Chapter 8

Attenuation of SSRI-induced increases in extracellular brain 5-HT by benzodiazepines.
Abstract

Enhanced serotonergic neurotransmission is generally thought to be the neurochemical basis of the antidepressant effects of Selective Serotonin Reuptake Inhibitors (SSRIs). The anxiolytic benzodiazepines, on the other hand, have been shown to decrease serotonergic neurotransmission. Since depressed patients are frequently treated with a combination of SSRIs and benzodiazepines, we investigated the effects of co-administration of these drugs on extracellular levels of serotonin (5-HT) in guinea pig brain.

Using microdialysis of 5-HT in ventral hippocampus of freely moving guinea pigs, we investigated the effects of the typical benzodiazepines oxazepam and temazepam, alone and in combination with the SSRI paroxetine on extracellular levels of 5-HT.

Paroxetine alone increased extracellular 5-HT levels in hippocampus to about 350% of control values, whereas oxazepam and temazepam each produced small decreases to 90% and 70% of control levels, respectively when administered alone. The combined administration of paroxetine with oxazepam as well as temazepam significantly attenuated the increase of 5-HT levels induced by paroxetine.

Since the co-administration of SSRIs with benzodiazepines attenuates the serotonergic transmission, but enhances the clinical effects of SSRI’s in depressed patients, it might be questioned whether the mechanism of the antidepressant action of SSRI’s is completely due to enhanced serotonergic neurotransmission.
1. Introduction

Selective Serotonin Re-uptake Inhibitors (SSRIs) are generally believed to exert their antidepressant effects by enhancing serotonergic neurotransmission (Blier et al. 1987). Upon acute administration of SSRIs, extracellular brain 5-HT levels are increased (Fuller 1994), but these 5-HT enhancing effects are restricted by the counteraction of release modulating serotonergic autoreceptors. It has been shown that during chronic treatment these autoreceptors are desensitized, thereby potentiating the effect of SSRIs on brain 5-HT levels (Blier et al. 1987; Invernizzi et al. 1994). Since the gradual desensitization of autoreceptors is thought to produce, at least partly, the antidepressant effects of SSRIs, it was hypothesized that co-administration of an SSRI with an autoreceptor antagonist would instantaneously mimic this desensitization, and therefore reduce the time until onset of antidepressant action (Artigas 1993, Hjorth 1993). Indeed, clinical trials investigating the effects of co-administration of an SSRI with the putative 5-HT$_{1A}$ antagonist pindolol have shown promising results, indicative of the beneficial effects of augmented 5-HT levels in the treatment of depression (McAskill et al. 1998).

Depressed patients are often simultaneously treated with benzodiazepines because of comorbidity of depression and anxiety. Although patients who were being treated with other drugs are excluded from clinical trials that investigate the effect of SSRIs or combinations of SSRIs with pindolol, patients on benzodiazipines are usually included in these studies (Bordet et al. 1998; Perez et al. 1997). Previous preclinical studies had shown that administration of benzodiazepines decreases the extracellular levels of 5-HT in a wide variety of brain structures in rats and guinea pigs (Rex et al. 1993; Pei et al. 1989, Gibson et al. 1996). Since this 5-HT decreasing effect could possibly counteract the 5-HT increase produced by SSRIs, we investigated the effect of co-administration of the SSRI paroxetine with two commonly used benzodiazepines on extracellular 5-HT levels in guinea pig brain.
2. Materials and Methods

2.1 Animals and drug administration

Male albino guinea pigs of a Dünkin Hartley strain (300-400 g; Harlan, Zeist, The Netherlands were housed in cages (32 x 40 x 40 cm), and had free access to food and water. The experiments are concordant with the declarations of Helsinki and were approved by the animal care committee of the faculty of mathematics and natural science of the University of Groningen.

The following drugs were used: paroxetine (SKB, West Sussex, UK), Oxazepam and Temazepam (Bufa, the Netherlands). Paroxetine was dissolved in ultrapure water. Oxazepam and temazepam were dissolved in 10 % v/v solutol and ultrapure water. In experiments with a drug alone, the appropriate vehicle for the combination was injected before or after the drug.

