A study of the brain structures involved in the acute effects of fluoxetine on REM sleep in the rat

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Abstract
The effects of acute administration of fluoxetine, a selective serotonin reuptake inhibitor on spontaneous sleep, were studied in adult rats implanted for chronic sleep recordings. Fluoxetine was administered systemically or infused directly into the dorsal raphe nucleus (DRN), the right laterodorsal tegmental nucleus (LDT) or the medial pontine reticular formation (mPRF). Systemic administration of fluoxetine (3.0–12.0 μmol/kg) significantly reduced rapid-eye-movement sleep (REMS) and the number of REM periods; REMS latency was augmented. Direct infusion of fluoxetine (1.0 nmol) into the DRN induced a significant increment of REMS and of the number of REM periods whereas REMS latency was reduced. Microinjection of fluoxetine into the LDT (1.0 nmol) or the mPRF (0.8 nmol) decreased REMS and the number of REM periods whereas REMS latency was augmented. Pre-treatment with the selective 5-HT₁A receptor antagonist WAY 100635 prevented the reduction of REMS induced by the microinjection of fluoxetine into the LDT. Our results indicate that the fluoxetine-induced suppression of REMS is related to the inhibition of brainstem structures involved in the promotion and the induction of REMS. The decrease of REMS would be dependent upon the activation of several 5-HT receptor subtypes, including the 5-HT₁A receptor.

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Key words: Fluoxetine, REM sleep, sleep, serotonin, WAY 100635.

Introduction
Polysomnographic changes in depression are well documented. They include sleep continuity changes; slow-wave sleep deficits, and rapid-eye-movement sleep (REMS) abnormalities (decrease of REMS latency, increase of REMS percentage, and a significant longer first REM period) (Kupfer, 1984; Reynolds and Kupfer, 1987). A number of hypotheses have been proposed to account for the pathophysiological mechanisms underlying REMS abnormalities in depression. The cholinergic-aminergic imbalance hypothesis originally put forward by Janowsky et al. (1972) proposes that either enhanced cholinergic neurotransmission or diminished serotonergic and noradrenergic neurotransmission accounts for the REMS changes in the depressed patient.

The effects of acute administration of selective serotonin (5-HT) reuptake inhibitors (SSRIs) on sleep have been studied in laboratory animals, healthy adults, and depressed patients (for review see Armitage, 1996; Oberndorfer et al., 2000; Sharpley and Cowen, 1995; Staner et al., 1999). SSRIs are potent REMS suppressors, prolonging the latency to the first REM period. The effect of SSRIs on REMS has been proposed to result from enhancement of central nervous system serotonergic neurotransmission. In this respect, it is currently accepted that cholinergic neurons in the laterodorsal tegmental (LDT) and the pedunculopontine tegmental (PPT) nuclei act to promote REMS. All these neurons are inhibited by serotonergic afferents from the dorsal raphe nucleus (DRN) (Hobson et al., 1998; McCarley and Hobson, 1975). In turn, the REMS induction region of the medial pontine reticular formation (mPRF) is activated by efferent connections of the LDT/PPT nuclei. In addition, the
REMS induction zone receives an inhibitory input of
the DRN (Semba, 1993).

The present experiments were undertaken to eluci-
date the mechanism of action of acutely administered
fluoxetine on REMS occurrence. This was accom-
plished by injecting fluoxetine either systemically
or into the DRN, the LDT or the mPRF of animals
prepared for chronic sleep recordings. In addition, the
role of the 5-HT₁₅ receptor in fluoxetine-induced
suppression of REMS was assessed by treating the
animals with the selective 5-HT₁₅ receptor antagonist
WAY 100635 [N-(2-(4-(2-methoxyphenyl)-1-piper-
azinyl) ethyl-N-(2-pyridinyl) cyclohexanecarboxamide
trihydrochloride] prior to the microinjection of fluox-
etine into the LDT.

