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The anxiogenic drug FG-7142 increases serotonin metabolism in the rat medial prefrontal cortex

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Abstract

The neural mechanisms underlying anxiety states are believed to involve interactions among forebrain limbic circuits and brainstem serotonergic systems. Consistent with this hypothesis, FG-7142, a partial inverse agonist at the benzodiazepine allosteric site of the $GABA_A$ receptor, increases c-Fos expression within a subpopulation of brainstem serotonergic neurons. Paradoxically, FG-7142 has no effect on extracellular serotonin concentrations, as measured using in vivo microdialysis, in certain anxiety-related brain structures. This study tested the hypothesis that FG-7142 alters serotonin metabolism within one or more nodes of a defined anxiety-related forebrain circuit. Rats received one of four treatments (vehicle, 1.9, 3.8, or 7.5 mg/kg FG-7142, i.p.) and brains were collected 1 h following treatment. Thirteen forebrain regions were microdissected and analyzed for L-tryptophan, serotonin, and 5-hydroxyindoleacetic acid concentrations using high pressure liquid chromatography with electrochemical detection. FG-7142 (7.5 mg/kg) increased L-tryptophan, serotonin, and 5-hydroxyindoleacetic acid concentrations in the prelimbic cortex but not in several other regions studied including subdivisions of the amygdala and bed nucleus of the stria terminalis. These data demonstrate that FG-7142 alters brain tryptophan concentrations and serotonin metabolism in specific components of an anxiety-related forebrain circuit including the medial prefrontal cortex, an important structure involved in executive function and the regulation of emotional behavior.

Keywords: Anxiety; FG-7142; Serotonin; L-tryptophan; Medial prefrontal cortex

1. Introduction

The neural mechanisms underlying the physiological and behavioral effects of anxiogenic drugs are not thoroughly understood. However, recent studies suggest that multiple anxiogenic drugs with diverse pharmacological properties have convergent effects on a distributed anxiety-related neural system. Drugs, including *N*-methyl-beta-carboline-3-carboxamide (FG-7142), a partial inverse agonist at the benzodiazepine allosteric site of the $GABA_A$ receptor (Dorow et al., 1983), *m*-chlorophenylpiperazine (mCPP), a non-selective 5-HT_{2C} receptor agonist (Whitton and Curzon, 1990), caffeine, an adenosine receptor antagonist (Baldwin and File, 1989), and yohimbine, an α_2-adrenoceptor antagonist (Charney et al., 1983), increase the expression of the protein product of the immediate-early gene, *c-fos*, in multiple forebrain structures widely acknowledged to be part of a network mediating anxiety-related behavioral and physiological responses (Singewald et al., 2003). These structures include the basolateral and central amygdaloid nuclei, bed nucleus of the stria terminalis, dorsomedial hypothalamus, cingulate cortex, and infralimbic and prelimbic cortices (Gray and McNaughton, 2000; Millan, 2003; Walker et al., 2003). In addition, these anxiogenic drugs induce c-Fos expression in a subpopulation of serotonergic neurons within the dorsal raphe nucleus (Singewald and Sharp, 2000; Abrams et al., 2005).

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One mechanism through which anxiogenic drugs may influence anxiety states and anxiety-related behavior is via actions on brainstem neuromodulatory systems including serotonergic systems. Serotonin is widely implicated in the modulation of anxiety states (Andrews and File, 1993; Graeff et al., 1996a; Gray and McNaughton, 2000; Millan, 2003; Maier and Watkins, 2005; Lowry et al., 2005). In addition, recent studies suggest that anxiety-related stimuli, including treatment with anxiogenic drugs (Abrams et al., 2005), the anxiety-related neuropeptide urocortin 2, (Staub et al., 2005) or exposure to stress-or anxiety-related challenges such as social defeat (Gardner et al., 2005) or inescapable stress (Grahn et al., 1999) have selective actions on topographically organized subpopulations of serotonergic neurons within the mid-rostrocaudal and caudal part of the dorsal raphe nucleus. Subpopulations of serotonergic neurons in these regions of the brainstem raphe complex give rise to projections to specific forebrain regions involved in the regulation of anxiety states and anxiety-related behavior and physiology (Lowry et al., 2005). However, there is very limited knowledge on the effects of anxiogenic drugs such as FG-7142 on serotonin metabolism or neurotransmission within anxiety-related forebrain structures.

FG-7142 induces an increase in anxiety state as measured in a variety of experimental paradigms in rats, mice, cats, and primates, including humans (Ninan et al., 1982; Ongini et al., 1983; Skolnick et al., 1984; Dorow, 1987; File and Baldwin, 1987). Serotonergic systems arising from the dorsal raphe nucleus may play an important role in these effects as microinjections of FG-7142 directly into the rat dorsal raphe nucleus increase passive avoidance behavior in an elevated T-maze test of anxiety-related behavior (Graeff et al., 1996b; Sena et al., 2003). In addition, understanding interactions between FG-7142 and serotonergic systems may be relevant to neural mechanisms underlying the behavioral consequences of uncontrollable or inescapable stress, called "behavioral depression" (Weiss et al., 1981) or "learned helplessness" (Maier and Seligman, 1976). Serotonergic neurons within the mid-rostrocaudal and caudal regions of the dorsal raphe nucleus appear to be critical for the potentiation of conditioned fear and induction of deficits in escape behavior that occur 24 h following exposure to inescapable stress in a learned helplessness paradigm (Maier and Watkins, 2005). These effects of uncontrollable stress and the associated serotonin release in the forebrain have been linked to neural mechanisms regulating anxiety states (Maier and Watkins, 2005) and can be blocked with the anxiolytic drug flumazenil (Maier et al., 1995b). FG-7142 and the full inverse agonist at the benzodiazepine allosteric site of the $GABA_A$ receptor, methyl 6,7-dimethoxy-4-ethyl-beta-carboline-3-carboxylate (DMCM), have been shown to mimic the effects of uncontrollable stress in that they induce a similar potentiation of conditioned fear and deficits in escape behavior measured 24 h later (Drugan et al., 1985; Maier et al., 1995a). Together, these findings implicate FG-7142 and a subpopulation of serotonergic neurons within the mid-rostrocaudal and caudal dorsal raphe nucleus in facilitation of anxiety- or fear-related behavior.

