CNS Drug Reviews
Vol. 13, No. 4, pp. 475–501

Pharmacology of the β-Carboline FG-7142, a Partial Inverse Agonist at the Benzodiazepine Allosteric Site of the $GABA_A$ Receptor: Neurochemical, Neurophysiological, and Behavioral Effects

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Keywords: Anxiety — Benzodiazepine binding site — Carbolines — FG-7142 — $GABA_A$ receptors.

ABSTRACT

Given the well-established role of benzodiazepines in treating anxiety disorders, β-carbolines, spanning a spectrum from full agonists to full inverse agonists at the benzodiazepine allosteric site for the $GABA_A$ receptor, can provide valuable insight into the neural mechanisms underlying anxiety-related physiology and behavior. FG-7142 is a partial inverse agonist at the benzodiazepine allosteric site with its highest affinity for the $\alpha 1$ subunit-containing $GABA_A$ receptor, although it is not selective. FG-7142 also has its highest efficacy for modulation of GABA-induced chloride flux mediated at the $\alpha 1$ subunit-containing $GABA_A$ receptor. FG-7142 activates a recognized anxiety-related neural network and interacts with serotonergic, dopaminergic, cholinergic, and noradrenergic modulatory systems within that network. FG-7142 has been shown to induce anxiety-related behavioral and physiological responses in a variety of experimental paradigms across numerous mammalian and non-mammalian species, including humans. FG-7142 has proconflict actions across anxiety-related behavioral paradigms, modulates attentional processes, and increases cardioacceleratory sympathetic reactivity and neuroendocrine reactivity. Both acute and chronic FG-7142 treatment are proconvulsive, upregulate cortical adrenoreceptors, decrease subsequent actions of GABA and β-carboline agonists, and increase the effectiveness of subsequent $GABA_A$ receptor antagonists and β-carboline inverse

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The authors have no conflict of interest.

agonists. FG-7142, as a partial inverse agonist, can help to elucidate individual components of full agonism of benzodiazepine binding sites and may serve to identify the specific $GABA_A$ receptor subtypes involved in specific behavioral and physiological responses.

INTRODUCTION

Despite many advances in anxiety-related research, the neural mechanisms underlying anxiety-related behavior and physiology are not well-understood. Clinically effective treatments for anxiety disorders classically include tricyclic antidepressants, barbiturates, and benzodiazepines, as well as novel selective serotonin or norepinephrine reuptake inhibitors. This review will focus on the role of benzodiazepine binding sites in anxiety-related neurophysiology and behavior as elucidated by N-methyl-β-carboline-3-carboxamide (FG-7142), a partial inverse agonist at the benzodiazepine allosteric site of the $GABA_A$ receptor. While focusing on anxiety, this review will cover benzodiazepine binding-site regulation of cognitive, physiological, and behavioral processes broadly related to regulation of normal and pathological states. The benzodiazepine binding site allosterically modulates the $GABA_A$ receptor; agonists facilitate GABA receptor-mediated increases in chloride conductance, whereas inverse agonists have the opposite effect. Antagonism of the benzodiazepine binding site has no intrinsic influence on the GABA receptor-mediated chloride conductance, yet blocks the effect of both agonists and inverse agonists. The availability of endogenous agonists and inverse agonists for the benzodiazepine binding site, in conjunction with its role in regulating anxiety states, provides a unique opportunity to increase our understanding of the neural mechanisms underlying anxiety-related behavior and physiology.

β-Carbolines were identified as potential endogenous ligands for the benzodiazepine binding site, identified as stress-sensitive compounds excreted in urine (also present in the rat brain), and found to have competitive affinity for benzodiazepine binding sites (Braestrup et al. 1980). The β-carboline family of compounds have since been found to span the complete range from full agonists to full inverse agonists at the benzodiazepine allosteric site for the $GABA_A$ receptor. FG-7142 is one of many β-carboline compounds including (1) the full inverse agonists β-carboline-3-carboxylic acid methyl ester (β-CCM) and 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylic acid methyl ester (DMCM); (2) other partial inverse agonists such as β-carboline-3-carboxylate ethyl ester (β-CCE), β-carboline-3-carboxylate propyl ester (β-CCP), ethyl-4-ethyl-5-methoxy-β-carboline-3-carboxylate (ZK 90886), and isopropyl-5-isopropoxy-β-carboline-3-carboxylate (ZK 132 553); (3) antagonists such as 4-methyl-5-methylethoxy-β-carboline-3-carboxylate ethyl ester (ZK 93426); (4) partial agonists such as ethyl-5-benzyloxy-4-methoxymethylene-β-carboline-3-carboxylate (ZK 91296), as well as (5) full agonists such as 6-benzyloxy-4-methoxymethylene-β-carboline-3-carboxylate (ZK 93423). Endogenous β-carboline inverse agonists include 6-methoxytetrahydro-β-carboline (pinoline) and methyl-β-carboline (harmane). For detailed structural analysis of FG-7142 and other β-carbolines see Ferretti et al. (2004). The structure of FG-7142 is shown in Figure 1.

FG-7142 is an amide derivative of β-CCE (Little et al. 1987a) and is formed naturally during the combustion process of many plant leaves, most notably tobacco, and is a component of cigarette smoke that is detectable generally in the environment (indoor air: increases from 1 to 50 pg per cubic meter following 1–6 cigarettes smoked) (Yuan and

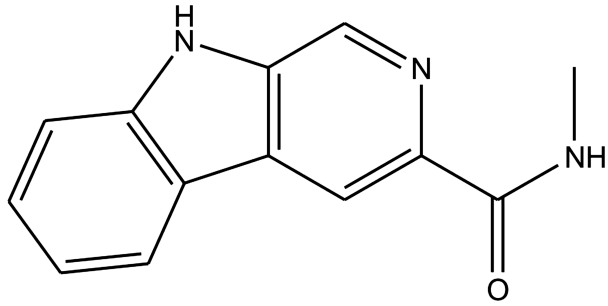

FIG. 1. Structure of FG-7142.

Manabe 1995). Consequently, FG-7142 is detectable in urine of both smokers (2.4 ng/day) and nonsmokers (0.5 ng/day) (Yuan and Manabe 1996). FG-7142 has been demonstrated to induce anxiety-related behavioral and physiological responses in a variety of experimental paradigms in rats, mice, cats, and primates (Ninan et al. 1982; Ongini et al. 1983; Skolnick et al. 1984; File and Baldwin 1987; Thiebot et al. 1988) as well as anxiety states in humans (Dorow et al. 1983; Dorow 1987). The following rewiew will address in vitro and *in vivo* pharmacological properties of FG-7142 and the associated neurochemical, physiological, and behavioral changes following FG-7142 administration.

RECEPTOR BINDING AND PHARMACOKINETICS

$GABA_A$ Receptor α-Subunit Specificity

In competition assays against a radiolabeled antagonist at the benzodiazepine-binding site of the $GABA_A$ receptor, flumazenil, FG-7142 has an effective concentration for a half-maximal response (EC_{50}) of 10–12 mg/kg *in vivo* and an *in vitro* binding affinity (K_i) of 265 nM for displacing flumazenil from cortical membranes. In comparison, the full agonist DMCM has an EC_{50} of 1–2 mg/kg and a K_i of 2.1 nM (Stephens et al. 1987; Cole et al. 1995).

