± 37	117 ± 22 *
Frontal cortex	474 ± 33	92 ± 21 *
Hypothalamus	505 ± 49	235 ± 42 *

5-HT concentration in three brain areas expressed as mean ± S.E.M. (for further explanations, see Methods). Differences between the groups are based upon t test.

* $P<.05$.

4. Discussion

Our experiments were focussed on the question of which role the DRN plays in the regulation of anxiety-related behaviour compared to the median raphe nucleus. The DRN is a major source of 5-HT neurons and projects to most parts of the forebrain as well as receives serotonergic afferents from the median raphe nucleus in the rat (Azmitia and Segal, 1978; Stamford et al., 2000). Neurons originating in the DRN project to brain structures involved in the regulation of anxiety, e.g., the hippocampus and the frontal cortex (Azmitia and Segal, 1978). Serotonergic neurons of the median raphe nucleus project to the hippocampus, too, but also solely to the hypothalamus and bulbus olfactorius (Azmitia and Segal, 1978; Vertes et al., 1999).

Studies exploiting microinjections of drugs acting at presynaptic 5-HT receptors into the DRN showed that a decreased firing rate of 5-HT neurons is accompanied with an anxiolytic-like behaviour in models of anxiety, e.g., the Geller–Seifter test (Cervo et al., 2000) and the social interaction test (Picazo et al., 1995). In general, these experiments confirmed that a drug-induced anxiolytic-like behaviour is associated with a reduced 5-HT release in projection areas of the DRN, as the hippocampus and the frontal cortex, while aversive conditions increase the 5-HT release in these areas (Marsden et al., 1995; Rex et al., 1993a; File et al., 1993; Wright et al., 1992).

Similar effects were achieved if 5-HT_{1A} agonists were applied into the median raphe, leading also to an anxiolytic-like behaviour, e.g., in the social interaction test (Andrews et al., 1994) and the conditioned suppression of drinking (Carli and Samanin, 1988).

Additionally, it has been shown that rat strains differing in the natural anxiety-related behaviour also differ in the function of the serotonergic system. Rats displaying a ‘‘low-anxiety’’ behaviour had lower 5-HT tissue levels in the brain and a diminished 5-HT release during exposure to the X-maze compared to ‘‘high-anxiety’’ rats (Bert et al., 2001; Rex et al., 1999).

It seems that both dorsal raphe and median raphe are implicated in the regulation of anxiety-related behaviour. To our knowledge a ‘differentiation’ in the functional importance of the two raphe nuclei has not described yet.

In an earlier study, we could show that the destruction of 5-HT neurons in the median raphe nucleus has no effect on the anxiety-related behaviour of rats, though the 5-HT levels in the hippocampus were drastically reduced (Thomas et al., 2000). A suspected compensation of the loss of the median raphe nucleus-derived serotonergic neurons by the DRN could be excluded by microdialysis experiments showing that the typical aversion-induced increase in hippocampal 5-HT release was totally abolished and a fenfluramine-induced stimulation of 5-HT release failed (Thomas et al., 2000).

Based on the open question about the functional importance of the median and DRN, respectively, our experiments were planned. The consequences of a neurotoxic destruction of 5-HT neurons in the DRN were investigated behaviourally and neurochemically to assess the consequences of the loss of a main source of serotonin in the CNS. We applied the same basic experimental design as in the assessment of the effects of the median raphe lesion (Thomas et al., 2000) to be able to compare the results.

No changes in anxiety-related behaviour were seen approximately one week after the destruction of the serotonergic neurons in the DRN.

Implantation of the microdialysis probes and surgery-related stress did not change the rats’ behaviour significantly. However, in our experiments, we found a tendency of anxiolytic-like behaviour on exposure to the X-maze in the animals with a microdialysis probe implanted. It could be argued that the implantation of the microdialysis probe and the subsequent isolation in single cages, which is supposedly a strong stressor in rodents, would it make more likely to see an anxiolytic-like consequence of the lesioned DRN. On the other hand, the necessary handling of the animals as post-surgery care and the microdialysis procedure prior exposure to the X-maze may have caused the slightly increased percentage of entries into the open arms and more time spent in the open arms in the microdialysis rats, seen also in the non-lesioned and sham-lesioned rats, compared to rats exposed solely to the behavioural test. Similar effects have been described in a microdialysis experiment during the social interaction test (Cadogan et al., 1994).

In a previous study, it was found that greater depletions of serotonin resulted in hypoactivity, rather than anxiolysis (Deakin et al., 1979). Determination of the number of closed arm entries, a reliable measure of activity in the plus maze, has revealed no change in the locomotor activity between sham-lesioned and lesioned rats in our study.

In contrast to the lesion of the MRN, we still found the typical increase in extracellular 5-HT seen in anxiety-related brain structures as the hippocampus when animals were exposed to aversive conditions in various animal models of anxiety (Rex et al., 1993a; Voigt et al., 1999; Matsuo et al., 1996).