2.2 Surgery

Preceding surgery, the animals were anaesthetised by means of an intraperitoneal injection of ketamine/xylazine (50/8 mg/kg), after premedication with midazolam (5 mg/kg s.c.). Lidocaine-HCl, 10 % (m/v) was used for local anaesthesia. The animals were placed in a stereotaxic frame (Kopf, USA), and home made I-shaped probes (polyacrylonitrile / sodium methyl sulphonate copolymer dialysis fibre; 4 mm open surface, i.d. 220 µm, o.d. 310 µm, AN 69, Hospal, Italy) were inserted into the ventral hippocampus (co-ordinates: IA: + 4.9 mm, lateral: +/− 6.5 mm, ventral: - 9.0 mm from the dura mater (Luparello, stereotaxic atlas) and secured with dental cement. Postoperative analgesia was accomplished by an intramuscular injection of 0.1 mg/kg buprenorphine.

2.3 Microdialysis experiments

Guinea pigs were allowed to recover for at least 24 h, after which the probes were perfused with artificial CSF (147 mM NaCl, 3.0 mM KCl, 1.2 mM CaCl₂, and 1.2 mM MgCl₂) at a flow-rate of 1.5 µl / min (Harvard apparatus, South Natick, Ma., USA). 15 minute samples were collected in vials containing 7.5 µl of 0.02 mM acetic acid.

2.4 5-HT assay

Concentrations of 5-HT in microdialysates were measured by HPLC with electrochemical detection. Twenty µl samples were injected onto a reversed phase column (Phenomenex Hypersil 3 : 3 µm, 100 x 2.0 mm, C18, Bester, Amstelveen, the Netherlands) by an autoinjector (CMA/200 refrigerated microsampler, Carnegie Medicine, Sweden). The mobile
phase consisted of 5 g/l di-ammoniumsulfate, 500 mg/l ethylene diamino tetra acetic acid (EDTA), 50 mg/l heptane sulphonic acid, 4 % methanol v/v, and 30 µl/l of triethylamine, at a pH of 4.65, and was delivered at a flow-rate of 0.4 ml/min (Shimadzu LC-10 AD liquid chromatograph). 5-HT was detected electrochemically at a glassy carbon electrode set at a working potential of 500 mV vs. Ag/AgCl (Antec Leyden, Leiden, the Netherlands). The detection limit was 0.5 fmol 5-HT per 20 µl sample (signal to noise ratio 3).

2.5 Data presentation and statistics

Four consecutive microdialysis samples with less than 20 % variation were taken as control and set at 100 %. Data are presented as percentages of control level (mean ± SEM). Statistical analysis was performed using Sigmastat for Windows (Jandel Corporation). Treatment effects were compared versus saline treatment using two way ANOVA for repeated measurements, followed by Student’s Newman Keuls post-hoc test. Treatment effects were compared versus basal values using one way ANOVA for repeated measurements on ranks. Level of significance level was set at $p<0.05$. 
3. Results

3.1 Serotonin basal levels:

Basal levels of 5-HT in dialysates from guinea pig ventral hippocampus were 8.87 ± 0.84 fmol/sample (mean ± S.E.M.) (n= 27). No significant differences were observed between the different treatment groups.

3.2 Oxazepam administration and paroxetine + oxazepam co-administration:

Figure 1 shows the effect of administration of oxazepam alone and in combination with paroxetine. Oxazepam alone significantly decreased extracellular levels of 5-HT in guinea pig ventral hippocampus to about 90 % of basal values (χ^2_{10} = 18.5, p<0.05). Paroxetine alone increased extracellular 5-HT levels to about 350 % of control values (χ^2_{10} = 28.9; p<0.05). Injection of oxazepam 30 minutes prior to administration of paroxetine significantly attenuated the 5-HT produced by paroxetine alone to 250% of basal levels (F(1,151) = 2.73; p<0.05).

Figure 1. Effect of administration of 5 mg/kg paroxetine (■, n = 10, vehicle t=0, paroxetine t=30), oxazepam 1µmol/kg (▲, n = 5 , oxazepam t=0, vehicle t=30), and paroxetine 5 mg/kg together with oxazepam (●, n = 4 ,oxazepam t=0, paroxetine t=30). * denote significant vs. paroxetine alone.
Figure 2. Effect of administration of 5 mg/kg paroxetine (■, n = 10, vehicle t=0, paroxetine t=30), temazepam 1µmol/kg (▲, n = 4 , temazepam t=0, vehicle t=30), and paroxetine 5 mg/kg together with temazepam oxazepam (●, n = 4 , temazepam t=0, paroxetine t=30). * denote significant vs. paroxetine alone.