The benzodiazepine binding site is thought to occur between the α and $\gamma 2$ subunits of $GABA_A$ receptors containing 2α, $2\beta 3$, and $1\gamma 2$ subunits (McKernan and Whiting 1996; Sieghart and Sperk 2002). The α-subtype is thought to differentiate the various sedative/convulsant [$\alpha 1$ (Rudolph et al. 1999; Crestani et al. 2000; McKernan et al. 2000; Rudolph et al. 2001)], anxiety-related [$\alpha 2$ and $\alpha 3$ (Low et al. 2000; Atack et al. 2005; Morris et al. 2006; Atack et al. 2006b]), and cognitive/amnesic [$\alpha 5$ (Chambers et al. 2003; Collinson et al. 2006; Dawson et al. 2006)] behavioral responses elicited by actions at the benzodiazepine allosteric binding site (Korpi et al. 2002; Rudolph and Mohler 2006). The different α-subunit-containing $GABA_A$ receptors are differentially distributed throughout the brain (Schwarzer et al. 2001). Among human recombinant $GABA_A$ receptors expressing $\beta 3$ and $\gamma 2$ subunits, FG-7142 (as a partial inverse agonist) has highest affinity for those expressing the $\alpha 1$ subunit [K_i (nM): $\alpha 1$, 91 $\pm$ 22; $\alpha 2$, 330 $\pm$ 62; $\alpha 3$, 492 $\pm$ 105, $\alpha 4$, N.A.; $\alpha 5$, 2150 $\pm$ 725 (Atack et al. 2005)]. Similarly FG-7142 has a higher efficacy in modulating GABA-induced chloride flux at $GABA_A$ receptors expressing the $\alpha 1$ subunit ($EC_{50} = 137$ nM) as compared to the other α subunits [EC_{50} (nM): $\alpha 2 = 507$, $\alpha 3 = 1021$, $\alpha 5 = 1439$

(Atack et al. 2005)]. See Table 1 for a comparison of efficacies, affinities, and percentage modulation of GABA-induced chloride flux for FG-7142 and related compounds.

Additional Binding Affinities

Several studies suggest that, similar to caffeine, β-carbolines, including FG-7142, have antagonist properties at adenosine receptors (Phillis and Oregan 1988; Mumford and Holtzman 1991).

IN VIVO PHARMACOLOGY

Actions within an Anxiety-Related Network

Studies using measurement of immediate-early gene expression, metabolic changes within the brain, and electrophysiological responses, support a role for FG-7142 in activation of specific anxiety-related circuits in the brain.

Immediate-Early Gene Expression Studies

Studies using FG-7142 and a cohort of pharmacologically diverse compounds have revealed that anxiogenic drugs activate a distributed anxiety-related neural system. FG-7142 (7.5 mg/kg i.p) and the drugs m-chlorophenylpiperazine (mCPP), a nonselective 5-HT_{2C} receptor agonist; caffeine, an adenosine receptor antagonist; and yohimbine, an α_2-adrenoceptor antagonist, increase the expression of the protein product of the immediate-early gene, *c-fos*, in multiple forebrain and hindbrain structures widely acknowledged to be part of a network mediating anxiety-related behavioral and physiological responses (Singewald and Sharp 2000; Singewald et al. 2003) (Fig. 2). These forebrain structures include the basolateral and central amygdaloid nuclei, bed nucleus of the stria terminalis, dorsomedial hypothalamus, cingulate cortex, and infralimbic and prelimbic cortices. Hindbrain regions include the periaqueductal gray, locus coeruleus, caudal part of the dorsal raphe nucleus, parabrachial nucleus, ventrolateral medulla, and nucleus of the solitary tract (Gray and McNaughton 2000; Millan 2003; Walker et al. 2003). A study examining *c-fos* messenger RNA expression, as opposed to c-Fos protein, following FG-7142 (10 mg/kg i.p.) administration, reported increases in *c-fos* mRNA expression in the nucleus accumbens core, medial septum, and ventral hippocampus, but did not observe increases in *c-fos* expression in the bed nucleus of the stria terminalis, central amygdaloid nucleus, basolateral amygdaloid nucleus, dorsal or median raphe nuclei, locus coeruleus, paraventricular nucleus of the hypothalamus, or lateral septum (Funk et al. 2006). Discrepancies between sites of action in these studies could reflect a different time course of *c-fos* mRNA expression versus c-Fos protein expression or differences in test species examined, although both studies identify specific components of recognized anxiety-related circuits that are activated by FG-7142.

In rats bred for high (HAB) and low (LAB) anxiety, FG-7142 (7.5 mg/kg) resulted in greater increases in c-Fos protein in HAB rats than in LAB rats in the anterior hypothalamus,

TABLE 1. Binding affinity and efficacy at the benzodiazepine allosteric site of GABA$_A$

	Human recombinant $GABA_A$ receptors containing $\beta3$, $\gamma2$ plus				
	$\alpha1$	$\alpha2$	$\alpha3$	$\alpha5$	References
FG-7412					
K_i (nM)*	91 ± 22	330 ± 62	492 ± 105	2150 ± 725	(Atack et al. 2005)
% modulation†	−47 ± 2	−38 ± 6	−40 ± 5	−35 ± 4	(Atack et al. 2005; Dawson et al. 2006)
EC_{50} (nM)†	137	507	1021	1439	(Atack et al. 2005)
DMCM					
K_i (nM)	10 ± 1	13 ± 5	7.5 ± 1.2	2.2 ± 1	(Chambers et al. 2003)
% modulation	−71 ± 1	−53 ± 3	−62 ± 2	−57 ± 1	(Chambers et al. 2003; Dawson et al. 2006)
EC_{50} (nM)	3.9	3.7	2.4	1.2	(Dawson et al. 2006)
β-CCM					
K_i (nM)	2.4 ± 0.3	7.4 ± 1.4	72 ± 14	44 ± 6	(Smith et al. 2001)
% modulation	−16 ± 3	−22 ± 5	−19 ± 2	−27 ± 2	(Smith et al. 2001)
EC_{50} (nM)	–	–	–	–	
α3IA					
K_i (nM)	1029 ± 118	323 ± 20	82 ± 12	410 ± 134	(Collins et al. 2002)
% modulation	−31 ± 4	−24 ± 4	−45 ± 5	−4 ± 4	(Atack et al. 2005)
EC_{50} (nM)	1300	185	70	–	(Atack et al. 2005)
α5IA					
K_i (nM)	0.88 ± 0.19	0.58 ± 0.17	0.61 ± 0.17	0.66 ± 0.14	(Dawson et al. 2006)
% modulation	−18 ± 3	+13 ± 3	−7 ± 4	−40 ± 1	(Dawson et al. 2006)
EC_{50} (nM)	–	–	–	2.2	(Dawson et al. 2006)
α5IA-II					
K_i (nM)	1.4 ± 0.7	2.7 ± 0.8	1.4 ± 0.4	0.80 ± 0.34	(Collinson et al. 2006)
% modulation	−14 ± 4	−7 ± 3	−17 ± 5	−45 ± 3	(Collinson et al. 2006)
EC_{50} (nM)	3.2	na	5.6	2.5	(Collinson et al. 2006)
Zolpidem					
K_i (nM)	52.1	255.1	608.5	>10000	(Street et al. 2004)
% modulation	+133 ± 31	+187 ± 23	+142 ± 25	na	(Street et al. 2004)
EC_{50} (nM)	86.6 ± 14.1	–	–	–	(Hadingham et al. 1995)
Ro 1788					
K_i (nM)	1.1 ± 0.04	1.5 ± 0.1	1.0 ± 0.1	0.50 ± 0.08	(Smith et al. 2001)
% modulation	6 ± 3	10 ± 3	19 ± 3	53 ± 12	(Smith et al. 2001)
EC_{50} (nM)	–	–	–	–	
Chloirdiazepoxide					
K_i (nM)	770 ± 170	460 ± 71	740 ± 160	520 ± 140	(Sternfeld et al. 2004)
% modulation	–	–	–	–	
EC50 (nM)	–	–	–	–	
Diazepam					
K_i (nM)	14	20	15	11	(Atack et al. 1999)
% modulation	103 ± 14	135 ± 11	118 ± 28	106 ± 3	(Dawson et al. 2006)
EC_{50} (nM)	27	19	48	17	(Dawson et al. 2006)

*Affinity measured as the ability of the compound to displace [^{3}H]Ro 15-1788 at $GABA_A$ receptors.
†Efficacy and % modulation measured in terms of the ability of the compound to modulate the calcium ion flux produced by an effective concentration of GABA resulting in a 20% maximal response.