Our results showed somewhat surprisingly that the magnitude of the aversion-induced increase in hippocampal 5-HT release was also not affected compared to sham-lesioned rats, although hippocampal 5-HT content was reduced by at least 60% in the lesioned rats. The efficacy of the lesion was comparable to lesion-induced losses in the literature (Hölzel et al., 1984; File et al., 1979; Hellweg et al., 2001) and to the lesion of the median raphe nucleus (Thomas et al., 2000).

Our results are in accordance with the marked loss of 5-HT neurons (for review, see Tabatabaie and Dryhurst, 1998) and NADH fluorescence as an important parameter of cellular metabolism (Rex et al., 2001) after application of 5,7-DHT. Altogether, in our study, the lesion of the DRN had no robust effect either on the anxiety-related behaviour or on the magnitude of the aversion-induced increase in extracellular 5-HT, whereas a lesion of the median raphe totally abolished the increased 5-HT release. These findings

suggest that the loss of 5-HT neurons in the DRN, but not of the MRN, can be compensated.

Both raphe nuclei have partially overlapping projection areas as the hippocampus and the median raphe nucleus projects additionally to the DRN, but not vice versa.

The present study suggests that the loss of DRN projection into the hippocampus may be functionally compensated by the MRN, since the loss of 5-HT and consequently of physiological function of the DRN is counterbalanced. It could be shown that a partial defect of the 5-HT system is not sufficient to unsettle the anxiety-related behaviour in group-housed and unstressed animals. Our results are supported by previous studies showing that just a reduction of 5-HT release by pharmacological manipulations does not necessarily induce an anxiolytic-like behaviour in an animal test of anxiety (File et al., 1987; Rex et al., 1993b). Thus, it is not possible to demonstrate a simple relationship between anti-aversive behaviour and inhibition in 5-HT release.

It has be taken into account that other transmission systems located in the DRN and/or in the projection area might also contribute to the compensation effect.

In conclusion, our results show how complex the relation and interdependence between central 5-HT activity and anxiety-related behaviour is.

Pharmacological findings in the literature are speaking in favour of a predominant role of the dorsal raphe. However, our results show that under pathophysiological conditions, e.g., the loss of 5-HT neurons in the dorsal raphe, and associated long-term changes in 5-HT activity, the dorsal raphe can be functionally completely compensated.

Based on these results, it is not certain that the dorsal raphe is the 'primus inter pares' of the raphe nuclei in the regulation of anxiety-related behaviour in rodents.

Acknowledgements

We are indebted to Mrs. Cornelia Tanneberger for the excellent technical assistance in the microdialysis experiments.

References

Andrade TG, Graeff FG. Effect of electrolytic and neurotoxic lesions of the median raphe nucleus on anxiety and stress. Pharmacol Biochem Behav 2001;70:1–14.

Andrews N, Hogg S, Gonzalez LE, File SE. 5-HT-1A receptors in the median raphe nucleus and dorsal hippocampus may mediate anxiolytic and anxiogenic behaviours respectively. Eur J Pharmacol 1994;264: 259–64.

Azmitia EC, Segal M. An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe. J Comp Neurol 1978;179:641–68.

Bert B, Fink H, Sohr R, Rex A. Different effects of diazepam in Fischer rats and two stocks of Wistar rats in tests of anxiety. Pharmacol Biochem Behav 2001;70:411–20.

Cadogan AK, Kendall DA, Fink H, Marsden CA. Social interaction increases 5-HT release and cAMP efflux in the rat ventral hippocampus in vivo. Behav Pharmacol 1994;5:299–305.

Carli M, Samanin R. Potential anxiolytic properties of 8-hydroxy-2-(di-*n*-propylamino)tetralin, a selective serotonin 1A receptor agonist. Psychopharmacology 1988;94:84–91.

Cervo L, Mocaer E, Bertaglia A, Samanin R. Roles of 5-HT_{1A} receptors in the dorsal raphe and dorsal hippocampus in anxiety assessed by the behavioral effects of 8-OH-DPAT and S 15535 in a modified Geller–Seifter conflict model. Neuropharmacology 2000;39:1037–43.

Critchley MAE, Njung'e K, Handley SL. Actions and some interactions of 5-HT-1A ligands in the elevated X-maze and effects of dorsal raphe lesions. Psychopharmacology 1992;106:484–90.

Deakin JF, File S.E, Hyde JR, MacLeod NK. Ascending 5-HT pathways and behavioural habituation. Pharmacol Biochem Behav 1979; 10:687–94.

File SE, Gonzalez LE. Anxiolytic effects in the plus-maze of 5-HT-1A receptor ligands in dorsal raphe and ventral hippocampus. Pharmacol Biochem Behav 1996;54:123–8.

File SE, Hyde JRG, MacLeod NK. 5,7-dihydroxytryptamine lesions of dorsal and median raphe nuclei and performance in the social interaction test of anxiety and in a home-cage aggression test. J Affective Disord 1979;1:115–22.

File SE, Curle PF, Baldwin HA, Neal MJ. Anxiety in the rat is associated with decreased release of 5-HT and glycine from the hippocampus. Neurosci Lett 1987;83:318–22.