3.3 Temazepam administration and paroxetine + temazepam co-administration:

Administration of temazepam alone induced a significant decrease of extracellular levels of 5-HT levels to about 70 % of basal values ($\chi^2_{10} = 20, p<0.05$). Similar to oxazepam, co-administration of temazepam with paroxetine induced a blunted response to paroxetine and extracellular 5-HT levels increased to only 225% ($F(1, 150) = 2.10; p<0.05$).
4. Discussion

The present data show that co-administration of benzodiazepines and the SSRI paroxetine attenuates the effect of the SSRI on brain extracellular 5-HT levels.

The few studies that investigated the effect of benzodiazepine administration on the serotonergic system showed that these drugs have an inhibitory influence on the firing rate of 5-HT neurons and decrease terminal 5-HT release in several brain structures of rats (Pei et al. 1989; Gibson et al., 1996) and guinea pigs (Rex et al. 1993). It has been suggested that this inhibitory effect of benzodiazepines on the serotonergic system is a consequence of enhanced GABAergic transmission, resulting from the binding of benzodiazepines to the benzodiazepine/GABA<sub>A</sub> receptor complex. (Pei et al. 1989).

As mentioned in the introduction, SSRIs are believed to exert their clinical effect by enhancing serotonergic neurotransmission. In support of this, clinical and preclinical studies have indeed shown evidence for the positive effects of enhanced serotonergic levels in treatment of depression. Analysis of literature revealed that patients treated for depression are frequently also treated with benzodiazepines and that in trials designed to evaluate the effects of enhanced serotonergic levels by co-administration of SSRIs with the autoreceptor antagonist pindolol, patients on benzodiazepines were included (Bordet et al. 1998; Perez et al. 1997).

It is clear from the present study that co-administration of benzodiazepines such as oxazepam and temazepam, attenuates the effect of SSRIs on extracellular 5-HT levels and thus the SSRI-induced effect on serotonergic transmission. Therefore if enhanced serotonergic neurotransmission is indeed responsible for the antidepressant action of SSRI’s, one would expect that co-administration of benzodiazepines to patients on SSRIs would also diminish the antidepressant effects. This effect however, is not observed in the clinic, in contrast, some authors have suggested that co-administration of an SSRI with a benzodiazepine is superior to SSRI treatment alone (Smith et al. 1998). Co-morbidity of anxiety with depression in addition to the well-known anxiolytic effect of benzodiazepine could be a feasible explanation for this apparent discrepancy (Keller et al. 1995). Furthermore, since some potent benzodiazepines have antidepressant activity of their own, this might further complicate the explanation of the overall clinical outcome of combined administration of both classes of compounds (Petty et al. 1995, Sussman 1998).

Theoretically, the observed pharmacological effects of co-administration of oxazepam and temazepam with paroxetine might be explained by pharmacokinetic interference (drug interactions) between the compounds. However, since oxazepam and temazepam are directly conjugated, interference does not seem likely between these specific benzodiazepines and paroxetine (Sproule et al. 1997).

Although the present findings seem to be at odds with the idea that enhanced serotonin levels are associated with an antidepressant response, the situation might be different after chronic treatment. Since chronic benzodiazepine treatment has been shown to result in subsensitivity of GABAergic receptors in the dorsal raphe nucleus (Wilson and Gallager 1988), these effects might counteract the inhibitory effects of GABA in the DRN and augment the SSRI induced 5-HT increases (Tao and Auerbach 1996).
It would require further preclinical studies to evaluate whether chronic benzodiazepine and SSRI co-administration attenuates the serotonergic effects of SSRIs (thus questioning the relevance of enhanced 5-HT levels for the antidepressant response), or that this combination rapidly desensitizes GABA-ergic mechanisms, leading to an augmented response to SSRIs.
References


Tao R., Ma Z., Auerbach S.B. (1996). Differential regulation of 5-hydroxytryptamine release by GABA\textsubscript{A} and GABA\textsubscript{B} receptors in midbrain raphe nuclei and forebrain of rats. British Journal of Pharmacology 119 : 1375-1384