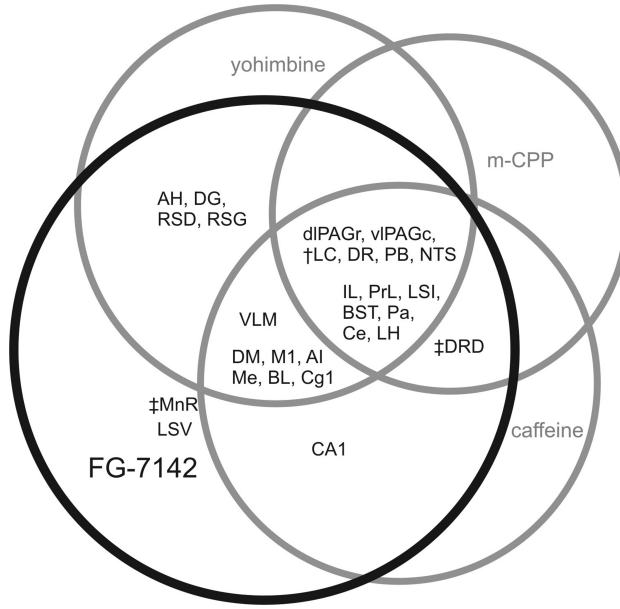

FIG. 2. A diagram illustrates the forebrain and hindbrain sites of convergent action of FG-7142 with three other anxiety-related compounds: (1) caffeine, an adenosine receptor antagonist; (2) m-chlorophenylpiperazine (mCPP), a nonselective 5-HT_{2C} receptor agonist; and (3) yohimbine, an α_2-adrenoceptor antagonist, as indicated by induction of c-Fos protein expression following systemic drug injection (Singewald and Sharp 2000; Singewald et al. 2003). † indicates coexpression of c-Fos and dopamine-β-hydroxylase immunostaining following systemic FG-7142 (Salchner et al. 2006). ‡ indicates coexpression of c-Fos and tryptophan hydroxylase immunoreactivity following systemic FG-7142 (Abrams et al. 2005). Abbreviations: AH, anterior hypothalamus; AI, agranular insular cortex; BL, basolateral amygdaloid nucleus; BST, bed nucleus of the stria terminalis; CA1, CA1 region of the hippocampus; Ce, central amygdaloid nucleus; Cg1, cingulate cortex; DG, dentate gyrus; DM, hypothalamus, dorsomedial part; DRD, dorsal raphe nucleus, midrostrocaudal and caudal, dorsal part; IL, infralimbic cortex; PrL, prelimbic cortex; LC, locus coeruleus; LH, lateral hypothalamus; LSI, lateral septal nucleus, intermediate part; LSV, lateral septum, ventral part; M1, primary motor cortex; Me, medial amygdaloid nucleus; MnR, median raphe nucleus; NTS, nucleus of the solitary tract; Pa, hypothalamus, paraventricular nucleus; dlPAGr, dorsolateral periaqueductal gray, rostral part; vlPAGc, ventrolateral periaqueductal gray, mid-rostrocaudal part; PB, parabrachial nucleus; RSD, retrosplenial dysgranular cortex; RSG, retrosplenial granular cortex; VLM, ventrolateral medulla.

medial preoptic area, lateral parabrachial nucleus, and locus coeruleus, and lower-Fos protein in HAB than in LAB rats in the prelimbic and infralimbic cortices as well as area 2 of the cingulate cortex (Salchner et al. 2006). The former areas are believed to play a role in the facilitation of anxiety-related behavior (Gray and McNaughton 2000; Millan 2003), whereas the latter are believed to play a role in inhibition of anxiety states (Rosenkranz et al. 2003; Amat et al. 2005). Double-immunostaining with dopamine-β-hydroxylase and c-Fos following treatment of rats with FG-7142 (7.5 mg/kg, i.p.) revealed that a majority of the FG-7142-induced increase in c-Fos protein in the locus coeruleus occurred in noradrenergic neurons (Salchner et al. 2006) (see *Neurochemical Effects*: *Norepinephrine*).

Metabolic Changes

Functional magnetic resonance imaging (fMRI) studies in rats have shown that FG-7142 (10 mg/kg i.p.), like the $5HT_{2C}$ agonist, mCPP, increases the blood oxygen level dependent

(BOLD) signal in the amygdala, dorsal hippocampus, and medial hypothalamus (Hackler et al. 2007), regions involved in processing affective and stress-related stimuli. These convergent effects of FG-7142 and mCPP on the BOLD signal within the amygdala and hypothalamus, as well as on anxiety-related behavioral inhibition in the social interaction test (see *Behavioral Effects*: *Social interaction test*) were blocked by pretreatment with the $5HT_{2C}$ receptor antagonist, SB 242084. Both drugs also had convergent effects to decrease BOLD signal in the medial prefrontal cortex, effects that were not blocked with the $5\text{-}HT_{2C}$ receptor antagonist. While the functional significance of changes in BOLD signal are not fully understood and may change across a time course in relation to changes in activity within a neural network, the medial prefrontal cortex is thought to be a key site of FG-7142 action. The medial prefrontal cortex is reciprocally connected to multiple nodes of an anxiety-related network including the amygdaloid complex, and is implicated in the modulation of activity within numerous neural networks (see *Electrophysiology*; and *Neurochemical Effects*: *Dopamine*).

A positron emission tomography (PET) study in conscious rhesus monkeys reported decreased regional cerebral blood flow in the thalamus, a major relay center for sensory information into the cortex, following FG-7142 administration (1 mg/kg i.m.) and dose-dependent decreases in regional cerebral metabolic rate of glucose utilization in cortical and motor regions (cingulate, frontal, parietal, temporal, and occipital cortices, and striatum, thalamus, and cerebellum) (Takamatsu et al. 2003). In another study, analysis of changes in local cerebral glucose utilization (LCGU) following FG-7142 administration (5–10 mg/kg i.v.) in rats revealed *increased* LCGU in limbic structures (lateral septal nucleus and anterior thalamic nuclei) as well as motor structures (substantia nigra pars reticulata, globus pallidus, ventral thalamic nuclei, and cerebellum). In addition, dramatic increases of up to 70% were observed in the mammillary bodies, structures that are integrated with septohipocampal circuits and involved in relaying information from the amygdaloid complex and hippocampal formation to the thalamus. These effects were reversed by pretreatment with the benzodiazepine antagonist Ro 15–1788 (Ableitner and Herz 1987). The functional implication of changes in regional cerebral blood flow and LCGU are not well-understood; cerebral blood flow may be regulated independent of changes in neuronal activity (Giardino et al. 2007), but regional changes serve to identify brain regions that may be involved in specific processes, such as anxiety, and can corroborate other lines of functional evidence.

Increases in energy (lactate) metabolism in the basolateral amygdaloid complex and medial prefrontal cortex are seen following footshock or psychological stress (Uehara et al. 2006). FG-7142 (5–10 mg/kg i.p.) replicates this effect in the basolateral amygdaloid complex (medial prefrontal cortex was not measured) with a maximum increase observed at 8 min and return to baseline within 60 min (Uehara et al. 2005). This effect is attenuated with diazepam pretreatment.