FG-7142 treatment increases c-Fos immunoreactivity in a subset of serotonergic neurons within the mid-rostocaudal and caudal parts of the dorsal raphe nucleus, a region of the brainstem raphe complex containing large numbers of neurons that project to forebrain structures implicated in the regulation of anxiety states (Van Bockstaele et al., 1993; Graeff et al., 1996a; Vertes et al., 1999; Lowry et al., 2005; Abrams et al., 2005). However, previous studies using microdialysis found no effect of systemic or intra-dorsal raphe nucleus injections of FG-7142 on extracellular serotonin concentrations in the ventral hippocampus, amygdala or dorsomedial periaqueductal gray region (Pei et al., 1989; Viana et al., 1997). The lack of previous evidence for FG-7142-induced increases in serotonergic neurotransmission in specific forebrain regions implicated in the regulation of anxiety states led us to conduct a comprehensive survey of multiple components of an anxiety-related forebrain network as defined by Singewald et al. (2003). The current study was designed to test the hypothesis that FG-7142 treatment increases indices of serotonin metabolism within one or more components of an anxiety-related forebrain circuit. In this study, we investigated the effects of intraperitoneal (i.p.) injections of FG-7142 on tissue concentrations of L-tryptophan (tryptophan; the amino acid precursor to serotonin), 5-hydroxytryptamine (serotonin; 5-HT), and 5-hydroxyindoleacetic acid (5-HIAA; the main catabolite of serotonin) in microdissected regions of an anxiety-related network in the forebrain. We predicted that FG-7142 treatment would induce increases in serotonin and 5-HIAA concentrations within components of this forebrain neural network that receive serotonergic innervation from a subpopulation of FG-7142-sensitive serotonergic neurons within the brainstem raphe complex.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (300–400 g) were obtained from B&K Universal (Hull, UK) and single-housed under standard environmental conditions in the testing environment for 2 weeks prior to the experiment (21±2 °C; 55±10% humidity; illumination of approximately 100 lx or less at 1 m). Animals were housed in RC1 cages (50 cm length×33 cm width×23 cm height; North Kent Plastics, Dartford, UK) on a 14:10 h light–dark cycle with lights on at 5 AM. Food (B&K CRM rat chow; B&K Universal) and water were provided ad libitum. All animals were handled for 2 min daily for 5 days prior to the experimental treatment. Housing conditions were replicated from previous work in our lab in which FG-7142 (7.5 mg/kg) increased c-Fos immunoreactivity in a subpopulation of midbrain serotonergic neurons, as well as behavioral arousal and vigilance behaviors in a home-cage environment (Abrams et al., 2005). All procedures were approved by the Ethical Review Group at the University of Bristol and were conducted in accordance with

UK Home Office guidelines and the Scientific (Animal Procedures) Act 1986.

2.2. Drug treatment

Each rat received an intraperitoneal injection of 0, 1.9, 3.8, or 7.5 mg/kg ($n=5$) of the partial inverse agonist at the benzodiazepine allosteric site of the $GABA_A$ receptor, *N*-methyl-beta-carboline-3-carboxamide (FG-7142) (Tocris, Avonmouth, UK), dissolved in 0.9% saline/40% 2-hydroxy-propyl-β-cyclodextrin (Tocris, Avonmouth, UK) to increase solubility. Solutions were made up for 2 mL/kg injections and volumes were adjusted to animal weight. Rats were taken from their home cage, given an injection in an adjacent room and were returned to their home cage. After a period of 1 h, rats were taken to a dissection room, rapidly decapitated, and brains were dissected, rapidly frozen on dry ice, and stored at −80 °C.

2.3. Brain microdissection

Brain microdissection combined with high pressure liquid chromatography and electrochemical detection of tryptophan, serotonin, and 5-HIAA was based on a previously described procedure (Renner and Luine, 1984). Although not without limitations, this approach allowed for simultaneous measurement of tryptophan, serotonin, and 5-HIAA concentrations in multiple components of a defined anxiety-related network of brain structures (Singewald et al., 2003) with a high degree of neuroanatomical resolution, a combination that is not possible using other techniques. Frozen brain tissue was sectioned using a cryostat (Leica CM1900, Milton Keynes, UK) and serial 300 μm sections were thaw-mounted onto glass microscope slides, rapidly re-frozen, and stored at −80 °C until microdissection. Individual brain regions were microdissected at −10 °C following the Palkovits punch technique (Palkovits, 1988) using a stainless steel microdissection needle (690 or 410 μm diameter, Neuropunch #18036-19 and #18036-22, Fine Science Tools, Foster City, CA, USA). Microdissected tissues from individual brain regions from individual rats were expelled into separate tubes containing 100 μl acetate buffer (0.3% sodium acetate and 0.43% glacial acetic acid; pH 5.0), and then stored at −80 °C until they were analyzed for tissue concentrations of tryptophan, serotonin and 5-HIAA.

