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EFFECTS OF THE VASOPRESSIN (V_{1B}) RECEPTOR ANTAGONIST, SSR149415, AND THE CORTICOTROPIN-RELEASING FACTOR 1 RECEPTOR ANTAGONIST, SSR125543, ON FG 7142-INDUCED INCREASE IN ACETYLCHOLINE AND NOREPINEPHRINE RELEASE IN THE RAT

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Abstract—Arginine vasopressin and corticotropin-releasing factor are two neuroactive peptides that regulate hypothalamic–pituitary-axis and associated stress response. While the potential antidepressant and anxiolytic profiles of corticotropin-releasing factor 1 antagonists have been well studied, the concept of blockade of vasopressin system as another approach for the treatment of emotional processes has only been made available recently by the synthesis of the first non-peptide antagonist at the V_{1b} receptor, SSR149415. In the present study SSR149415 has been compared with the corticotropin-releasing factor 1 antagonist SSR125543 and with anxiolytic and antidepressant drugs on the response of hippocampal cholinergic and cortical noradrenergic systems to the anxiogenic benzodiazepine receptor inverse agonist FG 7142. Acute (0.3–10 mg/kg, i.p.) and long-term administration (10 mg/kg, i.p., 21 days) of SSR149415 and SSR125543 reduced the FG 7142-induced increase in extracellular concentrations of acetylcholine in the hippocampus of anesthetized rats measured by microdialysis. By contrast acute and long-term administration of SSR149415 failed to reduce the FG 7142-induced increase in the release of norepinephrine in the cortex of freely moving rats. The present results demonstrate that the two compounds have similar profiles in a model of activation by an anxiogenic drug of the hippocampal cholinergic system and they suggest that SSR149415 and SSR125543 may have anti-stress anxiolytic and antidepressant effects via a mechanism of action different from classical benzodiazepine ligands and noradrenergic antidepressants.

Key words: microdialysis, stress, depression, antidepressant drugs, acetylcholine, norepinephrine.

The neural circuitry involved in stress responses includes several brain monoaminergic, GABAergic, neuropeptide systems and the hypothalamic–pituitary–adrenal (HPA) axis whose hyperactivity is a robust characteristic of major depression (Holsboer, 1999). Arginine vasopressin (AVP) and corticotropin-releasing factor (CRF) are two main neuroactive peptides regulating the activity of stress sensitive brain circuitry of the anterior pituitary (for review see Griebel et al., 2003). At this pituitary level AVP synergizes with CRF to stimulate the adrenocorticotropin (ACTH) release. Abnormalities in both AVP and CRF levels have been detected in biological fluids of depressed patients (van Londen et al., 1997; de Winter et al., 2003), however animal studies have mainly focused on the antidepressant and anxiolytic effects of CRF_1 receptor antagonists (Lejeune and Millan, 2003; Lelas et al., 2004; Chaki et al., 2004).

The biological effects of AVP are mediated by three G protein-coupled receptor subtypes: the V_{1a} and V_{1b} receptors, which are present in the CNS and activate phospholipases, and the V_2 receptor, mostly found in the kidney and activating adenylyl cyclase (for review see Birnbaumer, 2000). Many selective vasopressin-related compounds are available for characterization of the V_{1a} or V_2 vasopressin receptor subtypes, but until recently, pharmacological studies dealing with vasopressin receptor isoforms were severely hampered by the lack of selective agonists or antagonists recognizing the pituitary V_{1b} vasopressin receptor. A selective, nonpeptide vasopressin V_{1b} receptor antagonist, SSR149415, has recently been characterized (Serradeil-Le Gal et al., 2002) and used to demonstrate that blockade of these receptors induces antidepressant and anxiolytic-like activity in several behavioral models and may represent a new therapeutic approach for the treatment of major depression and of some forms of anxiety (Griebel et al., 2002a).

SSR149415, and also the CRF_1 receptor antagonist SSR125543 (Gully et al., 2002) are able to attenuate stress-related behavioral changes and neurochemical responses in rodents (Alonso et al., 2004; Griebel et al., 2003; Louis et al., in press) as well as enhancement of tail pinch-induced outflow of cortical norepinephrine (NE) in freely moving rats (Griebel et al., 2002b). As stressful events often occur before symptoms of anxiety and depression, the anti-stress action of these drugs may be important in both their anxiolytic and antidepressant effects.

Various experimental conditions, including pharmacological stressors that mimic stressful events, increase the activity of central cholinergic neurons (Inglis and Fibiger,

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Abbreviations: ACh, acetylcholine; ANOVA, analysis of variance; AVP, arginine vasopressin; CRF, corticotropin-releasing-factor; DA, dopaminergic; HPA, hypothalamic-pituitary-adrenal; HPLC, high-performance liquid chromatography; NE, norepinephrine.

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1995; Moore et al., 1995b) and modulation of the cholinergic response to stress might contribute to the therapeutic action of antidepressant drugs.