Electrophysiology

Electrophysiological oscillations of the hippocampal formation, specifically theta oscillations, have been shown to be positively correlated with anxiety-related behavioral inhibition. Classic and novel anxiolytic drugs, including diazepam, share the common feature of reducing hippocampal theta rhythm (Gray and McNaughton 2000). GABAergic projections from the medial septum to the hippocampal formation (Vertes and Kocsis

1997; Buzsaki 2002) and serotonergic innervation from the median raphe nucleus (Leranth and Vertes 1999) are believed to be involved in regulating hippocampal theta rhythm. *In vivo* electrophysiological studies from anesthetized rats revealed that systemic administration of diazepam (0.03–0.1 mg/kg) inhibited, while FG-7142 (1 mg/kg i.p.) enhanced hippocampal theta rhythm, consistent with reported anxiolytic and anxiogenic actions of these compounds (Hajos et al. 2004). The $\alpha 5$ subunit-containing $GABA_A$ receptor is preferentially located in the hippocampus (Sur et al. 1999; Pirker et al. 2000) and is implicated in hippocampal-sensitive behavioral tasks (Crestani et al. 2002; Collinson et al. 2006) and enhancement of long-term potentiation induced by a theta burst stimulation in the hippocampus (Atack et al. 2006a). FG-7142 has a low affinity for the $\alpha 5$ subunit, and, consequently, the effects of FG-7142 on desynchronization of hippocampal theta rhythm are likely to be mediated in regions that modulate hippocampal theta rhythm, such as the medial septum or median raphe nucleus, both of which have been identified as sites of action following FG-7142 administration (Abrams et al. 2005; Funk et al. 2006), and both of which express $\alpha 1$ subunit-containing $GABA_A$ receptors (Gao et al. 1993).

The medial prefrontal cortex and basolateral amygdaloid nucleus have reciprocal connections and display a synchronized pattern of neuronal firing. The projections from the medial prefrontal cortex to the basolateral amygdaloid complex are thought to play an important role in fear extinction (Quirk et al. 2003; Milad et al. 2004), as well as modulation of sensory driven output from the basolateral amygdaloid complex (Rosenkranz and Grace 2001). Extracellular single unit and local field potential recordings used to simultaneously examine basolateral amygdaloid and medial prefrontal cortical neuronal activity revealed that systemic FG-7142 injections (0.3–10 mg/kg i.v.) disrupt synchronized unit firing between the two regions (Stevenson et al. 2007) (see *Neurochemical Effects: Dopamine*). Flumazenil blocked these effects. Additionally there is evidence that FG-7142 injections (10 mg/kg i.v.) induce long-term potentiation in amygdalar efferents to the ventromedial hypothalamus and periaqueductal gray, changes which have been shown to be involved in chronic anxiety-related behavioral effects of FG-7142 (Adamec 2000a; Adamec 2000b).

Neurochemical Effects

Serotonin

Serotonergic systems arising from the dorsal raphe nucleus may play an important role in the anxiogenic effects of FG-7142. A study looking at the convergent action of multiple anxiogenic drugs in the dorsal raphe nucleus revealed that FG-7142 (7.5 mg/kg i.p.), the $5\text{-}HT_{2C}$ agonist mCPP, and caffeine induced *c-Fos* expression in serotonergic neurons, specifically in the caudal part of the dorsal raphe nucleus. Other studies have revealed that the anxiety-related peptide urocortin 2 (Staub et al. 2006), or exposure to anxiety-related stimuli, such as social defeat (Gardner et al. 2005) or inescapable shock (Grahn et al. 1999) also increase *c-Fos* expression in serotonergic neurons in this region of the dorsal raphe nucleus. Increases in *c-Fos* expression were also observed in serotonergic neurons of the median raphe nucleus following systemic FG-7142 (7.5 mg/kg i.p.) (Abrams et al. 2005), although this response was not observed following treatment with other anxiogenic drugs. However, serotonergic projections from the median raphe nucleus to the medial septum or hippocampal formation are believed to be involved in desynchronization of hippocampal theta rhythm (Leranth and Vertes 1999), an anxiety-related effect observed

following FG-7142 (7.5 mg/kg i.p.) administration (see *in vivo Pharmacology*: Actions within an Anxiety-Related Network). Systemic FG-7142 injections (7.5 mg/kg i.p.) result in increases in indices of serotonin metabolism in components of the forebrain anxiety-related network, most notably the prelimbic region of the prefrontal cortex (Evans et al. 2006); 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 (Marek and Aghajanian 1998; Puig et al. 2005). Systemic FG-7142 (10 mg/kg i.p.), or microinjections of the related β-carboline, DMCM (1 μg), directly into the dorsal raphe nucleus, have been shown to mimic the effects of uncontrollable stress in the induction of deficits in escape behavior measured 24 h later in a model of learned helplessness (Drugan et al. 1985; Maier et al. 1995a), a behavioral response that is dependent on increases in serotonin neurotransmission within components of an anxiety-related network including the medial prefrontal cortex (Maier and Watkins 2005b). Microinjections of FG-7142 (40 pmol) directly into the rat dorsal raphe nucleus increase passive avoidance behavior in an elevated T-maze test of anxiety-related behavior (Graeff et al. 1996; Sena et al. 2003) but have no effect on serotonin concentrations in either the amygdala or periaqueductal gray (Viana et al. 1997). FG-7142 (10 mg/kg i.p.) and mCPP have convergent actions on anxiety-related behavioral responses in the social interaction test, which can be reversed with a $5HT_{2C}$ receptor antagonist (Hackler et al. 2007). In summary, anxiogenic effects of FG-7142 appear to be dependent in part on activation of serotonergic systems and $5\text{-}HT_{2C}$ receptor-signaling mechanisms.

Dopamine

Systemic administration of FG-7142 activates mesolimbocortical dopaminergic projections, leading to increases in dopamine in the prefrontal cortex in monkeys (0.2 mg/kg i.m.) (Murphy et al. 1996b) and rats (15–30 mg/kg i.p.) (Bradberry et al. 1991a; Horger et al. 1996; Bassareo et al. 1996; Murphy et al. 1996b; Dazzi et al. 2001; Atack et al. 2005) and in the nucleus accumbens in rats (10–30 mg/kg i.p.) (Brose et al. 1987; McCullough and Salamone 1992; Horger et al. 1996), although see Bassareo et al. (1996).

FG-7142 (15 mg/kg i.p) increases tyrosine hydroxylase activity and dopamine turnover in the medial prefrontal cortex and ventral tegmentum *in vivo* and *in vitro*, but effects were not detected in mesolimbic or nigrostriatal areas (Knorr et al. 1989). FG-7142-induced increases in dopamine turnover in the prefrontal cortex may be mediated via actions at the α3-subunit-containing $GABA_A$ receptor subtype as a pyridone selective inverse agonist, α3IA, or FG-7142 (15 mg/kg i.p), or immobilization stress all increase dopamine metabolism in the medial prefrontal cortex (Atack et al. 2005). Increases in dopamine neurotransmission in the prefrontal cortex following FG-7142 administration (20–30 mg/kg i.p.) in rats can be blocked by glycine/NMDA receptor antagonists administered systemically or into the ventral tegmental area (Horger et al. 1996; Murphy et al. 1996b), by α2 adrenoceptor agonists administered systemically (Murphy et al. 1996b), or by chronic but not acute pre-administration of either imipramine or mirtazapine (Dazzi et al. 2001). The functional implication of increases in dopamine neurotransmission in the prefrontal cortex is not well-established. There is evidence that increases in dopamine in the prefrontal cortex can increase sensory driven output within another component of the anxiety-related network, the basolateral amygdaloid nucleus (Rosenkranz and Grace 2001). Excessive dopamine receptor stimulation in the prefrontal cortex has been implicated in FG-7142 impairment

of spatial working memory in both rats and monkeys (Murphy et al. 1996a; Murphy et al. 1996b).

Increases in dopamine observed in the nucleus accumbens are controversial. In contrast to previously reported increases in accumbal dopamine concentrations following systemic FG-7142 (10–30 mg/kg i.p.) (McCullough and Salamone 1992), Bassareo and colleagues (1996) demonstrate that with use of microdialysis they can detect increases in dopamine concentrations in the nucleus accumbens, but not the prefrontal cortex, following morphine, ethanol, and nicotine administration. In contrast, they do not detect increases in dopamine concentrations in the nucleus accumbens, but detect increases in the prefrontal cortex, following FG-7142 (10 mg/kg i.p.), picrotoxin, or pentylentetrazol administration. Analysis of tissue concentration of dopamine and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) in microdissected subregions of the nucleus accumbens 30 min following FG-7142 administration (15 mg/kg i.p.) reveals a small increase in the ratio of DOPAC to dopamine in the shell, but not the core of the nucleus accumbens, an effect that is blocked with systemic antagonism of the glycine/NMDA receptor (Horger et al. 1996). Increases in dopamine concentrations in the nucleus accumbens have also been shown to be temporally delayed (Brose et al. 1987). While the functional implication of increases in dopamine concentrations in the shell of the nucleus accumbens is often attributed to a reward-related pathway, FG-7142 is not self-administered in rats, and this association is debatable (see *Behavioral Effects*: *Brain stimulation*). A functional implication of FG-7142-induced increases in dopamine concentrations in the nucleus accumbens is disinhibition of cholinergic projections to the cortex resulting in increases in cortical acetylcholine release (Moore et al. 1999), which are thought to mediate increases in attention or vigilance processes (Sarter et al. 2001b).