Method

Five different groups of male Wistar rats weighing
350–400 g were included in the study. All rats were
cared for and used in strict accordance with the Eu-
ropean Community Guidelines for the Use of Exper-
imental Animals. All procedures were approved by
the Institutional Animal Care and Use Committee of
the Medical School, Montevideo, Uruguay. The ani-
mals were anaesthetized with sodium pentobarbital
(40.0 mg/kg, i.p.) and implanted with Nichrome®
(Driver-Harris Co., Newark, NJ, USA) electrodes
(200-μm diameter) for chronic sleep recordings of
electroencephalogram and electromyogram activities,
through placement on the frontal and occipital cortex
for the former, and on the dorsal neck musculature for
the latter. In addition, a stainless-steel guide tube was
stereotaxically implanted above the DRN, the right
LDT or the mPRF; the final coordinates for the guide
tube implantations were: DRN: AP 7.64–8.0, L 0.0,
V —5.8 below the cortex; LDT: AP 8.72–8.8, L 0.6, V
—6.0 below the cortex; mPRF: AP 8.3–8.72, L 1.0,
V —7.4 below the cortex (Paxinos and Watson, 1986).
The tubular guide (26 gauge) for drug injection was
implanted 2 mm above the middle of the correspond-
ing neuroanatomical structure to minimize cellular
damage at the injection site. The animals were treated
postoperatively for 4 d with 50 mg/kg of the antibiotic
cefradine. A topical antibiotic (neomycin) was also
applied to the implant incision. Drug or vehicle was
injected into the DRN, the LDT or the mPRF with an
injection cannula (29 gauge) which extended 2 mm
beyond the guide, in a 0.25 μl volume over a 2-min
period. On completion of the study, the rats were
sacrificed under pentobarbital anaesthesia, and can-
nulae placements were defined histologically. Correct
cannula/injection sites were assessed using the Atlas
of Paxinos and Watson (1986) following a 0.25 μl in-
jection of Sky-Blue dye into the DRN, the LDT or the
mPRF. All data presented in this report are derived
from animals whose injection site was within the lim-
its of the corresponding neuroanatomical structure.

The animals were housed individually in a tem-
perature-controlled room (23 ± 1 °C) under a 12 h
light/dark cycle and with food and water ad libitum.
Ten days after surgery the animals were habituated to
a sound-proof chamber fitted with slip-rings and cable
connectors, and to the injection procedures. The drugs
were always administered during the light phase of
the 12 h light/dark cycle, at approx. 07:30 hours. A
balanced order of drug and control injections was
always used to merge the effects of both the drug and
the time elapsed during the protocol. Electrographic
activity of 25-s epochs was scored by a trained rater
(H.J.) blind to the treatment received by the animals.
The predominant activity of each epoch was assigned
to one of the following categories: wakefulness (W),
light sleep (LS), slow-wave sleep (SWS), or REMS.
SWS and REMS latencies, and the number of REM
periods were also determined (Monti et al., 1988).

The doses of fluoxetine selected for the present
study were based on pilot work in our laboratory and
the limited previous research in which the anti-
 depressant drug was administered to laboratory ani-
mals, and its acute effects on electroencephalographic
sleep were recorded.

The effects of fluoxetine were studied in five differ-
ent groups of animals according to the following
experimental paradigms:

Experiment 1 (group 1)

Fluoxetine hydrochloride (Sigma, St. Louis, MO, USA)
3.0, 6.0 or 12.0 μmol/kg (1.0, 2.0 or 4.0 mg/kg) or
vehicle (saline) was administered subcutaneously. Six
animals were used for each dose.

Experiment 2 (group 2)

Fluoxetine hydrochloride 0.6, 0.8 or 1.0 nmol (210.0,
280.0 or 350.0 ng) or vehicle (saline) was infused into
the DRN. Four microinjections were administered to
each animal. Six animals were used for each dose.

Experiment 3 (group 3)

Fluoxetine hydrochloride 0.6, 0.8 or 1.0 nmol or saline
was infused into the LDT. Four microinjections were
administered to each animal. Six animals were used
for each dose.
Experiment 4 (group 4)

Fluoxetine hydrochloride 0.4, 0.6 or 0.8 nmol (140.0, 210.0 or 280.0 ng) or saline was infused into the mPRF. Four microinjections were administered to each animal. Six animals were used for each dose.