File SE, Zangrossi HJ, Andrews N. Social interaction and elevated plus-maze tests: changes in release and uptake of 5-HT and GABA. Neuropharmacology 1993;32:217–21.

Handley SL, Mithani S. Effects of alpha-adrenoceptor agonists and antagonists in a maze—exploration model of "fear"-motivated behaviour. Naunyn-Schmiedeberg's Arch Pharmacol 1984;327:1–5.

Hellweg R, Thomas H, Arnswald A, von Richthofen S, Kay S, Fink H, et al. Serotonergic lesion of median raphe nucleus alters nerve growth factor content and vulnerability of cholinergic septohippocampal neurons in rat. Brain Res 2001;907:100–8.

Hogg S. A review of the validity and variability of the elevated plus-maze as an animal model of anxiety. Pharmacol Biochem Behav 1996;54: 21–30.

Hogg S, Andrews N, File SE. Contrasting behavioural effects of 8-OH DPAT in the dorsal raphe nucleus and ventral hippocampus. Neuropharmacology 1994;33:343–8.

Hölzel B, Pfister C, Fink H. Morphologische Veränderungen an Neuronen des Nucleus raphe dorsalis der Ratte nach Applikation von 5,7-Dihydroxytryptamine. Eine Golgi-rapid-Imprägnationsstudie. J Hirnforsc 1984;3:343–9.

König JFR, Klippel RA. A stereotactic atlas. Baltimore: Williams Wilkins; 1963.

Marsden CA, Beckett SRG, Wilson W, Bickerdike M, Fink H, Rex A, et al. Serotonin involvement in animal models of anxiety and panic. In: Takade A, Curzon G, editors. Serotonin in the central nervous system and periphery. Amsterdam: Elsevier; 1995. p. 135–43.

Matsuo M, Kataoka Y, Mataki S, Kato Y, Oi K. Conflict situation increases serotonin release in rat dorsal hippocampus: in vivo study with microdialysis and Vogel test. Neurosci Lett 1996;215:197–200.

Paxinos G, Watson C. The brain stereotaxic coordinates. New York: Academic Press; 1997.

Pellow S, Chopin P, File SE, Briley M. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 1985;14:149–67.

Picazo O, López-Rubalcava C, Fernández-Guasti A. Anxiolytic effect of the 5-HT-1A compounds 8-hydroxy-2-(di-*n*-propylamine)tetralin and ipsapirone in the social interaction paradigm: evidence of a pre-synaptic action. Brain Res Bull 1995;37:169–75.

Rex A, Marsden CA, Fink H. Effect of diazepam on cortical 5-HT release and behavior in the guinea pig on exposure to the elevated plus maze. Psychopharmacology 1993a;110:490–6.

Rex A, Marsden CA, Fink H. 5-HT1A receptors and changes in extracel-

lular 5-HT in the guinea pig prefrontal cortex: involvement in aversive behaviour. J Psychopharmacol 1993b;110:490–6.

Rex A, Voigt JP, Fink H. Behavioral and neurochemical differences between Fischer 344 and Harlan–Wistar rats raised identically. Behav Genet 1999;29:187–92.

Rex A, Pfeifer L, Fink H. Determination of NADH in frozen rat brain sections by laser-induced fluorescence. Biol Chem 2001;382:1727–32.

Rodgers RJ, Cole JC. The elevated plus-maze: pharmacology, methodology and ethology. In: Cooper SJ, Hendrie CA, editors. Ethology and Psychopharmacology. London:Wiley; 1994. p. 9–44.

Stamford JA, Davidson C, McLaughlin DP, Hopwood SE. Control of dorsal raphe 5-HT function by multiple 5-HT(1) autoreceptors: parallel purposes or pointless plurality. Trends Neurosci 2000;23:459–65.

Tabatabaie T, Dryhurst G. Molecular mechanisms of action of 5,6- and 5,7-dihydroxytryptamine. In: Kostrzewa RM, editor. Highly selective neurotoxins: basic and clinical applications. Totowa: Humana Press; 1998. p. 269–91.

Thomas H, Fink H, Sohr TR, Voits M. Lesion of the median raphe nucleus: a combined behavioral and microdialysis study in rats. Pharmacol Biochem Behav 2000;65:15–21.

Verbanac JS, Commissaris RL, Pitts DK. An electrophysiological evaluation of serotonergic dorsal raphe neurons in Maudsley rats. Life Sci 1996;58:245–50.

Vertes RP, Fortin WJ, Crane AM. Projections of the median raphe nucleus in the rat. J Comp Neurol 1999;407:555–82.

Voigt J-P, Rex A, Sohr R, Fink H. Hippocampal 5-HT and NE release in the transgenic rat TGR(mREN2)27 related to behavior on the elevated plus maze. Eur Neuropsychopharmacol 1999;9:279–85.

Wright IK, Upton N, Marsden CA. Effect of established and putative anxiolytics on extracellular 5-HT and 5-HIAA in the ventral hippocampus of rats during behavior on the elevated X-maze. Psychopharmacology 1992;109:338–46.