Acetylcholine

While benzodiapezines decrease cortical acetylcholine transmission, FG-7142 (8 mg/kg i.p.) increases cortical acetylcholine concentrations, an effect which can be blocked by selective lesions of the cortical-projecting basal forebrain cholinergic neurons (Berntson et al. 1996), by systemic haloperidol administration, or by microinjections of D2 antagonists in the nucleus accumbens (Moore et al. 1999) (see *Neurochemical Effects*: *Dopamine*). As discussed later (*Behavioral Effects*: *Cognition*), this increase in cholinergic transmission in the cortex is thought to mediate an anxiety-related increase in vigilance (Himmelheber et al. 2000), which is seen following FG-7142 administration. This basal forebrain-cortical cholinergic system is also thought to mediate cardioacceleratory responses associated with FG-7142 administration [for review see (Berntson et al. 1998)].

Norepinephrine

It has been demonstrated that FG-7142 (10–20 mg/kg i.p.) has no effect on baseline noradrenaline release in the frontal cortex of freely moving rats, but results in an enhanced release in rats subsequently placed into a novel environment (Mason et al. 1998). A higher dose of FG-7142 (30 mg/kg) induces a 90% increase in norepinephrine output in the prefrontal cortex of freely moving rats, and this result is blocked by chronic but not acute pre-administration of either imipramine or mirtazapine (Dazzi et al. 2002a) or the

serotonin/norepinephrine reuptake inhibitor, venlafaxine (Dazzi et al. 2002b). Other studies report FG-7142-induced (15 mg/kg, i.p.) norepinephrine release in the hypothalamus, amygdala, and locus coeruleus but not hippocampus or cortex (Ida et al. 1991) [see (Tanaka et al. 2000) for review].

Acute administration of FG-7142 (40 mg/kg i.p.) resulted in an increase in cortical β-adrenoceptor density measured 7 days after treatment (Stanford et al. 1987), while FG-7142-induced kindling (40 mg/kg i.p. for 12 days) increased both cortical $\alpha 2$- and β-adrenoceptor binding sites measured 7 days after treatment (Stanford et al. 1986).

In summary, these different methods of assessing effects of FG-7142 within a neuroanatomical network on various neurochemical systems within the brain reveal that FG-7142 administration results in changes in serotonergic, dopaminergic, cholinergic, and noradrenergic transmission within a distributed anxiety-related neural circuit-including the medial prefrontal cortex and amygdaloid complex.

Behavioral Effects

FG-7142 has been demonstrated to induce anxiety-related behaviors in a variety of experimental paradigms in rats, mice, cats, and primates (Ninan et al. 1982; Ongini et al. 1983; Skolnick et al. 1984; File and Baldwin 1987; Thiebot et al. 1988) and to induce an anxiety state in humans (Dorow et al. 1983; Dorow 1987). Generally, FG-7142 has been demonstrated to have proconflict (File and Baldwin 1987) and proconvulsant (Little et al. 1984) effects, and to increase attentional processes (Sarter et al. 2001a). Specific behavioral and physiological effects of FG-7142 are summarized below.

Home cage behavior

Systemic administration of FG-7142 in rats (7.5 mg/kg i.p.) induces spontaneous nonambulatory motor activity, including increased visual scanning of the cage and nonambulatory limb movements, between 30 and 90 min upon being returned to the home cage environment (Abrams et al. 2005). In this study, FG-7142 was one of multiple pharmacologically diverse anxiogenic drugs tested, and spontaneous nonambulatory motor activity was the only behavioral response common to all drugs. This behavioral response is thought to represent an increase in vigilance and risk assessment in the absence of any novelty or clearly identifiable threat (Butler et al. 1990).

Vogel punished drinking

FG-7142 suppressed punished drinking in rats at doses that had no effect on unpunished drinking ($IC_{30} = 1.8$ mg/kg i.v.), but was the least potent of other β-carbolines tested [β-CCM, β-CCE, and DMCM (Corda et al. 1983; Stephens et al. 1987)]. The effects of FG-7142 on punished drinking are blocked or reversed by pretreatment with either the benzodiazepine antagonists Ro 1501788 or CGS 8216 or the agonist diazepam (Corda et al. 1983). FG-7142 and the other β-carboline-induced increases in anxiety-related behavioral responses in this test were evident only when the frequency of punished drinking was increased by reducing the intensity of the shock from 0.8 mA to 0.35 mA. Chronic FG-7142 treatment (15 mg/kg i.p. twice daily for 10 days) suppresses punished drinking, 5 and

15 days following the last treatment, an effect that coincides with a decrease in the density of GABA binding sites (Biggio et al. 1987).

Punished locomotor (four plate test)

In an experimental test of conflict between exploration of a novel chamber and receiving a shock when crossing into a new area (plate) in a chamber, FG-7142 (25 and 100 but not 6.25 mg/kg i.p.) reduced exploratory behavior both in punished and unpunished conditions, which could either reflect a general reduction in locomotor activity or a reduction in exploration of a novel environment (Stephens and Kehr 1985).

Elevated plus-maze test

Numerous studies in rats and mice have demonstrated that FG-7142 (10–100 mg/kg i.p.) decreases the amount of time spent in the open arms and increases the time spent in the closed arms of an elevated plus-maze (Pellow and File 1986; Pellow et al. 1987; Cole et al. 1995; Rodgers et al. 1995; Atack et al. 2005; Dawson et al. 2006). The anxiogenic effects of FG-7142 in the elevated plus-maze are similar to those of an inverse agonist, α3IA, specific to the α3-subunit-containing $GABA_A$ receptor (Atack et al. 2005) but not of α5IA, an inverse agonist with specific efficacy at the α5-subunit-containing $GABA_A$ receptor (Dawson et al. 2006), suggesting that this behavioral component of FG-7142 may be mediated via actions at the α3-subunit-containing $GABA_A$ receptor. In contrast to FG-7142, and DMCM, other β-carbolines β-CCE, ZK 132553, and ZK 90886 did not alter the time spent exploring the open arms of the elevated plus-maze at any of a range of doses (Cole et al. 1995).

Elevated T-maze test

A variation of the elevated plus-maze, the elevated T-maze, has one closed arm perpendicular to two opposing open arms. Inhibitory avoidance can be measured by placing rats in the closed arm and measuring the amount of time it takes for the test subject to venture into the open arm. Active escape latencies can also be measured by placing rats in the open arm and measuring the time it takes the test subject to retreat to the closed arm. FG-7142 microinjected into the dorsal periaqueductal gray facilitated both inhibitory avoidance (40 pmol) and decreased escape latencies (20–80 pmol), suggesting panicogenic and anxiogenic effects (Bueno et al. 2005). On the other hand, the benzodiazepine agonist, midazolam, had no effect on inhibitory avoidance but increased escape latency. Intradorsal raphe nucleus microinjections of FG-7142 (40 pmol) facilitated inhibitory avoidance, but either did not affect escape latencies (Graeff et al. 1996) or impaired escape (Sena et al. 2003).