Experiment 5 (group 5)

In the fifth set of experiments 1.0 nmol fluoxetine hydrochloride was injected into animals pre-treated with 0.06 nmol (25.0 ng) WAY 100635. The drugs were microinjected into the LDT 20 min apart in the interaction experiments. There were six animals in the experimental group, and each received three microinjections. A 6-h recording was started 10 min after vehicle or drug(s) administration.

Recently, we tested the effects of WAY 100635 given systemically or microinjected into the LDT on REMS in the rat (Monti and Jantos, 2004). Systemic injection of the 5-HT$_{1A}$ receptor antagonist augmented W and REMS latency and reduced REMS and the number of REM periods. On the other hand, direct infusion of WAY 100635 into the LDT increased REMS and the number of REM periods. Therefore, WAY 100635 was injected directly into the LDT in the interaction experiments to avoid non-specific effects on sleep variables.

The half-life of the 5-HT transporter recovery after irreversible inhibition amounts to 48–72 h in the rat (Kimmel et al., 2001). However, the elimination half-life of fluoxetine and its N-demethylated byproduct is approx. 50 and 200 h respectively (Baldessarini, 2001). Thus, in all experiments at least 9 d were allowed to elapse between experiments to avoid long-lasting and rebound effects of fluoxetine on sleep. In addition, the animals were readapted to the recording procedure the day prior to drug(s) administration.

A one-way analysis of variance (ANOVA) using dose as the between-subject factor was performed, with multiple post-hoc comparisons carried out with the Dunnnett Multiple Comparisons test when the ANOVA indicated significance ($p<0.05$).

Results

The histological analysis of the injection sites showed that 30 of the 35 animals originally included in the study received microinjections of fluoxetine that were confined within the limits of the corresponding neural structure. In those animals where microinjections of fluoxetine were not confined within the limits of the corresponding neural structure REMS values remained almost unchanged or showed erratic changes.

There was considerable variability in the control values, particularly for REMS latency in the various tables. However, differences were not significant (ANOVA, with multiple post-hoc comparisons carried out with the Bonferroni Multiple Comparisons test).

Following subcutaneous administration of 12.0 µmol/kg fluoxetine, REMS was reduced during the first and the second 2-h periods after treatment ($F_{3,15}=7.88$, $p=0.002$, and $F_{3,15}=4.09$, $p=0.02$ respectively). The 3.0 or 6.0 µmol/kg dose induced a similar effect on REMS during the first 2 h of recording ($F_{3,15}=7.88$, $p=0.002$). W, LS, and SWS were slightly but not significantly modified (Figure 1). REMS latency showed a significant increase after the two largest doses of fluoxetine ($F_{3,15}=3.80$, $p=0.03$), whereas the number of REM periods was reduced during the first 2-h period after the entire range of doses given ($F_{3,15}=5.62$, $p=0.008$), and by the 12.0 µmol/kg dose during the second 2-h period ($F_{3,15}=4.47$, $p=0.01$) (Table 1).

After fluoxetine was microinjected into the DRN, REMS was increased by the 1.0 nmol dose during the second 2 h of recording ($F_{3,15}=3.83$, $p=0.03$). Values of W, LS, and SWS showed inconsistent changes that did not attain significance (Figure 2). When injected at the DRN fluoxetine (1.0 nmol) significantly reduced REMS latency ($F_{3,15}=3.28$, $p=0.05$), and increased the number of REM periods during the second 2-h period ($F_{3,15}=4.48$, $p=0.02$) (Table 2).

Following the microinjection of 1.0 nmol fluoxetine into the LDT, REMS was suppressed during the first and the second 2-h period ($F_{3,15}=4.14$, $p=0.02$, and $F_{3,15}=3.74$, $p=0.03$ respectively). W, LS, and SWS showed slight but inconsistent changes, that did not attain significance (Figure 3). REMS latency was increased after the 1.0-nmol dose ($F_{3,15}=3.21$, $p=0.05$), whereas the number of REM periods showed a significant decrease after 0.8 or 1.0 nmol during the first 2-h recording period ($F_{3,15}=4.91$, $p=0.01$) (Table 3).

