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Oxazepam and temazepam attenuate paroxetine-induced elevation of serotonin levels in guinea-pig hippocampus

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Abstract

Selective serotonin (5-HT) reuptake inhibitors (SSRIs) are used as a first-line treatment in depression. However, many depressed patients are also treated with benzodiazepines to alleviate increased anxiety and sleep disturbances normally associated with depression. Since benzodiazepines inhibit 5-HT neuronal firing activity, they might attenuate SSRI-induced increase in extracellular 5-HT levels. This study aimed to assess, using *in-vivo* microdialysis, the effects of the benzodiazepines oxazepam or temazepam on the SSRI paroxetine-induced 5-HT increase in the hippocampus of freely moving guinea-pigs. It was found that the acute systemic administration of paroxetine increased extracellular 5-HT levels. Pre-administration of oxazepam or temazepam significantly diminished the paroxetine-induced elevation of extracellular 5-HT levels (from 350% to 200% of baseline). It was concluded that benzodiazepines attenuate the ability of SSRIs to elevate hippocampal 5-HT levels. Thus, co-administration of benzodiazepines might affect the therapeutic efficacy of SSRI treatment.

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Key words: Hippocampus, microdialysis, oxazepam, selective serotonin reuptake inhibitors (SSRIs), temazepam.

Introduction

Selective serotonin (5-HT) reuptake inhibitors (SSRIs) and benzodiazepines are used as antidepressants and sedative medications, respectively. SSRIs are currently considered as a first-line treatment in depression (Kennedy, 2006). Benzodiazepines are given, in addition to SSRIs to about 35% of depressed patients, to alleviate increased sleep disturbances, anxiety and irritation normally associated with depression (Carvalho *et al.* 2007; Youssef & Rich, 2008).

The main pharmacological effect of SSRIs is an increase in extracellular 5-HT levels by selective blocking of 5-HT transporters (SERT). The increased extracellular 5-HT level results in the activation of

5-HT_{1A} and 5-HT_{1B} autoreceptors, in turn leading to suppression of the firing activity of 5-HT neurons. This suppression of firing activity of 5-HT neurons challenges the ability of SSRIs to stimulate 5-HT transmission. After 2–3 wk of SSRI administration, 5-HT_{1A/1B} autoreceptors desensitize and the firing activity of 5-HT neurons recovers to pre-treatment levels. This explains, at least in part, the delay between start of the treatment with SSRIs and the onset of their clinical effect (Mongeau *et al.* 1997; Pineyro & Blier, 1999).

It has been previously shown that benzodiazepines inhibit 5-HT transmission via a γ -aminobutyric (GABA)_A receptor-mediated mechanism (Lista *et al.* 1989, 1990). Since the inhibition of 5-HT neurons attenuates 5-HT release, it is possible that benzodiazepines, co-administered with SSRIs, will challenge the ability of SSRIs to elevate extracellular 5-HT levels. The present study aimed to assess, using *in-vivo*

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microdialysis, the effects of the benzodiazepines oxazepam or temazepam on the SSRI paroxetine-induced 5-HT increase in the hippocampus of freely moving guinea-pigs. Paroxetine was chosen for this study since it is a widely used and very potent SSRI (Mertens & Pintens, 1988). Oxazepam and temazepam represent the newest generation of benzodiazepines. These fast-acting sedative drugs are widely used because of their high safety and low side-effects (Breimer *et al.* 1980; McElnay *et al.* 1982).

Methods

Animals

Male albino Dunkin Hartley guinea-pigs (300–400 g; Harlan, The Netherlands) were used in this study. The animals were housed in cages (60 × 40 × 40 cm) with food and water available *ad libitum*. The experiments were in accord with the Declarations of Helsinki and were approved by the Institutional Animal Care and Use Committee of the University of Groningen.

Drugs

Paroxetine hydrobromide (GlaxoSmithKline, UK) was dissolved in ultrapurified water and injected subcutaneously (s.c.) at a dose of 5 mg/kg. The paroxetine dose was chosen based on our previous studies (Cremers *et al.* 2001). Oxazepam and temazepam (Bufa BV, The Netherlands) were dissolved in 10% (v/v) solutol (a polyoxyethylene ester of 12-hydroxystearic acid) and ultrapurified water and administered (s.c.) at a dose of 0.3 mg/kg each. In the control experiments where compounds were administered individually, the appropriate vehicle control for the combination was co-administered.

