Acute and chronic treatment with serotonin reuptake inhibitors exert opposite effects on respiration in rats: possible implications for panic disorder

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Abstract
Prompted by the suggested importance of respiration for the pathophysiology of panic disorder, we studied the influence of serotonin reuptake inhibitors (SRIs) as well as other serotonin-modulating compounds on respiration in freely moving rats. The effect on respiration after acute administration of compounds enhancing synaptic levels of serotonin, that is, the serotonin reuptake inhibitors paroxetine and fluoxetine, the serotonin-releasing agents m-chlorophenylpiperazine and d-fenfluramine, and the selective 5-HT1A antagonist WAY-100635, were investigated. All serotonin-releasing substances decreased respiratory rate in unrestrained, awake animals, suggesting the influence of serotonin on respiratory rate under these conditions to be mainly inhibitory. In line with a previous study, rats administered fluoxetine for 23 days or more, on the other hand, displayed an enhanced respiratory rate. The results reinforce the assumption that the effect of subchronic administration of a serotonin reuptake inhibitor on certain serotonin-regulated parameters may be opposite to that obtained after acute administration. We suggest that our observations may be of relevance for the fact that acute administration of SRIs, d-fenfluramine, or m-chlorophenylpiperazine often is anxiogenic in panic disorder patients, and that weeks of administration of an SRI leads to a very effective prevention of panic.

Keywords
serotonin, panic disorder, respiration, fluoxetine, paroxetine, m-chlorophenylpiperazine, WAY-100635, d-fenfluramine

Introduction
The theory that panic disorder is due to an aberration in the control of respiration (Klein, 1993, Nardi et al., 2009) is supported by the following observations: i) panic attacks – in contrast to normal fear – are often characterized by respiratory symptoms, such as hyperventilation and shortness of breath; ii) panic attacks can be elicited by respiratory stimulants such as carbon dioxide, sodium lactate, caffeine and doxapram (Abelson et al., 1996; Gorman et al., 1984; Kent et al., 2001; Lee et al., 1993; Liebowitz et al., 1984; Nardi et al., 2008; Papp et al., 1997; Pitts and McClure, 1967); iii) patients with panic disorder display enhanced respiratory variability between panic attacks both while awake and during sleep (Abelson et al., 2001; Gorman et al., 1988; Martinez et al., 2001; Papp et al., 1993; Perna et al., 1994; Stein et al., 1995; Wilhelm et al., 2001); and iv) twin studies suggesting that the same genetic determinants cause both CO₂ hyper-reactivity and increased risk for panic disorder (Battaglia et al., 2008, 2009). Whether the respiratory abnormalities characterizing panic disorder patients are best explained in terms of chemoreceptor hyper-responsiveness (Klein, 1993) or an irregularity of respiration unrelated to chemoreceptor function (Sinha et al., 2000; Struzik et al., 2002) is, however, a matter of controversy.

Chronic treatment with serotonin reuptake inhibitors (SRIs) is an effective way of preventing panic attacks, suggesting that brain serotonergic neurons are parts of – or influence – those neuronal circuits that generate panic anxiety (Den Boer and Westenberg, 1988; Modigh et al., 1992). The influence of brain serotonergic activity on anxiety in panic disorder patients is, however, complex, as illustrated by the fact that acute administration often elicit a paradoxical increase in anxiety, and that serotonin releasing drugs, such as d-fenfluramine (Garattini et al., 1987) and m-chlorophenylpiperazine (mCPP) (Eriksson et al., 1999), may trigger panic attacks (Charney et al., 1987; Mortimore and Anderson, 2000). Why acute facilitation of serotonergic activity elicits anxiety in responsive subjects, whereas long-term treatment with SRIs prevents panic attacks, remains unknown.

The idea that serotonergic neurons participate in the regulation of respiration gains support from the presence of serotonergic nerve terminals and serotonergic receptors in respiratory nuclei such as the nucleus tractus solitarii,
the hypoglossal nucleus, and the preBötzingер complex (Feldman et al., 2003; Richerson et al., 2005; Richter et al., 2003). How these neurons influence baseline respiration, and/or the response to hypercapnea and hypoxia, however, remains a matter of controversy, different studies yielding conflicting results. Partly the divergences in outcome of studies seems related to whether the studied animals have been i) awake and under restraint stress; ii) awake and freely moving; iii) asleep; or iv) anaesthetized or otherwise pretreated during the experiment. Moreover, the possibility that serotonin may exert different effects on different components of the breathing cycle, that is respiratory rate and tidal volume, respectively, and that different serotonergic receptors may exert different – and even opposite – effects on the regulation of ventilation, may also contribute to the lack of consensus on this issue.