The microinjection of 0.8 nmol fluoxetine into the mPRF significantly reduced REMS during the first and second 2-h recording period ($F_{3,15}=4.32$, $p=0.02$, and $F_{3,15}=6.57$, $p=0.004$). Values of W, LS, and SWS showed no significant differences from control values (Figure 4). The 0.8 nmol dose of the compound augmented REMS latency ($F_{3,15}=3.61$, $p=0.03$) and reduced the number of REM periods during the second 2-h recording period ($F_{3,15}=3.57$, $p=0.02$) (Table 4).

WAY 100635 (0.06 nmol) prevented the suppression of REMS induced by fluoxetine (1.0 nmol) during the first and second 2-h recording periods ($F_{3,15}=6.40$, $p=0.01$, and $F_{3,15}=4.40$, $p=0.04$ respectively). W, LS, and SWS were not significantly different from control
values (Figure 5). In addition, the 5-HT$_{1A}$ receptor antagonist prevented the fluoxetine-induced increase of REMS latency ($F_{3,15} = 4.62, p = 0.04$) and reduction of the number of REM periods during the first and the second 2-h recording periods ($F_{3,15} = 3.41, p = 0.05$, and $F_{3,15} = 3.39, p = 0.05$ respectively) (Table 5).

**Discussion**

As shown in the present study, the acute subcutaneous administration of fluoxetine reduced REMS in the rat. In common with fluoxetine, acute injection of several other SSRIs including sertraline and
citalopram have been shown to decrease REMS in the rat, the hamster or the cat (Gao et al., 1992; Hilakivi et al., 1987; Monaca et al., 2003; Pastel and Fernstrom, 1987; Ross et al., 1990; Slater et al., 1978). Notwithstanding, systemic drug studies have neither elucidated the site of action of fluoxetine nor characterized the 5-HT receptor subtype(s) involved in the suppressive effect on REMS. Results from a variety of experiments support the hypothesis that serotonergic inhibition of cholinergic neurons in the LDT/PPT nuclei or glutamatergic cells in the mPRF suppresses REMS (Hobson et al., 1998; Horner et al., 1997; Monti  

### Table 2. Effects of fluoxetine administered directly into the dorsal raphe nucleus (DRN) on sleep latencies and number of REM periods

<table>
<thead>
<tr>
<th>Group</th>
<th>SWS latency (min)</th>
<th>REMS latency (min)</th>
<th>Number of REM periods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1–2 h</td>
</tr>
<tr>
<td>Control</td>
<td>15.4±5.7</td>
<td>69.4±14.6</td>
<td>2.2±0.7</td>
</tr>
<tr>
<td>Fluoxetine 0.6</td>
<td>11.8±2.3</td>
<td>88.6±19.4</td>
<td>2.0±0.8</td>
</tr>
<tr>
<td>Fluoxetine 0.8</td>
<td>14.8±2.2</td>
<td>93.6±16.5</td>
<td>1.2±0.7</td>
</tr>
<tr>
<td>Fluoxetine 1.0</td>
<td>12.4±2.2</td>
<td>35.8±5.3*</td>
<td>2.2±0.4</td>
</tr>
</tbody>
</table>

All values are the means ± s.e.m. Six animals were in each experimental group. The doses are in nmol. Compared with control values: *p<0.05; **p<0.01 (Dunnett Multiple Comparisons test).