Table 1
Forebrain regions selected for analysis of tryptophan, serotonin, and 5-HIAA concentrations

Brain region	Rostrocaudal levels (mm Bregma)	Microdissections (# [diameter (μm)])
Infralimbic cortex (IL)[a]	2.89 to 2.59	2 [690]
Prelimbic cortex (PrL)[a]	2.89 to 2.59	2 [690]
Cingulate cortex (Cg1)[b]	2.89 to 2.59	2 [690]
Lateral septum, intermediate part (LSI)[a]	0.49 to 0.19 0.19 to −0.11	4 [690]
Bed nucleus of the stria terminalis, laterodorsal part (BSTLD)[a]	0.19 to −0.11 −0.11 to −0.41	4 [410]
Primary motor cortex (M1)[a]	−0.11 to −0.41	2 [690]
Agranular insular cortex, posterior part (AIP)[a]	−1.61 to −1.91	2 [690]
Paraventricular nucleus of the hypothalamus, medial parvocellular part (PaMP)[a]	−1.61 to −1.91 −1.91 to −2.21	4 [410]
Lateral hypothalamus (LH)[a]	−1.61 to −1.91 −1.91 to −2.21	4 [690]
Basolateral amygdaloid nucleus, anterior and posterior parts (BL)[b]	−2.51 to −2.81 −2.81 to −3.11 −3.11 to−3.41	6 [690]
Central amygdaloid nucleus (Ce)[a]	−2.81 to −3.11 −3.11 to −3.41	4 [410]
Medial amygdaloid nucleus, anterior and posterior ventral parts (Me)[b]	−2.81 to −3.11 −3.11 to −3.41	4 [410]
Dorsomedial hypothalamic nucleus, dorsal part (DMD)[b]	−2.81 to −3.11 −3.11 to −3.41	4 [410]

[a] Demonstrated increased c-Fos expression with all drugs (FG-7142, yohimbine, caffeine and mCPP) (Singewald et al., 2003).
[b] Demonstrated increased c-Fos expression with all drugs excluding mCPP (Singewald et al., 2003).

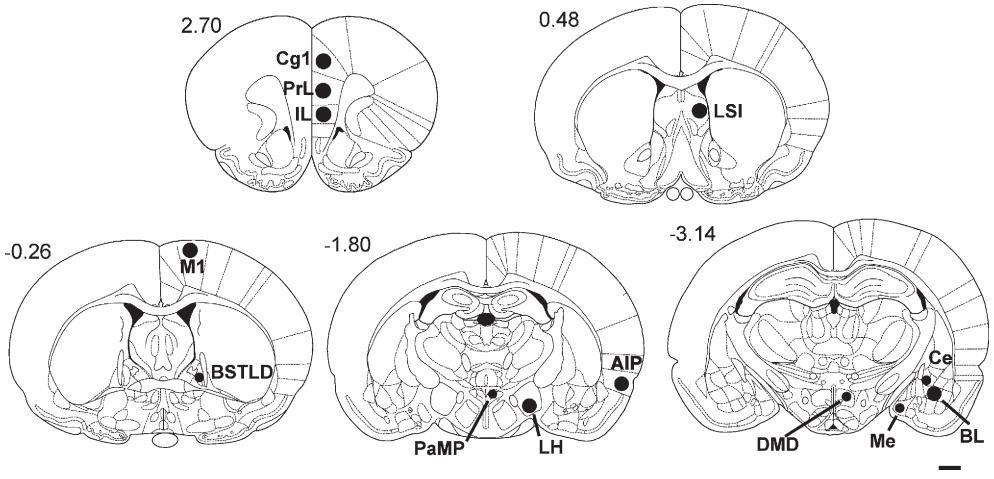

Fig. 1. Diagrams of coronal sections from a standard rat brain atlas (Paxinos and Watson, 1998) illustrate the thirteen microdissected brain regions analyzed for tryptophan, serotonin, and 5-HIAA concentrations. All regions were sampled bilaterally and when sampled from multiple levels, as indicated in Table 1, are only represented in the diagrams at one of the indicated levels. The numbers on the upper left of the diagrams indicate the distance from Bregma (mm). Scale bar, 1 mm.

2.4. Selection of forebrain regions for analysis

In order to investigate the effects of FG-7142 on serotonin metabolism in forebrain regions involved in physiological or behavioral responses to anxiogenic drugs, thirteen forebrain regions were selected based on a previous study showing increased c-Fos expression in these forebrain regions following anxiogenic drug treatment (Singewald et al., 2003; also see Fig. 1, Table 1). Regions were selected for analysis if they showed increased numbers of c-Fos-immunoreactive cells following treatment with each of a range of pharmacologically diverse anxiogenic drugs (FG-7142, yohimbine, mCPP, and caffeine; excluding mCPP in some cases). In the case of the bed nucleus of the stria terminalis (BST) we specifically microdissected the laterodorsal part of the bed nucleus of the stria terminalis (BSTLD); this was based on the striking pattern of c-Fos immunoreactivity within this subdivision of the BST following anxiogenic drug treatment (see Fig. 1 from Singewald et al., 2003). Anterior–posterior levels of sections selected for specific microdissections were identified by comparison with a standard rat brain stereotaxic atlas (Paxinos and Watson, 1998). The number and diameter of punches for each region are included in Table 1. All regions were dissected bilaterally, with 1 punch per side per rostrocaudal level with multiple rostrocaudal levels sampled for some regions as detailed in Table 1.