Social interaction test

In the social interaction test, an experimental paradigm involving social interaction with a novel conspecific, FG-7142 (10 mg/kg i.p.) and mCPP have convergent effects on

anxiety-related behavior. The effects of both drugs can be reversed with the $5HT_{2C}$ receptor antagonist, SB-232084 (Hackler et al. 2007). Several other studies have reported anxiety-related effects of FG-7142 in the social interaction test in rats and mice. FG-7142 increases avoidance behavior, decreases aggression, and decreases time spent in social interaction (File and Pellow 1984; Beck and Cooper 1986; Rawleigh and Kemble 1992), effects that are reversed by either chlordiazepoxide (5 mg/kg) or Ro 15–1788 (10 mg/kg) (File and Pellow 1984). The effects of FG-7142 on social interaction are similar to the effects of inescapable shock (Short and Maier 1993).

Resident intruder paradigm

The effects of FG-7142 on aggression in a resident intruder paradigm have been shown to be context dependent. Systemic administration of FG-7142 in mice increases aggression when a nesting box is available but decreases aggression when a nesting box is not available (Rawleigh and Kemble 1992).

Drug discrimination

In the drug discrimination test, rats trained to discriminate between a training drug and saline are tested with other drugs to determine if the test drug can substitute for the training drug in terms of interoceptive cues to elicit the normal response to the training drug, usually a specific lever press. When rats have been trained to discriminate a DMCM injection, they can be induced to respond to FG-7142 at 78% similarity to the DMCM response (Nielsen et al. 1985). Rats trained to discriminate diazepam or chlordiazepoxide were not induced to respond to FG-7142 (2.5–20 mg/kg i.p.); FG-7142 (2.5–10 mg/kg i.p.) antagonized the ability to recognize chlordiazepoxide and was able to substitute for a pentylenetetrazol cue (Stephens et al. 1984a; Stephens et al. 1984b).

Place preference test

File and Baldwin (1987) reported unpublished observations that rats given an injection of FG-7142 (2.5 mg/kg i.p.) then confined to one compartment of a box, will spend less time in the FG-7142 compartment on subsequent trials given a choice between the FG-7142 compartment and another compartment, suggesting an aversive association with the FG-7142 injection.

Brain stimulation

Systemic administration of FG-7142 (5–20 mg/kg i.p) in rats can mimic the effects of a social defeat paradigm, decreasing the frequency of self-stimulation to the lateral hypothalamus in rats. This effect of FG-7142 or social defeat is presumably due to a reduction in the rewarding effects of self-stimulation (Kureta and Watanabe 1996). Other studies have confirmed that administration of FG-7142 (1–20 mg/kg i.p.) or β-CCE reduces the response rate of lateral hypothalamic self-stimulation, an effect blocked by and opposite to that of chlordiazepoxide (Pellow et al. 1984; Mumford and Holtzman 1991). These

findings are incongruous with the idea that drugs that increase dopamine transmission in the nucleus accumbens lead to an increase in lateral hypothalamic self-stimulation (Colle and Wise 1988). However, it has been hypothesized that dopamine release in the nucleus accumbens is not sufficient to potentiate reward, but may be involved in aspects of appetitive motivation such as cost–benefit analysis and reward prediction (Neill et al. 2002; Salamone et al. 2007) (see *Neurochemical Effects*: *Dopamine*).

When examining electrical stimulation of the prefrontal cortex, FG-7142 (10 mg/kg i.p.) mimics the effects of restraint stress, increasing rates of self-stimulation for up to 24–48 h (McGregor and Atrens 1990). Recent studies suggest that stimulation of the medial prefrontal cortex inhibits the anxiety state generated by uncontrollable stress (Amat et al. 2005) and may suppress sensory-driven affective output of the basolateral amygdaloid complex (Rosenkranz and Grace 2001).

Startle response

Whereas the inverse agonist DMCM has been shown to enhance fear-potentiated startle responses (Hijzen and Slangen 1989), more recent studies have shown that FG-7142 (8 mg/kg i.p.) has convergent actions with the benzodiazepine chlordiazepoxide in suppression of both the basal and fear-potentiated somatic startle response, also eliminating the fear potentiation seen in vehicle-treated groups (Hart et al. 1998). Additionally, FG-7142 (8 mg/kg i.p.) had no effect on the cardiovascular component of the startle response (Hart et al. 1998). Risbrough and Geyer confirm that FG-7142 (10–20 mg/kg i.p.) suppresses fear-potentiated startle, but report that it has no effect on the baseline startle response in mice (Risbrough and Geyer 2005). While not enhancing either fear-potentiated or basal startle responses, FG-7142 (8 mg/kg i.p.) increases the cardiovascular defensive response to nonstartle acoustic stimulation during a startle paradigm, termed a *cardioacceleratory defensive response* (Quigley et al. 1994; Hart et al. 1999) (see *Neuroendocrine Effects*: on the autonomic nervous system). Hart and colleagues (1999) propose a model in which FG-7142 has actions within a cortical system modulating cardioacceleratory defensive responses that are separate from systems modulating basal startle responses.

Conditioned fear

In a conditioned fear paradigm in which a danger cue signals an aversive stimulus and a safety signal indicates omission of the aversive stimulus, FG-7142 (4 and 8 mg/kg i.p.) enhances behavioral suppression in response to withdrawal of a safety signal without reducing appetitively motivated responding in the presence of the safety signal (Thiebot et al. 1991). This behavioral suppression in response to withdrawal of a safety signal occurs even when the danger cue is absent (a cue that control animals associate with a safe period). In a similar conditioning paradigm in which 22-kHz ultrasonic vocalizations from rats were measured as an index of emotionality, control rats vocalized between trials and during the safety signal, but suppressed vocalizations during the danger cue. FG-7142 (5 mg/kg i.p.) resulted in a suppression of vocalizations during the safety signal, but had no effect on inter-trial vocalizations (Jelen et al. 2003). These two studies depict situations in which FG-7142 impairs the ability to suppress fear responses in the presence of a safety cue recognized by control rats, suggesting that FG-7142 may disinhibit fear-related behavior. In

support of this hypothesis, FG-7142 (5–10 mg/kg i.p.) has been demonstrated to facilitate recovery of extinguished fear (Harris and Westbrook 1998; Kim and Richardson 2007) and likewise recovery of fear cues acquired at a very young age and extinguished with maturity (infantile amnesia) in rats (Kim et al. 2006).

Learned helplessness

In a behavioral paradigm examining the consequences of uncontrollable or inescapable stress, called "behavioral depression" (Weiss et al. 1981) or "learned helplessness" (Maier and Seligman 1976), FG-7142 (10 mg/kg i.p.) and DMCM (1 μg dorsal raphe nucleus microinjection) 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; Short and Maier 1993; Maier et al. 1995a). These effects of uncontrollable stress, that are reproduced with FG-7142, have been linked to neural mechanisms regulating anxiety states (Maier and Watkins 2005a) and can be blocked with the anxiolytic drug flumazenil or the selective benzodiazepine antagonist Ro 151788 (Drugan et al. 1985; Maier et al. 1995b).