![Figure 2. Effects of fluoxetine microinjected into the dorsal raphe nucleus on sleep and wakefulness. Six animals were in each experimental group. Ordinate and abscissa as in Figure 1. Compared with control values: *p<0.05 (Dunnett Multiple Comparisons test). □, Control; ■, 0.6 nmol; ▼, 0.8 nmol; □, 1.0 nmol.](http://ijnp.oxfordjournals.org/).
and Jantos, 2003; Thakkar et al., 1998). Based on these findings we infused fluoxetine directly into the LDT or the mPRF. Moreover, it was of interest to determine the effects of direct microinjection of fluoxetine into the DRN. This study shows for the first time that the direct application of fluoxetine into the LDT or the mPRF decreases REMS in the rat. On the other hand, microinjection of the antidepressant drug into the DRN induces the opposite effect. The finding that WAY 100635 prevented the suppression of REMS

Table 3. Effects of fluoxetine administered directly into the laterodorsal tegmental nucleus (LDT) on sleep latencies and number of REM periods

<table>
<thead>
<tr>
<th>Group</th>
<th>SWS latency (min)</th>
<th>REMS latency (min)</th>
<th>Number of REM periods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1–2 h</td>
</tr>
<tr>
<td>Control</td>
<td>5.0±2.9</td>
<td>36.7±5.5</td>
<td>3.8±0.5</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td></td>
<td></td>
<td>3.3±0.6</td>
</tr>
<tr>
<td>0.6</td>
<td>9.2±2.6</td>
<td>54.5±7.4</td>
<td>2.0±0.6*</td>
</tr>
<tr>
<td>0.8</td>
<td>9.7±2.8</td>
<td>66.0±12.7</td>
<td>1.7±0.6*</td>
</tr>
<tr>
<td>1.0</td>
<td>9.2±2.3</td>
<td>80.3±14.9*</td>
<td></td>
</tr>
</tbody>
</table>

All values are the means ± S.E.M. Six animals were in each experimental group. The doses are in nmol. Compared with control values: *p<0.05 (Dunnett Multiple Comparisons test).

Figure 3. Effects of fluoxetine microinjected into the right laterodorsal tegmental nucleus on sleep and wakefulness. Six animals were in each experimental group. Ordinate and abscissa as in Figure 1. Compared with control values: *p<0.05 (Dunnett Multiple Comparisons test). I, Control; □, 0.6 nmol; ■, 0.8 nmol; ▲, 1.0 nmol.
induced by the direct administration of fluoxetine into the LDT tends to indicate that this effect could be, at least partly, mediated by 5-HT₁A receptors.

How can the effects of fluoxetine on REM sleep be understood? As is well known, the SSRIs inhibit the active reuptake (transport) of 5-HT into nerve terminals. In this respect, 5-HT uptake sites not only in terminal regions but also in the raphe nuclei are important targets for fluoxetine and related drugs (Asberg et al., 1986; Lemberger et al., 1985).

### Table 4. Effects of fluoxetine administration into the medial pontine reticular formation (mPRF) on sleep latencies and number of REM periods

<table>
<thead>
<tr>
<th>Group</th>
<th>SWS latency (min)</th>
<th>REMS latency (min)</th>
<th>Number of REM periods</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1–2 h</td>
</tr>
<tr>
<td>Control</td>
<td>3.0 ± 1.2</td>
<td>43.5 ± 11.1</td>
<td>4.0 ± 0.7</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>0.4</td>
<td>6.0 ± 1.9</td>
<td>47.3 ± 13.3</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>6.8 ± 1.7</td>
<td>55.8 ± 10.8</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>4.3 ± 1.9</td>
<td>71.3 ± 13.6*</td>
</tr>
</tbody>
</table>

All values are the means ± S.E.M. Six animals were in each experimental group. The doses are in nmol. Compared with control values: *p < 0.05 (Dunnett Multiple Comparisons test).

![Figure 4](http://ijnp.oxfordjournals.org/) Effects of fluoxetine microinjected into the medial pontine reticular formation on sleep and wakefulness. Six animals were in each experimental group. Ordinate and abscissa as in Figure 1. Compared with control values: **p < 0.01 (Dunnett Multiple Comparisons test). □, Control; ■, 0.4 nmol; †, 0.6 nmol; ♦, 0.8 nmol.
Quantitative autoradiography studies indicate that the 5-HT transporter has a somatodendritic location in the DRN (Burchett and Bannon, 1997).