Indeed, it has recently been shown that the increase in cortical or hippocampal acetylcholine (ACh) release evoked either by an exposure to stress stimuli or by a treatment with the anxiogenic β-carboline compound FG 7142 was prevented by acute administration of anxiolytics (Moore et al., 1995b; Dazzi et al., 1995) and that the increase in cortical ACh release induced by FG 7142 was also prevented by a chronic treatment with antidepressant drugs (Dazzi et al., 2001). There is no clear evidence concerning the rule of hippocampal cholinergic neurons in anxiety and depression but it may be suggested that hippocampal cholinergic system may be involved in the integration of anxiety and memory and that anxiolytics may reduce anxiety through a disruption of the association between emotion and cognition (Degroot and Nomikos, 2005).

To further evaluate the putative neurochemical mechanisms involved in the anti-stress, anxiolytic and antidepressant actions of SSR149415 and SSR125543, we compared the ability of their acute and chronic administration to modulate the excitatory effect of FG 7142 on the extracellular concentration of ACh in the hippocampus. This study was also extended to the noradrenergic system, another classical neurotransmitter system sensitive to stress stimuli and FG 7142 administration.

EXPERIMENTAL PROCEDURES

Animals

Experiments were performed on 143 adult male Sprague–Dawley rats (Charles River, St. Aubin les Elbeuf, France) weighing approximately 300 g. Rats were housed four per cage and maintained under a 12-h light/dark cycle (lights on at 07:00 h) with free access to food and water. They were acclimated for at least 4 days before use. All efforts were made to limit the number of rats used and to limit their suffering. Animal care and handling throughout the experimental procedures were approved by an animal ethics committee and were in accordance with French legislation (décret 87/848) that implemented the European Communities Council Directive (86/609/CEE).

Drugs and experimental protocol

SSR149415 and SSR125543 were synthesized by Sanofi-aventis (Montpellier, and Toulouse, respectively, France) and imipramine and diazepam were obtained commercially (Sigma, Saint-Quentin Fallavier, France). For acute and chronic administration, they were suspended extemporaneously in 0.1% Tween 80 in distilled water and were administered intraperitoneally (5 ml/kg of body weight). In acute administration experiments, SSR149415 and diazepam were given 30 min before FG 7142 (30 mg/kg, i.p., Sigma), while SSR125543 was given 180 min before FG 7142.

Surgery, microdialysis and experimental procedures

Hippocampal ACh release. Rats were anesthetized with urethane (1.4 g/kg, i.p.) and then placed in a stereotaxic frame. Their body temperature was monitored by a rectal probe and adjusted (37±1 °C) by a homeothermic blanket. A microdialysis probe (CMA 12, length 3 mm and outer diameter 0.5 mm, Carnegie Medicine AB, Stockholm, Sweden) was stereotaxically implanted in the ventral hippocampus. The coordinates were 5.3 mm posterior to bregma, 4.8 mm lateral to the midline and 7 mm down from the dura surface (Paxinos and Watson, 1998). The probe was perfused with a gassed Ringer's solution containing 125 nM NaCl, 3 mM KCl, 1.3 mM $CaCl_2$, 1.0 mM $MgCl_2$, 23 mM $NaHCO_3$ and 1.5 mM KH_2PO_4, pH 7.4, at a flow rate of 2 μl/min by using a microinjection pump (CMA-100, Carnegie Medicine AB). To reduce ACh degradation in the dialysate, 10 μM neostigmine (Sigma) was added to the Ringer's solution perfused in the hippocampal probe. Microdialysis sampling started 90 min after the probe was placed into the hippocampus. Serial samples were collected at 30 min intervals. The position of the probe was verified histologically at the end of each experiment.

Assay of ACh. ACh levels were measured in 30 min dialysate samples (60 μl) by using a high-performance liquid chromatography (HPLC) system (Waters, Milford, MA, USA) as previously described by (Steinberg et al. 1995). Briefly, the analytical system for ACh included a trapping pre-column and immobilized enzyme reactor (BASMF-6151, Bas Technicol, Congleton Cheshire, UK). The mobile phase, 35 mM phosphate buffer (pH 8.5) supplemented with the antibacterial reagent Kathon (5 ml/l; BASDF-2150, Bas Technicol) was pumped at a flow rate of 0.8 ml/min and replaced with a fresh preparation every 3 days. The enzyme post-column reactor converted ACh to hydrogen peroxide, which was electrochemically detected using a platinum electrode (ESA P/N 55–0183, Eurosep, Cergy-Pontoise, France) set at 0.3 V. The chromatography column and enzyme reactor were kept at 35 °C. The detection sensitivity was 200 fmol/60 μl. The HPLC system consisted of a Wisp 717 delivery system (Waters Associates, CA, USA) connected to a dual-piston HPLC pump (ESA model 580, Chelmsford, MA, USA) and an external pulse dampener.