According to one theory, serotonergic neurons serve as central chemoreceptors, responding to acidosis or enhanced CO₂ levels (Johnson et al., 2005; Richerson, 2004; Severson et al., 2003) and exert mainly a stimulatory influence on respiration. In line with this view, which is, however, not undisputed (Mulkey et al., 2004), electric activation of raphe cell bodies (where serotonergic neurons originate) or local exposure of raphe neurons to acidosis or enhanced CO₂ levels have been reported to stimulate respiration, as has administration of certain serotonin receptor agonists (Manzke et al., 2003). Moreover, baseline respiration, as well as the respiratory response to CO₂, is reported to be reduced in rats in which serotonergic transmission has been impaired by administration of a serotonergic neurotoxin (Martin-Body and Grundy, 1985). When interpreting these results, it should however be considered that many of these studies have been performed on anaesthetized or otherwise pretreated animals, rather than in animals that are intact, awake, and freely moving.

Supporting the alternative view that serotonin may serve mainly to inhibit respiration, rather than to stimulate it, serotonin synthesis inhibitors have been shown to induce hyperventilation without affecting the response to CO₂ (Annerbrink et al., 2003; Bach et al., 1993, Mitchell et al., 1983; Olson, 1987). In line with this, tryptophan depletion in humans, which leads to impaired serotonergic transmission, enhance ventilation without influencing CO₂ sensitivity (Struzik et al., 2002).

Given that serotonergic neurons influence the regulation of respiration, and that panic disorder is characterized by dysregulated respiration, it is tempting to speculate that an influence of SRIs on ventilation may contribute to the marked effects of these drugs when administered to panic disorder patients, including both the anxiety-provoking effect observed at acute treatment and the impressive anti-panic effect obtained by subchronic administration. In a previous study, we showed that administration of the SRI paroxetine for 5 or 15 weeks increased respiratory rate, but not ventilation, in awake, unrestrained, freely moving rats (Olsson et al., 2004). However, this study did not address the possible effect of acute administration. The aim of the present study was to compare the respiratory effect of acute SRI administration with that of chronic treatment, that is, that it would reduce respiratory rate, just as acute SRI administration exerts the opposite effect to that of chronic treatment on anxiety in panic disorder patients. In addition, we assessed the influence of acute administration of the serotonin-releasing, panic-provoking agents mCPP and d-fenfluramine, the a priori hypothesis being that these drugs too would exert an effect on respiration opposite to that of subchronic SRI administration, that is that they would reduce respiratory rate. Finally, the effects of acute administration of 5-HT1A receptor agonists and antagonists on respiration were assessed.

Materials and methods
Ethics
The study has been carried out in accordance with the Guide for the Care and Use of Laboratory Animals, as adopted and promulgated by the NIH, and was approved by the Ethics Committee for Animal Experiments, University of Gothenburg, Sweden.

Animals
Male Wistar rats were obtained from B&K (Sollentuna, Sweden), and housed in cages with 3–4 rats per cage under controlled conditions: temperature 21–2°C, humidity 55–65%, and reversed 12/12 h light/dark cycle. Food and water were available ad libitum at all times except during the experiments.

Respiratory measurement
All experiments took place in a silent room with lights on and an observer placed approximately 1 m from the rat. Two different pieces of equipment were used to measure respiratory rate and tidal volume in freely moving rats. For experiments 1, 2, 4, 5, and 7, a plethysmograph, allowing the animals to move freely, that was built within the department and described in detail previously (Annerbrink et al., 2003) was used. For experiments 3 and 6, the Unrestrained Whole Body Plethysmograph from Buxco Research Systems (Wilmingtom, NC, USA) was used. Before the day of the acute experiment, the animals were habituated to the plethysmograph on three different days (15 min + 15 min + 15 min). Before recording started on the day of the experiment, the animals were rated by means of gross observation with respect to motor activity. Animals that were not still after having spent 10–15 minutes in the plethysmograph were disqualified, as were those moving during the 2 minutes of registration of respiratory rate (breaths per min = BPM), tidal volume (ml/breath), and minute ventilation (ml/min). The rater was blind as to whether the rat was given saline or active substance.