Extracellular 5-HT has been shown to increase in several brain regions after the systemic administration of fluoxetine or citalopram to the rat. Increased

Table 5. Prevention by WAY 100635 of the effect of fluoxetine infused into the laterodorsal tegmental nucleus (LDT) on REMs latency and the number of REM periods

<table>
<thead>
<tr>
<th>Group</th>
<th>SWS latency (min)</th>
<th>REMS latency (min)</th>
<th>Number of REM periods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.6 ± 2.0</td>
<td>42.4 ± 7.0</td>
<td>4.2 ± 0.6 6.4 ± 0.7 5.2 ± 0.8</td>
</tr>
<tr>
<td>Fluoxetine 1.0</td>
<td>9.5 ± 2.4</td>
<td>71.5 ± 16.7*</td>
<td>1.8 ± 0.5 3.3 ± 0.6 3.4 ± 0.4</td>
</tr>
<tr>
<td>WAY 100635 0.06 + fluoxetine 1.0</td>
<td>11.0 ± 2.2</td>
<td>33.7 ± 7.1</td>
<td>3.2 ± 0.7 4.5 ± 0.5 5.2 ± 0.5</td>
</tr>
</tbody>
</table>

All values are the means ± S.E.M. Six animals were in each experimental group. The doses are in nmol. Compared with control values: *p < 0.05 (Dunnett Multiple Comparisons test).

Figure 5. Effects of WAY 100635 pre-treatment on the suppression of REM sleep induced by the microinjection of fluoxetine into the right laterodorsal tegmental nucleus. Six animals were in each experimental group. Ordinate and abscissa as in Figure 1. Compared with control values: *p < 0.05 (Dunnett Multiple Comparisons test). □ Control; ◆ 1.0 nmol fluoxetine; ■ 0.6 nmol WAY 100635 + 1.0 nmol fluoxetine.
synaptic availability of 5-HT activates a large number of post-synaptic 5-HT receptor types and 5-HT subtype autoreceptors (types 1A and 7 at raphe cell bodies and dendrites, type B/D at terminals). Stimulation of the somatodendritic 5-HT1A receptor after systemic injection of fluoxetine, fluvoxamine or sertraline inhibits the firing rate and the neurotransmitter release of 5-HT neurons (Arborelius et al., 1995; Evrand et al., 1999; Gartside et al., 1995; Hajós et al., 1995; Rigdon and Wang, 1991). However, in spite of the decreased firing of 5-HT neurons and decreased synthesis of the neurotransmitter, the inhibition of 5-HT reuptake at post-synaptic sites causes approx. 3- to 5-fold increases in extracellular 5-HT in several neuroanatomical structures including the hippocampus, the hypothalamus, the diencephalon, the striatum and the thalamus (Burchett and Bannon, 1997; Dailey et al., 1992; Gartside et al., 1995; Knobelman et al., 2001; Perry and Fuller, 1992, 1993; Rutter and Auerbach, 1993; Sabol et al., 1992). Thus, systemic administration of fluoxetine decreases the activity of 5-HT cells, but the amount of extracellular 5-HT, hence the activation of post-synaptic receptors is increased (Fuller, 1994). In this regard, the increased activation of post-synaptic 5-HT receptors located in the LDT and the mPRF results in the suppression of REMS in our experimental model. On the other hand, the increase of REMS after local infusion of fluoxetine into the DRN depends upon the selective activation of somatodendritic 5-HT1A receptors. As a result, the serotonergic activity is decreased and 5-HT levels are reduced at post-synaptic sites (Monti and Jantos, 2003). Interestingly, microinjection of the selective 5-HT1A receptor agonist flesinoxan into the DRN has been shown to induce similar effects on REMS in the rat (Monti et al., 2000, 2002). In addition, microdialysis administration of flesinoxan into the median raphe nucleus (MRN) induced a concentration-dependent reduction of dorsal hippocampus and MRN 5-HT levels (Van der Heyden et al., 1996).

It took 3–4 h before a significant effect on REMS became apparent after fluoxetine microinjection into the DRN. A similar delay occurred when flesinoxan was infused into the DRN of the rat (Monti et al., 2000). Moreover, in the study by Bjorvatn et al. (1997), where the 5-HT1A receptor partial agonist 8-OH-DPAT was perfused continuously for 6 h into the DRN of rats, a significant increase of REMS was only apparent during the third 2-h period.