2.5. HPLC analysis

Samples in acetate buffer were thawed at 4 °C and centrifuged at 13,000 RPM for 3 min at room temperature using a Sanyo MSE MicroCentaur centrifuge (Sanyo, Bensenville, IL, USA). The supernatant was drawn off and the pellet was reconstituted with 200 µL of 0.2 M NaOH for later assay of protein content (Pierce Protein Microassay Protocol, Pierce, Rockford, IL, USA). A 50 µL volume of the supernatant from each sample was then placed in an ESA 542 autosampler (ESA Analytical, Ltd., Huntington, UK) maintained at 4 °C. A 15 µL volume of supernatant from each sample was then injected onto the chromatographic system. Chromatographic separation was accomplished using an integrated precolumn/column system consisting of a guard cartridge (4.6×5 mm) attached to an Ultrasphere XL-ODS cartridge (4.6×70 mm; Beckman Coulter, Fullerton, CA, USA). The mobile phase consisted of 9.53 g/L KH_2PO_4, 200 mg/L 1-octane sulfonic acid, and 35 mg/L

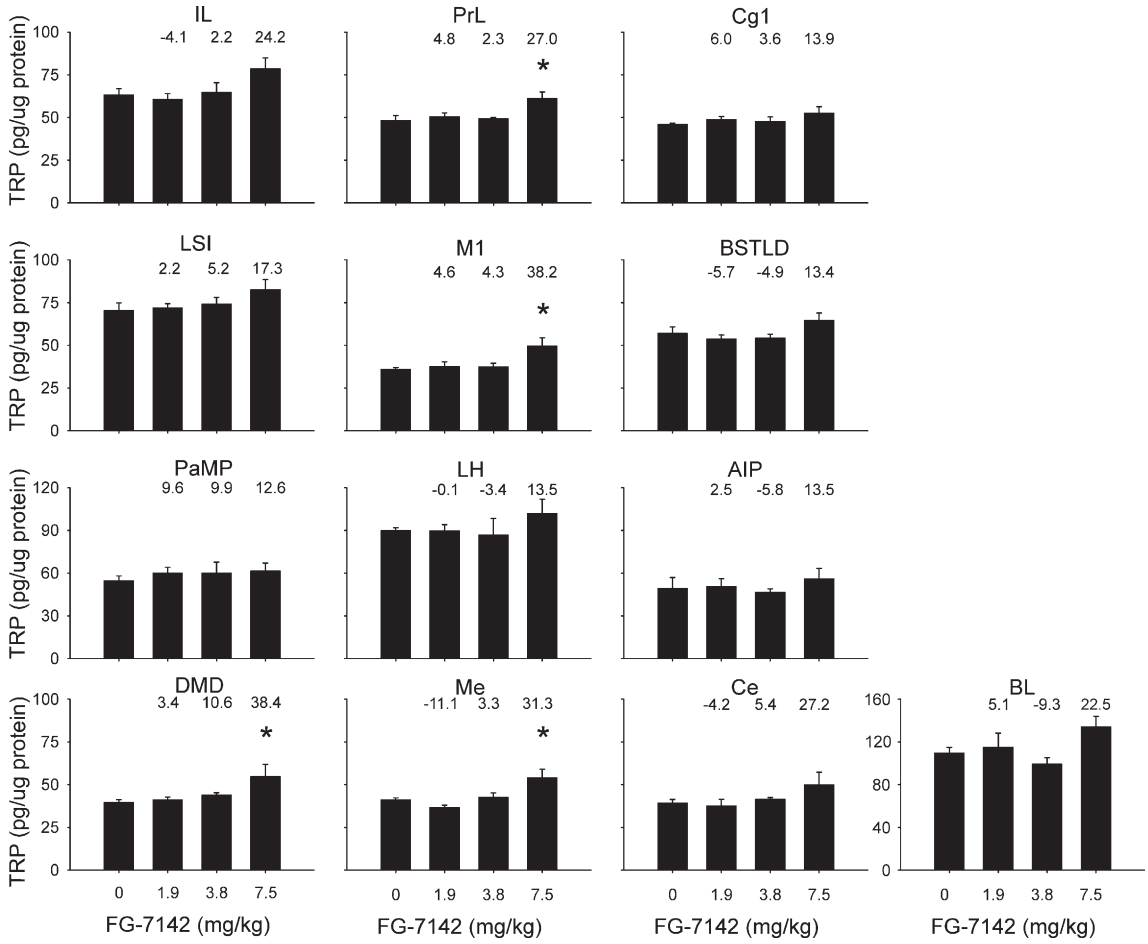

Fig. 2. FG-7142 dose-dependently increased tryptophan concentrations in specific microdissected brain regions. Graphs illustrate the tryptophan concentrations (pg/µg protein) 1 h following FG-7142 treatment (0, 1.9, 3.8, and 7.5 mg/kg; $n=5$ for each group). The percent change relative to the vehicle-treated control group is indicated above each bar. Tryptophan concentrations represent means±SEM. *$p<0.05$; Dunnett's test. For abbreviations see Table 1.