Drugs
All drugs were acquired from Sigma-Aldrich, Stockholm, Sweden, and administered via intraperitoneal injections.
Serum concentrations

Serum concentrations of fluoxetine and norfluoxetine were assessed using high-performance liquid chromatography followed by liquid chromatography tandem mass spectrometry at the Division of Clinical Chemistry and Pharmacology, Lund University Hospital.

Experiments

Experiment 1: 40 male Wistar rats were randomized into 4 groups and received saline or fluoxetine (1, 10, or 30 mg/kg). Measurements took place 1 h after the injection. After the recordings, the rats were decapitated so that trunk blood for analysis of fluoxetine and norfluoxetine levels in serum could be obtained.

Experiment 2: 15 male Wistar rats received saline and paroxetine (10 mg/kg) in a randomized cross-over design with a minimum wash-out period of 10 days. Measurements took place 1 h after the injection.

Experiment 3: 40 male Wistar rats were randomized into 4 groups and received saline or 8-OH-DPAT (0.01, 0.1, or 0.15 mg/kg). Measurements took place 1 h after the injection.

Experiment 4: 15 male Wistar rats received saline or mCPP (5, 10, or 20 mg/kg). Measurements took place 1 h after the injection. To reduce the number of rats used for this experiment, the same animal could receive more than one treatment, but no animal received more than two treatments, and there was always a wash-out period of 10 days between treatments. In total, 15 rats were used.

Experiment 5: 20 male Wistar rats were randomized into 2 groups and received either saline or d-fenfluramine (5 mg/kg).

Experiment 6: 40 male Wistar rats were randomized into 4 groups: saline + saline, saline + fluoxetine (10 mg/kg), WAY 100635 (1 mg/kg) + saline, and WAY 100635 (1 mg/kg) + fluoxetine (10 mg/kg). All rats received 2 injections with a 20 min interval. Measurements took place 1 h after the second injection.

Experiment 7: 30 male Wistar rats received daily injections of saline or fluoxetine (10 mg/kg) for 30 days. Respiration was measured on days 2, 4, 9, 16, 23, and 30, and took place 24 h after the latest injection. On day 30, the rats were decapitated so that trunk blood for analysis of fluoxetine and norfluoxetine in serum could be obtained. Eleven additional rats (that were not assessed with respect to respiration) were treated with fluoxetine (10 mg/kg) daily and decapitated on days 2 (n = 4), 8 (n = 4), and 14 (n = 3) for determination of fluoxetine and norfluoxetine levels in serum.

Statistics

Differences between groups in experiments 1, 3, 4, and 6 were statistically evaluated using ANOVA followed by Fisher’s PLSD test. In experiments 2 and 5, statistical differences were evaluated using paired and unpaired t-tests, respectively. Since rats not being still before the registration were disqualified, recordings were not obtained from all animals at all time points in experiment 7, the missing values precluding the use of two way ANOVA when analysing the effect of treatment. Instead, the two groups were compared for each time of assessment using unpaired t-test. All values are expressed as mean (±SD). While p-values from ANOVA are presented in the results section, p-values from Fisher’s PLSD test and t-tests are shown in the tables. p ≤ 0.05 was considered statistically significant.

Results

Experiment 1: administration of fluoxetine (1, 10, and 30 mg/kg) 1 h before recording reduced respiratory rate (ANOVA: F = 5.4, p = 0.005) and minute ventilation (ANOVA: F = 3.9, p = 0.02) but not tidal volume (ANOVA: F = 0.9, p = 0.5). Post hoc comparisons (Fisher’s PLSD) revealed a significant reduction of respiratory rate after fluoxetine administration in doses of 10 and 30 mg/kg but not in the dose of 1 mg/kg, and of minute ventilation in the dose of 30 mg/kg but not in the doses of 1 or 10 mg/kg (Table 1). Ten rats were disqualified (see Materials and methods). Mean serum fluoxetine and norfluoxetine concentrations (nmol/L) 1 h after fluoxetine administration were as follows: 1 mg/kg (n = 10): fluoxetine 50.1 ± 35.7, norfluoxetine 88.2 ± 15.2; 10 mg/kg (n = 9): fluoxetine 1092.8 ± 315.9, norfluoxetine 1203.2 ± 308.2; 30 mg/kg (n = 7): fluoxetine 3598.5 ± 425.4, norfluoxetine 1371.8 ± 245.9.