Surgery

Animals were pre-medicated with midazolam as analgesic (5 mg/kg s.c.) and anaesthetized by ketamine and xylazine (50 and 8 mg/kg, respectively, i.p.). Lidocaine-HCl, 10% (w/v) was used for local anaesthesia. The animals were placed in a stereotaxic frame (Kopf, USA), and homemade I-shaped probes (polyacrylonitrile/sodium methyl sulphonate co-polymer dialysis fibre; 4 mm open surface, i.d. 220 µm, o.d. 310 µm, AN69, Hospal, Bologna, Italy) were inserted into the ventral hippocampus (4.9 mm posterior and 6.5 mm lateral from bregma, 9.0 mm ventral to the brain surface) and secured with dental cement. Post-operative analgesia was accomplished by an intramuscular injection of 0.1 mg/kg buprenorphine.

Microdialysis

Microdialysis experiments were performed on freely moving guinea-pigs, as previously described (Cremers *et al.* 2001). Briefly, the animals were allowed to recover for at least 24 h after surgery, before the probes were perfused with artificial cerebrospinal fluid (aCSF; 147 mM NaCl, 3.0 mM KCl, 1.2 mM CaCl₂, 1.2 mM MgCl₂) delivered at flow rate of 1.5 µl/min (Harvard apparatus, USA). The samples were collected continuously in 15-min intervals into vials containing 7.5 µl of 20 µM acetic acid.

5-HT assay

Concentrations of 5-HT in microdialysates were measured by high-performance liquid chromatography with electrochemical detection, as previously described (Cremers *et al.* 2001). Briefly, samples of 20 µl were injected onto a reversed-phase column (Phenomenex Hypersil 3:3 µm, 100 × 2.0 mm, C18; Bester BV, The Netherlands) by an auto-injector (CMA/200 refrigerated microsampler, CMA Microdialysis, Sweden). The mobile phase consisted of 5 g/l di-ammonium sulfate, 500 mg/l ethylenediaminetetraacetic acid (EDTA), 50 mg/l heptane sulphonic acid (pH 4.65) plus 4% methanol (v/v) and 30 µl/l triethylamine. The mobile phase was delivered at a flow rate of 0.4 ml/min (Shimadzu LC-10 AD). 5-HT was detected electrochemically by a glassy carbon electrode set at a working potential of 500 mV *vs.* Ag/AgCl (Antec Leyden, The Netherlands). The detection limit was 0.5 fmol 5-HT per 20 µl sample (signal-to-noise ratio: 3).

Data presentation and statistics

Four consecutive microdialysis samples with <20% variation were taken as baseline and set at 100%. Data are presented as percentage of baseline level (mean ± s.e.m.). Statistical analysis was performed using Sigma-Stat for Windows (SPSS Inc., USA). Treatment effects were compared *vs.* saline treatment using two-way ANOVA for repeated measurements, followed by Bonferroni's *post-hoc* test. Level of significance was set at $p < 0.05$.

Results

The basal levels of 5-HT in the hippocampal dialysates were 8.87 ± 0.84 fmol/sample ($n = 27$). Figure 1 shows the effects of oxazepam and paroxetine on extracellular 5-HT levels. There were significant effects of time [$F(12, 246) = 89.59$, $p < 0.001$], treatment [$F(2, 246) = 60.39$, $p < 0.001$], and time × treatment interaction