ethylenediaminetetraacetic acid in 13% methanol; pH was adjusted to 3.4–3.5 using orthophosphoric acid. Electrochemical detection was accomplished using an ESA Coulochem II cell with electrodes set at −0.10 and +0.55 V. The mean peak heights (pg/cm) of known concentrations of tryptophan, serotonin and 5-HIAA standards were determined from the peak heights of two chromatographs run before and after each set of samples. Concentrations of tryptophan, serotonin and 5-HIAA in the microdissected samples were determined based on peak heights measured using a computerized analysis system (EZChrom Elite for Windows, version 2.8; Scientific Software, Inc., Pleasanton, CA, USA) while the analyst was blind to the nature of the treatment groups. Indole concentrations were expressed as pg/μg protein.

2.6. Statistics

All statistical analyses used Statistical Package for the Social Sciences (SPSS) version 11.5.0 (SPSS, Woking, UK), and all reported values are mean values and standard errors of the means (SEM). Effects of FG-7142 and REGION on indole concentrations were analyzed using a single multifactor analysis of variance (ANOVA) with repeated measures using FG-7142 as the between-subjects factor and REGION as the within-subjects factor for repeated measures analysis. The Greenhouse-Geisser correction was used for multifactor ANOVA in order to correct for differences in variance across repeated measures (Vasey and Thayer, 1987). A Grubb's test was used to eliminate outliers from the data set. Outliers (2.4%) and missing values (0.9%) were replaced by the method of Petersen (1985) prior to the multifactor ANOVA with repeated measures analysis, but the original data were used for post-hoc analysis and for graphical representation of the data. Main effects of FG-7142 and REGION or interactions between FG-7142 and REGION were further analyzed with post-hoc pair-wise comparisons using Dunnett's test for multiple comparisons with a single control (vehicle treatment). In all cases, significance was accepted at $p<0.05$.

3. Results

Compared to vehicle-treated control rats, rats treated with a systemic injection of 3.8 or 7.5 mg/kg FG-7142 had increased tissue concentrations of tryptophan, serotonin and 5-HIAA in