Experiment 2: administration of paroxetine (10 mg/kg) 1 h before recording significantly reduced respiratory rate. Neither tidal volume nor minute ventilation was significantly altered (Table 2). Seven rats were disqualified.

Experiment 3: administration of 8-OH-DPAT (0.01, 0.1, and 0.15 mg/kg) 1 h before registration did not influence respiratory rate (ANOVA: F = 0.4, p = 0.8), tidal volume (ANOVA: F = 1.4, n = 0.3) or minute ventilation (ANOVA: F = 1.1, p = 0.4) (Table 3). Four rats were disqualified.

Experiment 4: administration of mCPP (5, 10 and 20 mg/kg) 1 h before registration reduced respiratory rate (ANOVA: F = 44.6, p < 0.0001) and tidal volume (ANOVA: F = 4.9, p = 0.006), but not minute ventilation (ANOVA: F = 0.4, p = 0.7). Post hoc comparisons (Fisher’s PLSD)

Table 1. Respiratory response to fluoxetine

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Respiratory rate (mean ± SD)</th>
<th>Tidal volume (mean ± SD)</th>
<th>Minute volume (mean ± SD)</th>
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<tbody>
<tr>
<td>Saline</td>
<td>9</td>
<td>121.9 ± 9.6</td>
<td>1.4 ± 0.4</td>
<td>171.7 ± 47.7</td>
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<tr>
<td>Fluoxetine 1 mg/kg</td>
<td>6</td>
<td>114.8 ± 7.0</td>
<td>1.5 ± 0.4</td>
<td>165.3 ± 37.7</td>
</tr>
<tr>
<td>Fluoxetine 10 mg/kg</td>
<td>8</td>
<td>107.0 ± 15.4</td>
<td>1.3 ± 0.3</td>
<td>134.7 ± 32.7</td>
</tr>
<tr>
<td>Fluoxetine 30 mg/kg</td>
<td>7</td>
<td>96.7 ± 9.3</td>
<td>1.2 ± 0.3</td>
<td>114.4 ± 22.6</td>
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Fisher’s PLSD: 1 p = 0.03, 2 p = 0.0007, 3 p = 0.05, 4 p = 0.005 as compared with saline.
revealed significant effects of mCPP on respiratory rate (reduction) and tidal volume (increase) in the doses of 5, 10, and 20 mg/kg (Table 4). Ten rats were disqualified.

Experiment 5: administration of d-fenfluramine (5 mg/kg) decreased respiratory rate and increased tidal volume, but did not affect minute ventilation (Table 5). Two rats were disqualified.

Experiment 6: administration of fluoxetine (10 mg/kg), WAY 100635 (1 mg/kg), or WAY 100635 (1 mg/kg) + fluoxetine (10 mg/kg) 1 h before registration influenced respiratory rate (ANOVA: F = 11.3, p < 0.0001) and tidal volume (ANOVA: F = 5.6, p = 0.02), but not minute ventilation (ANOVA: F = 2.7, p = 0.06). Post hoc comparisons (Fisher’s PLSD) revealed a significant reduction of respiratory rate after fluoxetine, WAY 100635, and WAY 100635 + fluoxetine, and an increase in tidal volume after WAY 100635 (p = 0.01) (Table 6). No rats were disqualified.

Experiment 7: rats treated for 23 or 30 days with fluoxetine displayed a significant reduction in respiratory rate and increase in tidal volume as assessed 24 h after drug administration (ANOVA: F = 7.1, p = 0.001).
In line with our hypothesis, fluoxetine and paroxetine exerted an effect on respiration the opposite of that observed after chronic treatment, that is a reduction in respiratory rate. Rats receiving the high doses of fluoxetine also displayed a reduction in minute ventilation, but otherwise no such effect was observed, since the reduction in respiratory rate was usually accompanied by an increase in tidal volume. Our previous observation that subchronic administration of an SRI influences respiratory rate in the opposite direction, that is enhances it, was confirmed; a significant increase in respiratory rate was thus observed after 23 days of administration of fluoxetine and onwards.