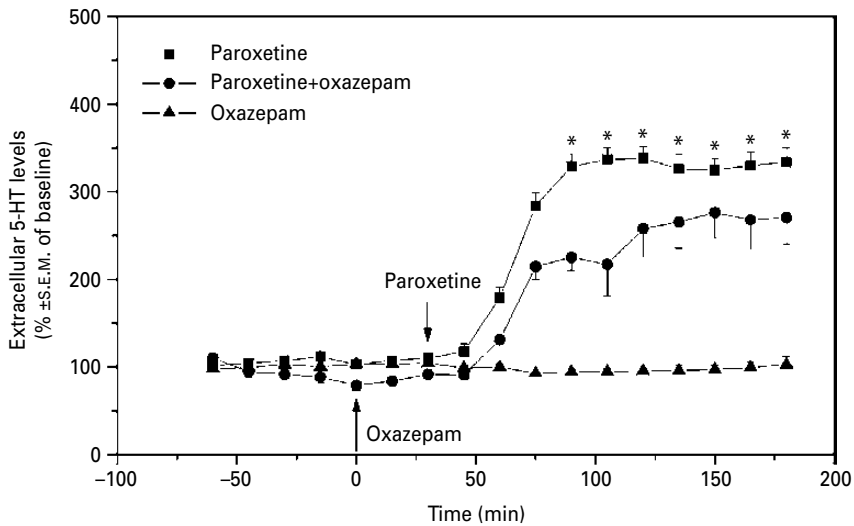


Fig. 1. Serotonin (5-HT) levels in hippocampal microdialysate of guinea-pigs administered oxazepam ($t=0$), paroxetine ($t=30$ min) or oxazepam + paroxetine (at $t=0$ and 30 min, respectively). * $p < 0.05$ compared to baseline, and compared between paroxetine- and paroxetine + oxazepam-treated animals (Bonferroni's *post-hoc* test).

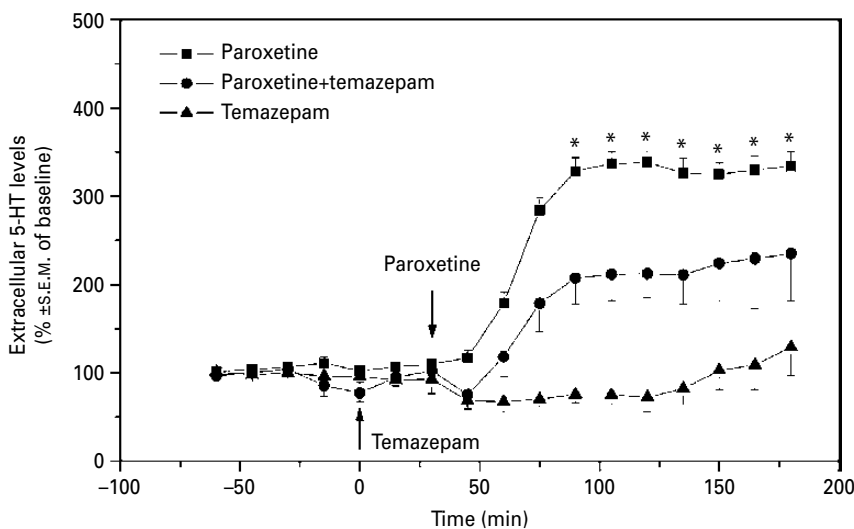


Fig. 2. Serotonin (5-HT) levels in hippocampal microdialysate of guinea-pigs administered temazepam ($t=0$), paroxetine ($t=30$ min) or temazepam + paroxetine (at $t=0$ and 30 min, respectively). * $p < 0.05$ compared to baseline, and compared between paroxetine- and paroxetine + oxazepam-treated animals (Bonferroni's *post-hoc* test).

[$F(24, 246) = 29.44$, $p < 0.001$]. There was no significant change in 5-HT levels in animals administered oxazepam alone ($n=5$). In animals administered paroxetine ($n=10$) and paroxetine + oxazepam ($n=4$) there was a significant increase in 5-HT levels compared to baseline ($p < 0.05$ for the time-points 50–180 min and 65–180 min after paroxetine administration). However, the elevation in 5-HT levels was significantly higher in animals administered

paroxetine alone than in animals co-administered paroxetine + oxazepam ($p < 0.05$ for the time-points 50–180 min after paroxetine administration).

Figure 2 shows the effects of temazepam and paroxetine on extracellular 5-HT levels. There were significant effects of time [$F(12, 233) = 49.34$, $p < 0.001$], treatment [$F(2, 233) = 36.50$, $p < 0.001$], and time \times treatment interaction [$F(24, 233) = 15.79$, $p < 0.001$]. There was no significant change in 5-HT levels in

animals administered temazepam alone ($n=4$). In animals administered paroxetine ($n=10$) and paroxetine+temazepam ($n=4$) there was a significant increase in 5-HT levels compared to baseline ($p<0.05$ for the time-points 65–180 min after paroxetine administration). However, the elevation in 5-HT levels was significantly higher in animals administered paroxetine alone than in animals co-administered paroxetine+temazepam ($p<0.05$, for the time-points 65–180 min after paroxetine administration).