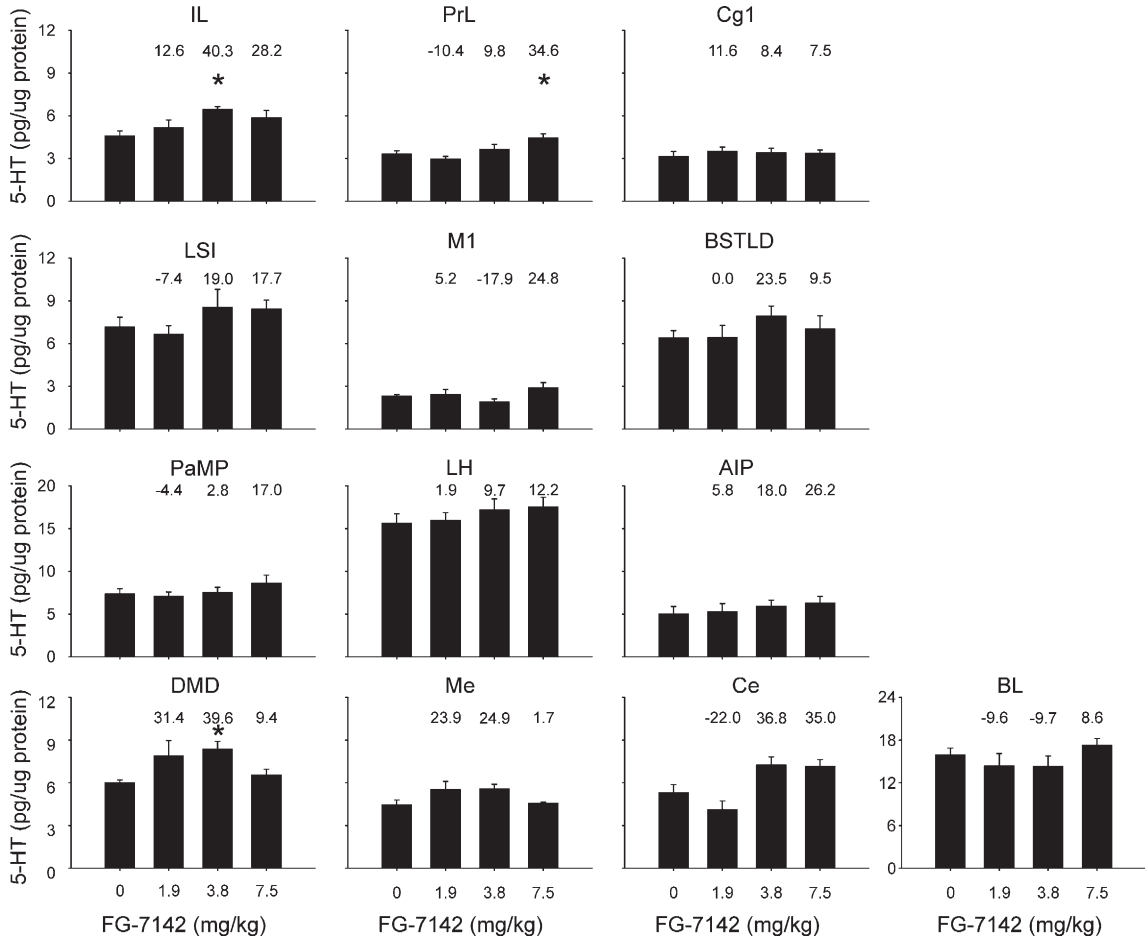

Fig. 3. FG-7142 dose-dependently increased 5-hydroxytryptamine (serotonin) concentrations in specific microdissected brain regions. Graphs illustrate the serotonin concentrations (pg/μg protein) 1 h following FG-7142 treatment (0, 1.9, 3.8, and 7.5 mg/kg; $n=5$ for each group). The percent change relative to the vehicle-treated control group is indicated above each bar. Serotonin concentrations represent means±SEM. *$p<0.05$; Dunnett's test. For abbreviations see Table 1.

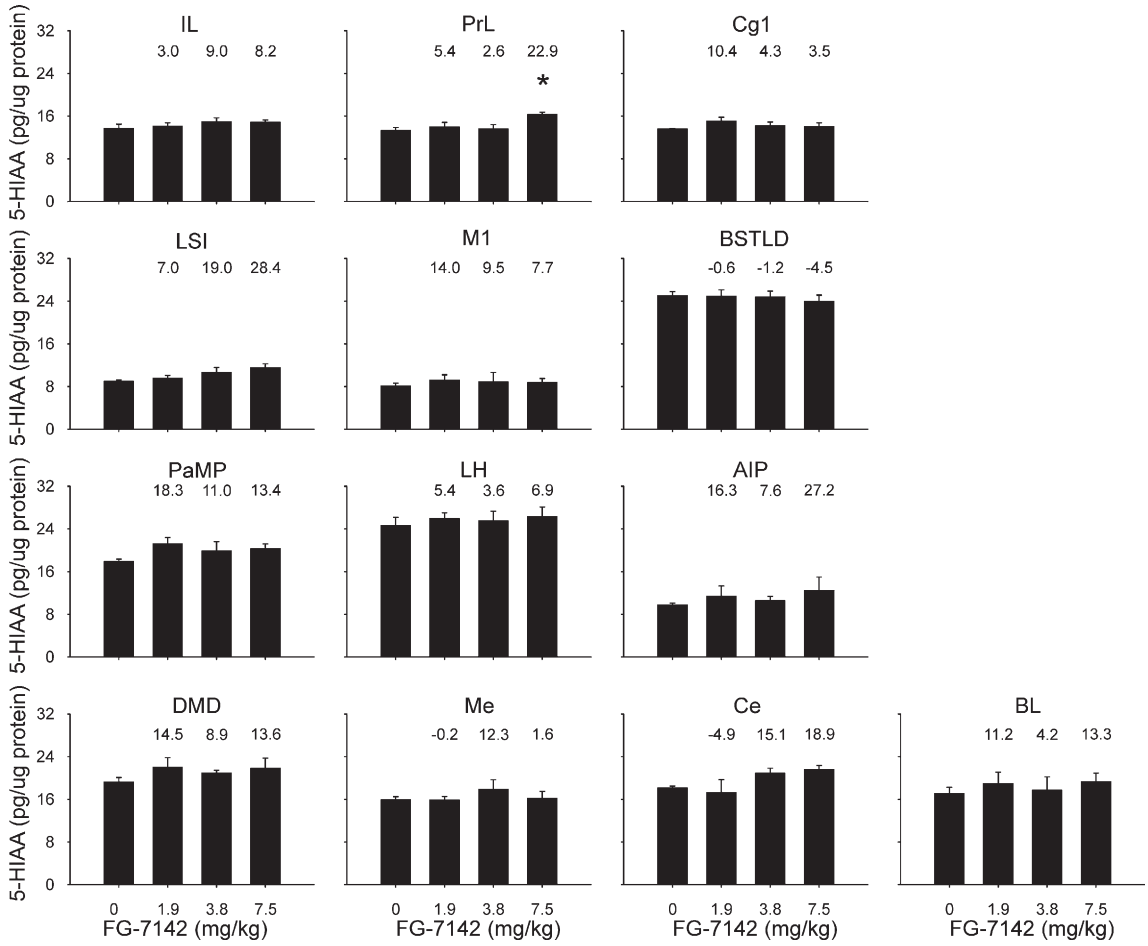

Fig. 4. FG-7142 dose-dependently increased 5-hydroxyindoleacetic acid (5-HIAA) concentrations in the prelimbic cortex. Graphs illustrate the 5-HIAA concentrations (pg/μg protein) 1 h following FG-7142 treatment (0, 1.9, 3.8, and 7.5 mg/kg; $n=5$ for each group). The percent change relative to the vehicle-treated control group is indicated above each bar. 5-HIAA concentrations represent means±SEM. *$p<0.05$; Dunnett's test. For abbreviations see Table 1.