Acute administration of SRIs increases synaptic levels of serotonin by blocking the reuptake inhibitor (Stahl, 1998). At the same time, however, these compounds induce an effective silencing of the serotonergic nerve cell activity, elicited by enhanced activation of autoreceptors of the 5HT1A subtype (Aghajanian et al., 1970; Blier and de Montigny, 1985). If serotonergic neurons serve as chemoreceptors, exerting a CO2-driven stimulation of respiration, such an inhibition of serotonergic cell firing may, tentatively, lead to a reduction in respiratory rate. The observed effect of acute administration of SRIs on respiratory rate could hence be due either to enhanced extracellular concentrations of serotonin, which would support the theory that serotonin mainly inhibits respiration, or to a feed-back inhibition of these neurons, impairing their capability to respond to CO2 fluctuations in a dynamic manner, which would support the opposite view, that is that serotonin is a respiratory stimulant.

<table>
<thead>
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<th>Table 7. Respiratory response to chronic administration of fluoxetine</th>
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<td><strong>A</strong></td>
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<td>Day 2</td>
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<td>Day 16</td>
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<td>Day 23</td>
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<td>Day 30</td>
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| **B**  | Day of experiment | n | Fluoxetine (10 mg/kg) (mean ± SD) | n | Saline (mean ± SD) | p-value (unpaired t-test) |
| Day 2  | 14              | 1.4 ± 0.4 | 12  | 1.8 ± 0.7 | 0.09 |
| Day 4  | 14              | 1.3 ± 0.3 | 13  | 1.3 ± 0.3 | 0.7 |
| Day 9  | 13              | 1.3 ± 0.3 | 15  | 1.4 ± 0.3 | 0.4 |
| Day 16 | 10              | 1.2 ± 0.3 | 10  | 1.4 ± 0.5 | 0.4 |
| Day 23 | 14              | 1.3 ± 0.3 | 13  | 1.6 ± 0.5 | 0.04 |
| Day 30 | 12              | 1.7 ± 0.3 | 9   | 2.3 ± 0.8 | 0.02 |

| **C**  | Day of experiment | n | Fluoxetine (10 mg/kg) (mean ± SD) | n | Saline (mean ± SD) | p-value (unpaired t-test) |
| Day 2  | 14              | 142.0 ± 27.9 | 12  | 159.1 ± 42.4 | 0.2 |
| Day 4  | 14              | 144.7 ± 32.7 | 13  | 133.7 ± 37.1 | 0.4 |
| Day 9  | 13              | 148.4 ± 29.2 | 15  | 138.6 ± 31.0 | 0.4 |
| Day 16 | 10              | 146.3 ± 36.6 | 10  | 152.8 ± 41.6 | 0.7 |
| Day 23 | 14              | 170.4 ± 38.3 | 13  | 162.5 ± 43.7 | 0.6 |
| Day 30 | 12              | 200.9 ± 26.7 | 9   | 207.0 ± 39.0 | 0.7 |

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<th>Table 8. Serum concentrations of fluoxetine and norfluoxetine after chronic fluoxetine administration</th>
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<td><strong>Group</strong></td>
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<td>Day 8</td>
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<td>Day 14</td>
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<td>Day 30</td>
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To address the latter of these alternative possibilities, we administered the 5-HT1A agonist 8-OH-DPAT, which effectively reduces the firing rate in serotonergic neurons (Blier and de Montigny, 1990). No support was, however, obtained for the theory that the effect of SRIs is secondary to 5-HT1A-mediated inhibition of serotonergic neurons, since there was no effect of 8-OH-DPAT, administered at doses activating 5-HT1A autoreceptors without influencing postsynaptic 5-HT1A receptors (Forster et al., 1995; Sharp et al., 1989). Notably, previously a dose-dependent increase in respiratory rate has been observed after administration of 8-OH-DPAT to awake, freely moving guinea pigs (Stone et al., 1993). Although partly different from our observation, probably due to the use of higher doses of the drug, this finding also argues against the notion that inhibition of serotonergic cell firing reduces respiratory rate. Also in rat, a stimulatory influence of 5-HT1A agonists on respiration has been reported, but these studies have been undertaken using animals that have been asleep or the subject of various pretreatments (Choi et al., 2005; Edwards et al., 1990; Mendelson et al., 1990; Valic et al., 2008; Wang et al., 2007).