Discussion

The present study aimed to assess, using *in-vivo* microdialysis, the effects of oxazepam or temazepam on the SSRI paroxetine-induced 5-HT increase in the hippocampus of freely moving guinea-pigs. It was found that paroxetine increased extracellular 5-HT levels. Oxazepam and temazepam, administered individually, did not alter hippocampal 5-HT levels. However, when these drugs were co-administered with paroxetine, they attenuated paroxetine-induced hippocampal 5-HT release. No statistical difference was observed between oxazepam and temazepam in their ability to diminish paroxetine-induced increase in hippocampal 5-HT levels.

The present study demonstrated that the SSRI paroxetine, given alone, results in a 350% increase in extracellular 5-HT levels in the guinea-pig hippocampus. This is probably due to inhibition of 5-HT reuptake by paroxetine. The paroxetine-induced increase in extracellular 5-HT, observed in the present study, is in agreement with previous studies using paroxetine and other SSRIs (Cremers *et al.* 2001; Dremencov *et al.* 2003). The paroxetine dose used was chosen based on a previous pharmacokinetic study with this drug (Cremers *et al.* 2001). In that study subcutaneous administration of 5 mg/kg paroxetine resulted in the steady-state presence of that drug in guinea-pig plasma, where lower doses failed to create a steady-state plasma concentration.

The present study demonstrated that oxazepam or temazepam, given alone, did not alter extracellular 5-HT levels in the guinea-pig brain. However, Pei *et al.* (1989) reported that benzodiazepines decrease extracellular 5-HT in the rat hippocampus. The difference between these findings might be caused by the different treatments used in the two studies. Here, oxazepam and temazepam were administered in low doses (0.3 mg/kg), whereas the study by Pei *et al.* (1989) was performed with flurazepam and diazepam administered at relatively high doses of 10 mg/kg each.

The main finding of the present study is the potency of benzodiazepines. When given in small doses they do not affect 5-HT transmission, but do attenuate SSRI-induced elevation in brain 5-HT levels. It is unlikely that the effects of oxazepam and temazepam on paroxetine-induced elevation of 5-HT levels are explained by pharmacokinetic interaction between these drugs: metabolism of paroxetine is mediated via 2D6, and metabolism of benzodiazepines via the subtype 3A4 of the enzyme cytochrome P450 (Nemeroff *et al.* 1996; Gregor *et al.* 1997).

It is possible that oxazepam and temazepam attenuate paroxetine-induced increase in 5-HT levels by suppressing the firing activity of 5-HT neurons. It was reported that the benzodiazepine diazepam, administered alone, does not affect the firing activity of 5-HT neurons in the rat dorsal raphe nucleus (DRN). However, pretreatment with 1 mg/kg diazepam potentiated the inhibitory effect of local GABA administration on the firing activity of 5-HT neurons in the DRN (Gallager, 1978). Another study demonstrated that the administration of 0.1 mg/kg diazepam reversed the inhibition of hippocampal neurons induced by the electrical stimulation of 5-HT pathways (Lista *et al.* 1989, 1990). This benzodiazepine-induced potentiation of GABA-mediated inhibition of 5-HT neurons might explain the oxazepam- and temazepam-induced attenuation of paroxetine-induced 5-HT release. On the other hand, this lack of a direct suppressive effect of benzodiazepines on the firing of 5-HT neurons possibly explains why oxazepam and temazepam do not totally reverse paroxetine-induced 5-HT release.

Since the ability to increase extracellular 5-HT levels suggests modulation of the clinical potency of SSRIs (Moore *et al.* 2005; Yevtushenko *et al.* 2007), co-administration of benzodiazepines to SSRI-managed patients may affect the therapeutic efficacy of the treatment. As many depressed patients receive benzodiazepines (Youssef & Rich, 2008), the dose of antidepressant should be carefully adjusted. In addition, inclusion of benzodiazepine-managed subjects into clinical trials with antidepressant drugs should be carefully considered (Bordet *et al.* 1998).

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Statement of Interest

None.

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