specific microdissected forebrain structures, measured 1 h following treatment. Multifactor repeated measures ANOVA revealed main effects of FG-7142 on tryptophan ($F_{(3,16)}=5.390$; $p=0.009$; Fig. 2), serotonin ($F_{(3,16)}=3.282$; $p=0.048$; Fig. 3) and 5-HIAA ($F_{(3,16)}=3.365$; $p=0.045$; Fig. 4) concentrations. Multifactor repeated measures ANOVA (with Greenhouse-Geisser correction) revealed highly significant main effects of REGION on tryptophan ($F_{(15,240)}=75.87$; epsilon=0.241; $p<0.001$), serotonin ($F_{(15,240)}=91.58$; epsilon=0.271; $p<0.001$), and 5-HIAA ($F_{(15,240)}=113.86$; epsilon=0.249; $p<0.001$) concentrations. Post-hoc pair-wise comparison of means revealed that rats treated with FG-7142 (7.5 mg/kg), compared to rats treated with vehicle, had increased tryptophan, serotonin and 5-HIAA concentrations in the prelimbic cortex. In addition, rats treated with FG-7142 (7.5 mg/kg) had increased tryptophan concentrations in the primary motor cortex, medial amygdala and the dorsomedial hypothalamic nucleus. Rats treated with an intermediate dose of FG-7142 (3.8 mg/kg), relative to vehicle-injected control rats, had increased serotonin concentrations in the infralimbic cortex and dorsomedial hypothalamus. No changes in tryptophan, serotonin, or 5-HIAA concentrations were detected in other brain regions studied.

4. Discussion

Although FG-7142 increases c-Fos expression in a subset of serotonergic neurons in the dorsal raphe nucleus (Abrams et al., 2005) and microinjections of FG-7142 into the dorsal raphe nucleus are known to increase anxiety-related behavior (Graeff et al., 1996b; Sena et al., 2003), microdialysis studies have not detected FG-7142-induced changes in extracellular serotonin concentrations in specific anxiety-related forebrain structures analyzed, i.e., the amygdala and hippocampus (Pei et al., 1989; Viana et al., 1997). In the present study analysis of 13 different components of an anxiety-related forebrain circuit revealed that systemic injection of FG-7142 increased tryptophan, serotonin, and 5-HIAA concentrations within the prelimbic region of the prefrontal cortex measured 1 h following treatment. FG-7142 also increased tryptophan concentrations in the primary motor cortex, medial amygdaloid nucleus and dorsomedial hypothalamus, but these effects were not associated with changes in either serotonin or 5-

HIAA concentrations. FG-7142 increased serotonin concentrations in the infralimbic cortex and the dorsomedial hypothalamus, but these effects were only observed at an intermediate dose. We did not detect any effects of FG-7142 treatment on tryptophan, serotonin, or 5-HIAA concentrations in some anxiety-related brain regions, including the basolateral and central amygdaloid nuclei, and the bed nucleus of the stria terminalis. It remains possible that more sensitive techniques that are able to detect temporally dynamic changes in serotonergic neurotransmission, for example in vivo microdialysis or in vivo voltammetry, would reveal more widespread effects of FG-7142 on indices of serotonergic neurotransmission. Nevertheless, these findings support the hypothesis that anxiogenic drugs alter serotonin metabolism within components of a forebrain neural circuit associated with modulation of anxiety states.

4.1. FG-7142 and serotonin metabolism in the medial prefrontal cortex

FG-7142 (7.5 mg/kg) increased tryptophan, serotonin, and 5-HIAA concentrations in the prelimbic region of the prefrontal cortex, a component of the ventral portion of the medial prefrontal cortex. This pattern of results is consistent with a previous study in rats in which systemic treatment with the same dose of FG-7142 increased the number of c-Fos-immunoreactive serotonergic neurons within regions of the raphe complex (median raphe nucleus and mid-rostrocaudal and caudal dorsal raphe nucleus; Abrams et al., 2005) which innervate the medial prefrontal cortex (Van Bockstaele et al., 1993; Vertes et al., 1999). The mechanisms underlying the effects of FG-7142 on tryptophan, serotonin, and 5-HIAA concentrations within the medial prefrontal cortex are not clear. While it is possible that the effects of FG-7142 on tissue concentrations of serotonin and 5-HIAA in the prelimbic cortex were associated with increased serotonergic neuronal firing rates (Hajos et al., 2003; Puig et al., 2005), it is also possible that these effects were due to local regulation of serotonin metabolism and release at terminal sites in the prefrontal cortex (Sari, 2004).

Increases in serotonin metabolism in the rat medial prefrontal cortex have been observed following exposure of rats to a variety of fear- and anxiety-related stimuli. For example, conditioned fear leads to increased freezing behavior with associated increases in serotonin release in the medial prefrontal cortex, responses that can be prevented by pretreatment with the anxiolytic drug diazepam (Yoshioka et al., 1995). Similarly, uncontrollable stress has been demonstrated to increase serotonin efflux in the medial prefrontal cortex (Bland et al., 2003), and pharmacological inhibition of neuronal activity in the medial prefrontal cortex can mimic the behavioral and neurochemical consequences of uncontrollable stress including induction of c-Fos expression in the caudal dorsal raphe nucleus and facilitation of learned helplessness behavior (Amat et al., 2005).