Proconvulsant and kindling effects

A seminal study (Little et al. 1984) on the convulsant effects of acute and chronic FG-7142 administration in mice revealed that acute administration (10–80 mg/kg i.p) does not induce seizures [although see (Nutt and Lister 1988) for mouse strain differences in this effect] but reduces seizure thresholds to convulsant compounds such as pentylenetetrazol (proconvulsant effect) (Little et al. 1984). The concentration–response curve of this proconvulsant effect is "U" shaped with a maximal reduction in seizure threshold at 40 mg/kg and elimination of the proconvulsive effect at 160 mg/kg (Little et al. 1984). Kindling is the ability of repeated subthreshold chemical or electrical stimulation to sensitize or induce convulsant effects with a subsequent subthreshold challenge. Little and colleagues (1984) using several chronic administration schedules (three times weekly, once daily, or three times daily), determined that, while all three schedules induced kindling, a once daily administration schedule (40 mg/kg i.p.) for 12 days was optimal for kindling effects. Under this schedule, FG-7142 sensitizes mice and rats to convulsant effects of a subsequent single dose of FG-7142 (40 mg/kg i.p.) after 4 days of administration with increasing sensitization occurring through day 12 and persisting one week and up to one month after the last kindling administration (Little et al. 1984; Little et al. 1987a; Dawson et al. 2006). While a range of doses of FG-7142 (10–80 mg/kg i.p) has proconvulsant effects, a dose of at least 40 mg/kg was necessary for kindling effects, as a lower dose of 20 mg/kg did not induce seizures at any point during the 12-day chronic administration schedule, or one week later. The kindling effects of chronic FG-7142 administration can be blocked with coadministration of a benzodiazepine antagonist, the imidazobenzodiazepine Ro 15–1788, administered either during kindling exposures or at the time of challenge following kindling (Little et al. 1984). Chronic FG-7142 treatment not only sensitizes the convulsant effects of subsequent administration of FG-7142 or other β-carboline inverse agonists such as β-CCM and DMCM, but also decreases the anticonvulsant effects of β-carboline (but not benzodiazepine) agonistic modulation of the $GABA_A$ receptor (Little et al. 1986; Petersen

and Jensen 1987; Little et al. 1987a; Little et al. 1987b). Chronic benzodiazepine (7 days flurazepam, 40 mg/kg i.p.) treatment leads to convulsive effects of a single dose of FG-7142 (10–80 mg/kg i.p.) 24 h later and lasting up to 1 week (Little et al. 1988). These dynamic changes that occur either at the allosteric binding site or at downstream sites (see *Neurochemical effects*: *Norepinephrine*) need to be considered when designing experiments involving chronic FG-7142 administration. See Little et al. (1987a) for a thorough review of the chronic effects of FG-7142 (40 mg/kg i.p for 12 days).

Cognitive effects

It has been suggested that anxiogenic effects of FG-7142 would be more accurately described as *hyperattentional effects resulting in overprocessing of conditioned and contextual stimuli* [for review see (Sarter et al. 2001a)]. In this context, FG-7142 administration has been suggested as a model to study neuropharmacological, behavioral, and cognitive aspects of psychosis believed to underlie disorders such as schizophrenia. It has been proposed that the psychotogenic and anxiogenic effects of FG-7142 may be different behavioral manifestations of a common cognitive action, that being induction of a hyperattentive state or overprocessing of conditioned and contextual stimuli (Sarter et al. 2001a), which would lead to increased vigilance and risk-assessment behavior. There is evidence to suggest that the anxiogenic actions of FG-7142 may be restricted to situations in which contextual information processing is required, such as in the case where FG-7142 (8 mg/kg i.p.) potentiates a cardioacceleratory defensive reactivity to a fear cue without affecting baseline cardiovascular responses to an unconditioned (and noncognitive) stimulus (Hart et al. 1998). As another example, FG-7142 (10–20 mg/kg i.p.) reduces fear-potentiated startle without affecting the baseline startle response (Risbrough and Geyer 2005). An anxiogenic response induced by FG-7142 may be attributed to an enhanced cognitive tendency to monitor punishment cues (or lack of safety cues) to determine the predictability of punishment delivery; a state of increased vigilance may lead to behavioral inhibition if it results in a biased attribution of significance to cues and contexts with potential aversive outcomes (Berntson et al. 1998). For example, in a paradigm in which animals were trained to press a lever for food and were presented with distinct cues signaling danger (potential shock) versus safety periods, withdrawal of the safety signal even in the absence of the danger cue reduces lever pressing, an effect that was exacerbated following FG-7142 (8 mg/kg i.p.) (Thiebot et al. 1991). It is possible that FG-7142 increases the salience of the aversive nature of withdrawal of the safety cue despite the absence of the danger cue.

Impulsivity may be a reflection of a lack of vigilance or a failure to monitor cues from the environment to determine an appropriate response. In a mouse 5-choice serial reaction time task providing an index of sustained and divided visuospatial attention, FG-7142 (10 mg/kg i.p.) reduced, while diazepam (2 mg/kg i.p.) increased, anticipatory (impulsive) responding in neuropeptide Y receptor 2 knockout mice (that have an anxiolytic-like impulsive phenotype) but had no effect on impulsivity in wild-type controls (Greco and Carli 2006). This reduction in impulsivity is consistent with an increase in vigilance and risk-assessment behavior, which could be attributed to increases in attention processing as described above. As is evident from this paradigm, anxiety-related effects of FG-7142 in reducing impulsivity may be more apparent in animals with a high baseline expression of impulsivity.

FG-7142 impairs spatial working memory in rats (20 mg/kg i.p.) and monkeys (0.2 mg/kg i.m.), an effect that can be reversed with the benzodiazepine receptor antagonist Ro 15–1788 or dopamine receptor antagonists, haloperidol, clozapine, and SCH23390 (Murphy et al. 1996a). Additionally, it results in impairments in a delayed alternation task in rats (20 and 30 mg/kg i.p.), an effect that can be prevented by pretreatment with α_2-adrenoceptor agonists (Birnbaum et al. 2000). Other studies have demonstrated that not only does FG-7142 impair performance in an delayed alternation working memory task, but females (ovariectomized with estrogen replacement) are more sensitive to the effects of FG-7142 than males, demonstrating impairments with a 2 mg/kg dose, whereas males were not impaired until receiving a 5 mg/kg dose (Shansky et al. 2004). Moreover, it was found that only ovariectomized females with estrogen replacement or ovary-intact females in proestrus, but not estrus, were impaired with a 2 mg/kg dose of FG-7142, implicating high-circulating estrogen levels in sensitizing the response to FG-7142 (Shansky et al. 2004).

The neurochemical basis of FG-7142-induced hyperattentional processes are thought to involve overactivity of mesolimbic dopaminergic systems (i.e., increased dopamine in the nucleus accumbens) resulting in disinhibition of cholinergic input to cortical areas. Increased cholinergic transmission in the cortex is thought to play a central role in FG-7142-induced increases in vigilance (Himmelheber et al. 2000). In addition, FG-7142-induced increases in dopamine in the prefrontal cortex (see *Neurochemical Effects*: *Dopamine*) may disinhibit prefrontal cortical suppression of sensory-driven output from an anxiety-related neural network (Rosenkranz and Grace 2001). Hyperattention-related cognitive and neurochemical effects of FG-7142 can be reversed with antipsychotic drugs (Murphy et al. 1996a; Sarter et al. 2001a).

Feeding

FG-7142 (2.5–10 mg/kg i.p.) has been demonstrated to reduce feeding behavior in male and female rats with free access to food, and may reduce food palatability (Cooper et al. 1985; Cottone et al. 2007); this effect is bidirectional with agonists at the benzodiazepine binding site increasing food consumption [for review see (Cooper 1987)].

Neuroendocrine Effects

FG-7142 (3–30 mg/kg i.p.) increases basal corticosterone concentrations and potentiates an increase in plasma corticosterone concentrations in a novel environment in rats (Pellow and File 1985; Stephens et al. 1987) and in rhesus monkeys (Takamatsu et al. 2003). An FG-7142-induced (12.5–25 mg/kg i.p.) rise in plasma concentrations of corticosterone in rats is minor compared to that induced by the selective *agonist* for the α1-subtype-containing $GABA_A$ receptor, imadazolpyridine zolpidem (Mikkelsen et al. 2005). Zolpidem also had a stronger effect on plasma corticosterone than two full nonselective agonists, diazepam, and zopiclone, suggesting that agonism at non-α1 (likely α2) subtype-containing $GABA_A$ receptors may oppose the effects of α1-subtype direct agonism on the hypothalamic–pituitary–adrenal (HPA) axis response. In this context, it may be the inverse agonistic effects of FG-7142 at non-α1-subunit-containing $GABA_A$ receptors that mediates the increase in plasma corticosterone. This needs to be studied further.