To assess the alternative explanation of the effect of acute administration of SRIs on respiratory rate, that is that it is secondary to enhanced synaptic output, two compounds known to effectively increase extracellular serotonin concentrations by reversing the direction of the serotonin transporters, that is mCPP and d-fenfluramine (Eriksson et al., 1999; Laferriere and Wurtman, 1989), were administered. The observation that both these compounds caused a robust decrease in respiratory rate, that is the same effect as that observed after fluoxetine and paroxetine, supports the notion that the effects of acute SRI administration on respiratory rate are indeed secondary to an increase in extracellular concentrations of serotonin.

WAY-100635 is a 5HT1A antagonist that enhances the firing of some but not all serotonergic neurons in awake animals (Fornal et al., 1996; Kasamo et al., 2001; Mlinar et al., 2005). If it is presumed that respiration-regulated serotonergic neurons are among those that are under tonic inhibitory influence of serotonin, and hence the subject of enhanced firing when exposed to WAY-100635, the observation that WAY-100635 elicited the same effect as fluoxetine, paroxetine, mCPP, and d-fenfluramine, that is a reduction in respiratory rate, lends further support for the notion that serotonin exerts an inhibitory influence on this parameter. The outcome of the WAY-100635 experiment may, however, seem contradictory to the lack of effect of the 5HT1A agonist 8-OH-DPAT on this parameter, the simplest explanation being that respiratory rate-modulating serotonergic neurons are equipped with inhibitory 5HT1A receptors, but that these are saturated by the endogenous transmitter in awake rats, and hence not further activated by the administration of an agonist.

When WAY-100635 is co-administered with an SRI, the former drug may counteract the inhibitory influence of the latter on serotonergic cell firing, and hence potentiate the stimulatory influence on extracellular levels of serotonin (Arborelius et al., 1996; Hjorth, 1993; Invernizzi et al., 1996). The finding that WAY-100635 plus fluoxetine did not reduce respiratory rate significantly more than did either drug alone (although the effect of the combination was numerically larger) suggests that respiratory rate-modulating serotonergic neurons are not inhibited by SRIs and hence is in line with the finding with 8-OH-DPAT. Notably, there was, however, a non-significant tendency for a reduction in minute ventilation in the group given both compounds (ANOVA: F = 2.7, p = 0.06; Fisher’s PLSD: p = 0.07), but not in the groups receiving only one of the two drugs. The fact that WAY-100635 did not counteract the effect of fluoxetine on respiratory rate suggests that SRI-induced reduction in respiratory rate is not mediated by postsynaptic 5-HT1A receptors.

Acute administration of various compounds having in common that they enhance extracellular serotonin concentrations thus reduces respiratory rate in freely moving, unrestrained, quiet but awake male rats. Since this effect is usually accompanied by an increase in tidal volume, it seldom leads to a significant reduction in minute ventilation; the results hence suggest that serotonin modulates the respiratory pattern rather than inhibits breathing. The effects on respiratory rate were, however, more clear-cut than those on tidal volume, and did sometimes lead to a reduction in minute ventilation, hence justifying the assumption that the treatment primarily influences respiratory rate, and that the effects on tidal volume are secondary to this influence, rather than vice versa. The present results thus challenge the notion that the influence of serotonin on respiration is predominantly stimulatory (see Introduction), but rather suggest that serotonin, in the awake, non-stressed rat, reduces respiratory rate but increases tidal volume, the net effect on minute ventilation being none or inhibitory. This notion also gains support from the previous observation that the serotonin synthesis inhibitor PCPA causes an increase rather than a decrease in minute ventilation (Annerbrink et al., 2003). Notably, this effect was, however, due to an increase in tidal volume rather than respiratory rate. One possible explanation would be that serotonin at normal synaptic concentrations mainly serves to keep tidal volume low, but at higher levels acts as an inhibitor of respiratory rate.

We have previously reported that administration of paroxetine for five weeks or more enhances respiratory rate in freely moving awake animals, that is the drug given subchronically exerts an effect opposite to that observed when it was given acutely in the present study (Olsson et al., 2004). This observation to some extent contrasts with a report by Henderson and co-workers suggesting that repeated administration of a low dose of fluoxetine do not influence baseline respiratory rate in goats (Henderson et al., 1999). Our present observation that also long-term administration of fluoxetine enhances respiratory rate in rat, however, confirms our previous finding with paroxetine. The effect of fluoxetine was significant when treatment had been ongoing for 23 days, but not seen after 16 days, and is hence consistent with a previous study failing to observe an effect on respiration in rats of 15 days of systemic fluoxetine administration (Taylor et al., 2004).