Numerous studies have demonstrated that serotonin modulates neuronal activity within the medial prefrontal cortex and this in turn may modulate the excitability of an anatomically distributed neural system regulating anxiety states. There is strong evidence for both excitatory (Marek and Aghajanian, 1998; Zhang and Arsenault, 2005) and inhibitory (Hajos et al., 2003; Amargos-Bosch et al., 2004; Puig et al., 2005) effects of serotonin on neuronal firing rates in the prefrontal cortex, including evidence for serotonin-receptor dependent inhibition of neuronal firing rates of prefrontal pyramidal neurons following electrical stimulation of the dorsal and median raphe nuclei (Hajos et al., 2003; Amargos-Bosch et al., 2004; Puig et al., 2005). The medial prefrontal cortex, in turn, has widespread influences on multiple components of forebrain circuits regulating anxiety states and anxiety-related behavior. For example, the ventral medial prefrontal cortex projects to the lateral septum, the bed nucleus of the stria terminalis, the medial, central, and basolateral amygdaloid nuclei, the dorsomedial hypothalamus, the nucleus accumbens, and the dorsal and median raphe nuclei of the brainstem (Vertes, 2004). These neuroanatomical connections are supported by electrophysiological studies showing that the medial prefrontal cortex modulates the excitability of neurons in the dorsal raphe nucleus (Hajos et al., 1998; Celada et al., 2001) as well as the central and basolateral amygdaloid nuclei (Rosenkranz and Grace, 2002; Quirk et al., 2003). Particularly relevant is evidence that inhibitory connections from the medial prefrontal cortex to interneurons driving the central nucleus of the amygdala play a role in the inhibition of conditioned fear (Berretta et al., 2005). These studies suggest that the medial prefrontal cortex exerts control over individual components of a distributed neural system modulating anxiety-related physiological and behavioral responses, including serotonergic neurons in the dorsal raphe nucleus (Amat et al., 2005; Robbins, 2005). Consequently, serotonin actions within the medial prefrontal cortex may modulate the excitability of anxiety-related neural systems including anxiety-related serotonergic neurons in the dorsal raphe nucleus.

4.2. Regional specificity of FG-7142-induced effects

The most consistent effects of FG-7142 on measures of tryptophan concentrations and serotonin metabolism were observed at the highest dose (7.5 mg/kg), a dose previously associated with FG-7142-induced increases in behavioral arousal and vigilance behaviors, increases in c-Fos immunoreactive neurons in an anxiety-related forebrain circuit, as well as increases in c-Fos expression in subpopulations of serotonergic neurons within the dorsal and median raphe nuclei (Singewald and Sharp, 2000; Abrams et al., 2005). The time point for these changes in serotonin metabolism, measured 1 h following FG-7142 injection, is consistent with the time course of the peak FG-7142-induced increases in behavioral arousal and vigilance behaviors in a home cage environment (Abrams et al., 2005). To our knowledge, the pharmacokinetics of FG-7142 in rats have not been well characterized; therefore it is not possible to determine if the changes in measures of serotonin metabolism that were observed 1 h following FG-7142 treatment were related to the anxiogenic effects of FG-7142, or to the recovery from these effects.

Treatment with FG-7142 increased indices of serotonin metabolism in some forebrain structures studied, but not others. We did not detect changes in measures of serotonin metabolism in the basolateral amygdaloid nucleus, the central amygdaloid nucleus or the bed nucleus of the stria terminalis, conspicuous targets of projections from serotonergic neurons within the mid-rostrocaudal and caudal dorsal raphe nucleus, a region implicated in the regulation of anxiety states and anxiety-related physiological and behavioral responses (Commons et al., 2003; Abrams et al., 2005). However, a lack of changes in serotonin metabolism in specific brain regions at the time corresponding to the peak of behavioral changes following FG-7142 treatment does not mean that serotonin metabolism might not change at a different time point. This is particularly the case if changes that we observed in the medial prefrontal cortex are either a prerequisite to or secondary to changes in other brain regions. It is also possible that alternative techniques that are able to detect temporally dynamic changes in measures of serotonergic neurotransmission, such as in vivo microdialysis or voltammetry, would reveal more widespread effects of FG-7142. On the other hand, the possibility that FG-7142 may affect serotonergic neurotransmission in some anxiety-related forebrain structures, but not others, is consistent with the finding that systemic injection of FG-7142 has no effect on extracellular concentrations of serotonin in the ventral hippocampus of rats, as measured using in vivo microdialysis (Pei et al., 1989). Likewise, this possibility is consistent with the findings that intra-dorsal raphe nucleus injections of FG-7142, although they alter anxiety-related behavior (Graeff et al., 1996b; Sena et al., 2003), have no effects on extracellular serotonin concentrations in the amygdala or the midbrain dorsal periaqueductal gray at any time point between 20 and 120 min following injection (Viana et al., 1997). It remains a possibility that local regulation of serotonin metabolism and release at presynaptic terminal sites in regions such as the basolateral amygdaloid nucleus, the central amygdaloid nucleus and the bed nucleus of the stria terminalis, could potentially explain a lack of effect of FG-7142 on measures of serotonin metabolism despite FG-7142 actions at serotonergic cell bodies projecting to those regions, described previously (Abrams et al., 2005). These are issues that need to be resolved in future studies.

5. Conclusions

These findings support the hypothesis that anxiogenic drugs alter serotonin metabolism within specific components of a neural network associated with modulation of anxiety states, including the prelimbic region of the medial prefrontal cortex. Together with previous studies these data are consistent with the hypothesis that FG-7142 alters anxiety states and anxiety-related physiological and behavioral responses in part by increasing serotonergic neurotransmission within the medial prefrontal cortex. This study provides a hypothetical framework for future studies of the effects of FG-7142 on temporally dynamic changes in serotonergic neurotransmission within the medial prefrontal cortex, part of an anxiety-related neural network regulating executive function and emotional behavior.

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