Effects on the autonomic nervous system

FG-7142 increases heart rate (tachycardia) and blood pressure in humans and monkeys (Ninan et al. 1982; Dorow et al. 1983). However, FG-7142 (8 mg/kg i.p.) results in baseline bradycardia and lengthened heart periods (duration between two consecutive R-peaks on an electrocardiogram) in rats (Hart et al. 1998). FG-7142 (8 mg/kg i.p.) potentiates and chlordiazepoxide attenuates the *cardioacceleratory defensive response* to acoustic stimuli in rats, which is characterized by the change in heart period in response to a nonstartle acoustic stimulation occurring during a startle paradigm as compared to the change in heart period in response to the same acoustic stimulation occurring independent from and prior to the startle paradigm (Quigley et al. 1994; Berntson et al. 1996; Hart et al. 1998; Berntson et al. 1998). This cardioacceleratory response is thought to reflect an increase in defensive reactivity during the startle paradigm and may be mediated by FG-7142-induced enhancement of basal forebrain-cortical cholinergic neurotransmission, as it can be blocked with selective lesions of cortical projecting basal forebrain cholinergic neurons (Berntson et al. 1996). These cardioacceleratory responses are also seen following intracerebroventricular (i.c.v.) carbachol administration, and the effects of both FG-7142 and carbachol administration can be blocked by pretreatment with the muscarinic acetylcholine receptor antagonist, atropine (Berntson et al. 1996). It is thought that the medial prefrontal cortex may be an important cortical recipient of the basal forebrain cholinergic projections mediating the cardioacceleratory response, as anxiogenic stimuli increase acetylcholine neurotransmission in the medial prefrontal cortex (Moore et al. 1999) and the medial prefrontal cortex sends projections to descending structures mediating autonomic reactivity. Indeed, Hart and colleagues (1999) have demonstrated that carbachol microinjections into the medial prefrontal cortex (but not the lateral prefrontal cortex or basolateral amygdaloid complex) increase the cardioacceleratory response to a novel acoustic stimulus without changing basal heart rate, and this effect is blocked by coadministration of the muscarinic receptor antagonist atropine. Furthermore, the FG-7142-induced potentiation of a cardioacceleratory response to an acoustic stimulus was blocked by (1) microinjections of atropine into the medial prefrontal cortex (but not the lateral prefrontal cortex or basolateral amygdaloid complex) and (2) selective lesions of the cholinergic input from the basal forebrain to the medial prefrontal cortex with microinjections of the immunotoxin 192 IgG saporin into the medial prefrontal cortex (Hart et al. 1999).

While FG-7142 (8 mg/kg, i.p.) potentiates the cardioacceleratory defensive response to acoustic stimulation (Berntson et al. 1996; Hart et al. 1998), it has no effect on the cardiovascular component of the baseline or fear potentiated acoustic startle response. It has been suggested that this dissociation between the effects on cardioacceleratory responsivity and on the cardiovascular startle response may be explained by selective actions of FG-7142 within basal forebrain-cortical circuits versus the brainstem basic startle circuitry (Hart et al. 1998). Hart and colleagues (1988) further suggest that FG-7142 may preferentially enhance cardiac sympathetic reactivity versus cardiac sympathetic tone.

Thermoregulation

FG-7142 (30–60, but not 10 mg/kg i.p.) induces a decrease in core body temperature in mice that occurs within 15 min and lasts from 30 to 60 min, depending on the dose (Jackson and Nutt 1991; Jackson and Nutt 1992). FG-7142 administration (40 mg/kg ip)

for 12 days attenuated the hypothermic effects of the $GABA_A$ receptor agonists muscimol and progabide, potentiated the hypothermic effects of pentobarbital, but had no effect on the hypothermic effects of bicuculline, picrotoxin, or pentylenetetrazol (Little et al. 1986).

Immune system

Whereas benzodiazepines have been reported to reverse the immunosuppressant effects of stress-related stimuli, FG-7142 (10 mg/kg i.p.) induces marked immunosuppression in rats within 24 h, an effect that can be blocked by pretreatment with flumazenil (Arora et al. 1987); for review see Zavala (1997).

Clinical Effects

Early human studies on the effects of FG-7142 describe severe anxiety induced by doses (200–400 mg i.v.) resulting in plasma concentrations of 500–1200 ng/mL (Dorow et al. 1983). Subjects reported increased muscle tension, flushing, inability to speak, increased blood pressure and heart rate, sweating, and a feeling of impending death. Subjects could not sit still and paced the room. Reported clearance rates from plasma in one human subject revealed a half-life of 30 min. Anxiety-related effects of FG-7142 were reversible with 1 mg lormetazepam i.v., with a time course of recovery within several minutes. Severe panic-like responses following FG-7142 administration in humans has led to the suggestion that further human experimentation be abandoned for ethical reasons (Malizia and Nutt 1995).

Clearance and half-life

Pharmacodynamic studies of FG-7142 (40 mg/kg i.p.) in mice demonstrate that binding-site occupancy is maximal within 30 min following injection, rapidly declines by 60 min, and returns to baseline between 120 and 240 min (Dawson et al. 2006). FG-7142 has been shown to have a high rate of clearance in rats (Atack et al. 2005) and humans (Dorow et al. 1983), where anxiety-related behavioral and physiological effects of the drug subside within 30 min. This is in agreement with the observation that acute proconvulsant effects of a single dose of FG-7142 (40 mg/kg i.p.) in rats are maximal between 5 and 30 min following injection and are gone by 90 min (Little et al. 1984). Increases in dopamine in the prefrontal cortex of rats are highest 20 min following FG-7142 injection (10 or 25 mg/kg i.p.) and are back to baseline between 60 and 100 min (Bradberry et al. 1991b; Bassareo et al. 1996). Increases in lactate metabolism in the basolateral amygdala following FG-7142 injection (5–10 mg/kg i.p.) peak at 8 min and are back to baseline within 60 min. Finally, hypothermic effects of FG-7142 (30–60 mg/kg i.p.) last between 30 and 60 min (Jackson and Nutt 1991).

Toxicology

The toxicology of acute FG-7142 application is not well-known. Kindling following chronic FG-7142 application (40 mg/kg i.p. for approximately 4–5 days) increases the occurrence of multiple or continuous seizures in mice, many of which are fatal (Little et al. 1986).

SUMMARY AND CONCLUSIONS

FG-7142 has well-characterized neurochemical, behavioral, and physiological effects that make it a valuable pharmacological tool for elucidating the neural underpinnings of behavior and physiology associated with modulation of the benzodiazepine allosteric site of the $GABA_A$ receptor. These effects can be studied as *components* of more complex states such as anxiety, fear, or psychoses. The construct validity of using FG-7142 to model these complex states is, therefore, a function of these components. In the context of anxiety, for example, FG-7142 results in robust activation of an anxiety-related neural network and activation of an anxiety-related subpopulation of serotonergic neurons in the dorsal raphe nucleus. Opposite to the effect of anxiolytic drugs, FG-7142 desynchronizes hippocampal theta rhythm, which may play a role in conflict resolution and behavioral inhibition. FG-7142 induces fear-related behavioral inhibition in situations involving conflicting goals, increases cardioacceleratory defensive reactivity, and can substitute for an uncontrollable footshock in inducing anxiety-related behavior in the social interaction test and in the induction of escape deficits 24 h later in a learned helplessness paradigm. FG-7142 application results in neurochemical changes observed following anxiogenic stimuli, such as (1) activation of cortically projecting basal forebrain cholinergic neurons, which may lead to increased attention or vigilance behaviors; and (2) activation of mesolimbocortical dopaminergic transmission and associated increases in extracellular dopamine concentrations in the prefrontal cortex which have been linked to an increase in sensory driven output from the basolateral amygdaloid nucleus. Increased dopamine neurotransmission in the prefrontal cortex is also thought to be an integral neurochemical component underlying psychoses, relevant to schizophrenic disorders. FG-7142 is likely to modulate $GABA_A$ receptor signaling in multiple functionally distinct neural circuits. Nevertheless, data support the hypothesis that the mechanisms underlying the effects of FG-7142 (allosteric modulation of $GABA_A$ receptor signaling) on cognitive, physiological, and behavioral processes are broadly related to regulation of normal and pathological states including those of anxiety and psychoses.

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