The delayed effect could be partly related to the slow diffusion of fluoxetine from the injection site to other subdivisions of the DRN. However, it should also be taken into consideration that the decrease of 5-HT release at post-synaptic sites may not be an immediate consequence of the suppression of serotonergic activity by fluoxetine, but could depend on the critical reduction of a terminal 5-HT releasable pool (Ferré et al., 1994). In this respect, Bonvento et al. (1992) found that the reduction of 5-HT output in the striatum and the frontal cortex was delayed after intra-DRN microinjection of 8-OH-DPAT in the rat. Further studies are, however, necessary to resolve this issue.

Moderate to dense projections of the DRN have been observed to reach the LDT and the PPT (Honda and Semba, 1994; Vertes and Kocsis, 1994). In addition, serotonergic afferents innervate non-cholinergic, presumptively glutamatergic, neurons of the REMS induction zone of the mPRF with the heaviest projections arising from the DRN and the MRN (Semba, 1993). Serotonergic receptors have been characterized in the LDT/PPT and the mPRF. In this respect, the binding density levels of 5-HT1A receptors in these structures vary from low to medium (Kia et al., 1996a,b; Sanford et al., 1994; Tohyama and Takatsuji, 1998). Recently, Strecker et al. (1999) quantified spontaneous levels of extracellular 5-HT in the PPT during sleep and wakefulness in the cat. Extracellular 5-HT levels were highest during wakefulness, and progressively lower during SWS and REMS. Furthermore, Thakkar et al. (1998) showed that local microdialysis perfusion of the 5-HT1A receptor partial agonist 8-OH-DPAT into areas where cholinergic LDT and PPT neurons are located almost completely suppressed the discharge activity of REM-on neurons. On the other hand, 8-OH-DPAT had minimal or no effect of W-REM-on cells.

Iwakiri et al. (1993) measured extracellular levels of endogenous 5-HT in the mPRF of intact cats and found that they were at their highest during wakefulness. As the animals entered SWS, 5-HT levels decreased to approx. 90%; during REMS the levels of this neurotransmitter were at their lowest (60–50%). Stevens et al. (1992) examined the action of 5-HT on mPRF neurons using intracellular recordings of rat brainstem slices in vitro. Serotonin induced a hyperpolarization associated with a decrease in input resistance in 34% of the neurons. This response was mimicked by 8-OH-DPAT and was blocked by the non-selective 5-HT1A receptor antagonist spiperone. Finally, microinjection of the 5-HT1A receptor agonist flesinoxan into the LDT or the mPRF reduced REMS in the rat (Monti and Jantos, 2003).

All these findings tend to support the proposal that the fluoxetine-related activation of post-synaptic 5-HT1A receptors after its systemic administration or its direct microinjection into the LDT or the mPRF is
responsible, at least partly, for the suppression of REMS. In agreement with our proposal, the citalopram-induced inhibition of REMS described in wild-type mice is impaired in 5-HT1A receptor knockout mice (Monaca et al., 2003). The finding that pre-treatment with the selective 5-HT1A receptor antagonist WAY 100635 prevented the reduction of REMS induced by the infusion of fluoxetine into the LDT further supports the proposal. Recently, we found that direct infusion of the 5-HT7 receptor antagonist SB-269970 into the LDT significantly increases REMS in the rat (J. M. Monti and H. Jantos, unpublished observations). Thus, several receptor subtypes could be involved in the fluoxetine suppression of REMS. Further studies with selective 5-HT7 receptor agonists (not available at the present time) might help to resolve this issue.

In conclusion, the systemic administration of fluoxetine or its direct microinjection into the LDT or the mPRF suppressed REMS. In contrast, the infusion of fluoxetine into the DRN induced an increase of REMS. Pre-treatment with WAY 100635 prevented the reduction of REMS induced by the direct application of the SSRI into the LDT.

Acknowledgements

None.

Statement of Interest

None.

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