As shown in this study, subchronic administration of fluoxetine led to a gradual and marked increase in serum levels of both the substance itself and its active metabolite norfluoxetine as measured 24 h after the latest injection (that is at the time of respiratory recording). The high concentrations obtained after subchronic treatment could not,
however, solely explain the increase in respiratory rate, since the fluoxetine and norfluoxetine levels observed 60 min after acute administration (1 h) were even higher, and did not result in enhanced respiratory rate, but had the opposite effect.

An issue that has been debated for a long time is why the symptom-reducing effect of SRIs in depression and in a number of anxiety disorders requires weeks of treatment to be observed. Some have argued that SRIs exert only weak effects on extra-cellular serotonin levels at acute administration due to the activation of an inhibitory feed-back influence on nerve cell activity and transmitter release, and that this feed-back is abated during long-term administration, leading to a gradual augmentation of the influence of the drug on serotonergic output (Stahl, 1998). An alternative theory, however, suggests that the stimulatory effect of acute administration of an SRI on serotonergic output after a few weeks of treatment is reversed to its opposite in synapses regulating, for example, anxiety, as the result of various adaptive mechanisms (such as down-regulation of postsynaptic receptors) (Stahl, 1998). The observation that SRIs in patients with panic disorder are not only devoid of anti-panic effects during the first days of treatment, but initially often aggravate anxiety (Nutt and Glue, 1989; Pohl et al., 1988), may be taken as support for the latter view, as may the observation that drugs causing a marked and immediate increase in synaptic serotonin concentrations, such as fenfluramine and mCPP, do not exert acute anti-anxiety effects, but, on the contrary, may elicit anxiety in patients with panic disorder (Charney et al., 1987; Mortimore and Anderson, 2000). The effect of subchronic administration of an SRI in anxiety-modulating synapses being opposite in direction to that of acute administration is also strengthened by the recent findings that acute SRI administration to man enhances amygdala reactivity (Bigos et al., 2008) whilst subchronic treatment reduces it (Harmer et al., 2006). The present observation, that the effect of acute administration of an SRI on respiratory rate in rat is opposite to that of subchronic administration, thus adds to an already existing body of data suggesting that the effect of subchronic treatment with SRIs on serotonergic output, at least in some neuronal circuits, is opposite in direction to that observed after acute administration. Of interest in this context is a recent finding suggesting that tryptophan depletion does not increase proneness to panic in SRI-treated panic disorder patients (Toru et al., 2006); however, the literature on this issue is not unanimous (Bell et al., 2002).

As discussed above, numerous observations support the notion that panic attacks may be caused by dysregulated respiration (see Introduction). To what extent the effects of serotonin-modulating drugs on respiration revealed by our study are of importance for the influence of these drugs on anxiety in panic disorder patients remains an open question, but it is notable that the acute effect of SRIs on respiratory rate in rat, just as in the effect on anxiety in panic disorder patients, was found to be opposite to that of subchronic treatment, and that panic-provoking serotonin-releasing agents exerted an effect similar to that of acute SRI exposure. In this context, the lack of effect of low doses of 8-OH-DPAT on respiratory rate is well in line with the observation that 5HT1A agonists neither reduce panic attacks nor elicit them (Sheehan et al., 1993; Van Vliet et al., 1996). Clarifying the possible role of respiration for the anxiogenic and anti-panic effects of serotonin modulators will, however, probably require further insight into the possible role of respiratory control for the generation of panic attacks. Notably, not only serotonin, but also endogenous opioids (Preter and Klein, 2008) and acetylcholine (Battaglia, 2002) have recently been attributed importance in this context.

In conclusion, acute administration of the SRIs fluoxetine and paroxetine, the 5HT1A-antagonist WAY-100635, and the serotonin releasing agents d-fenfluramine and mCPP all caused a significant reduction in respiratory rate when given to freely moving rats. Our observation that subchronic administration of an SRI, on the other hand, enhanced respiratory rate, lends further support to the notion that the effect of long-term administration of SRIs on the serotonergic output in certain brain regions is opposite to that observed after acute administration.

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Statement of interest
None.

References