Cortical NE release. Twenty-four hours before dialysis measurements rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and placed in a stereotaxic frame. Cannula guides were implanted in the prefrontal cortex. The coordinates were 3.2 mm anterior to bregma, 0.8 mm lateral to the midline and 1.5 mm down from the dura surface (Paxinos and Watson, 1998). Rats were placed in a Plexiglas cage and a microdialysis probe (CMA 12, length 3 mm and outer diameter 0.5 mm, Carnegie Medicine AB) was inserted into the prefrontal cortex through the guide (4.5 mm under the dura surface). The probe was perfused overnight with a gassed Ringer's solution containing 147 nM NaCl, 4 mM KCl, 1.2 mM $CaCl_2$, 1.0 mM $MgCl_2$ at a flow rate of 1 μl/min by using a microinjection pump (CMA-100, Carnegie Medicine AB) and then changed to 2 μl/min 2 h before the experiment started. Serial samples were collected at 20 min intervals. The position of the probe was verified histologically at the end of each experiment.

Assay of NE. NE levels were measured in 20 min dialysate samples (40 μl) by using HPLC coupled with an amperometric detection (Decade, Antec, Leyden, Netherlands). The separation was performed on a SymetryShield RP18 3 μm column (Waters) L=150 mm and 4.6 mm i.d. coupled to a pre-column (20 mm×4.6 mm i.d.) with a flow rate of 1 ml/min. Mobile phase was prepared by mixing 45.6 g K_2HPO_4, 21.5 g citric acid, 0.9 g octane sulfonic acid, 500 mg EDTA in 3 l distilled water. After filtration on 0.22 μm filter under vacuum, 240 ml of methanol was added to the mobile phase. In some cases, the amount of octane sulfonic acid was adjusted until 1.0 g to improve the separation of NE from other unidentified parameters. The temperature of column and electrochemical cell was maintained at 30 °C. For detection of NE, a glassy carbon work electrode was used at 0.55V vs. Ag/AgCl reference electrode. Under these conditions, the analysis was performed in less than 12 min and detection limit of NE was about 0.2 pg for 30 μl of injected samples.

Data analysis

Changes in ACh or NE levels were expressed as a percentage of the mean value of the two basal samples before FG 7142 treatment. Statistical analysis of the FG 7142 effect was carried out by a one-way analysis of variance (ANOVA) with repeated measures followed by Dunnett's test (# $P<0.05$). Analysis of drug antagonism of the FG 7142 effect was carried out either by a two-way ANOVA with repeated measures followed by Dunnett's test (* $P<0.05$) for individual time comparisons or by an ANOVA followed by Dunnett's test (* $P<0.05$) for area under the curve comparisons.

RESULTS

Effect of acute and chronic administrations of SSR149415 and SSR125543 on hippocampal ACh release

The average baseline outflow of ACh (mean±S.E.M.) over all animals ($n=31$), and not corrected for *in vitro* recovery, was 0.99±0.04 pmol/30 min in the hippocampus of anesthetized rats. An acute administration of SSR149415 (10 mg/kg, i.p.) did not affect hippocampal ACh release ($F(5,15)=0.881$, up to 150 min after administration). After a chronic treatment of either SSR149415 (10 mg/kg, i.p., q.d. for 21 days) or vehicle, extracellular basal levels of hippocampal ACh were similar (1.16±0.13 pmol/30 min and 1.05±0.22 pmol/30 min, respectively. $F(1,16)=0.197$) (results not shown). An acute administration of SSR125543 (20 mg/kg, i.p.) did not affect hippocampal ACh release ($F(5,25)=2.897$ up to 150 min after administration). After a chronic treatment of either SSR125543 (10 mg/kg, i.p., q.d. for 21 days) or vehicle, extracellular basal levels of hippocampal ACh were similar (1.90±0.26 pmol/30 min and 1.61±0.24 pmol/30 min, respectively, $F(1,20)=0.630$) (results not shown).

Effect of acute administration of SSR149415 and SSR125543 on FG 7142-induced increase in hippocampal ACh release

The administration of FG 7142 (30 mg/kg, i.p.) reached its maximal effect between 30 and 60 min following injection, increasing hippocampal ACh release from 38% to 51% over basal values ($P<0.05$), depending on the experiment. An acute administration of SSR149415 (30 min before FG 7142) dose-dependently (0.3–10 mg/kg, i.p.) and markedly inhibited the FG 7142-induced increase in hippocampal ACh release with a significant effect for doses of 3 and 10 mg/kg, i.p. ($F(4,21)=5.308$, $P<0.05$) (Fig. 1B). In time course representation, a significant inhibition was obtained at 60 and 150 min for the SSR149415 dose of 10 mg/kg i.p. ($F(1,8)=8.221$, $P<0.05$) (Fig. 1A).

SSR125543 was also tested in the same dose range, but 180 min before FG 7142 owing to kinetic data. It totally suppressed the FG 7142-induced increase in hippocampal ACh release beginning at the dose of 3 mg/kg, i.p. with a significant effect for 3 and 10 mg/kg, i.p. ($F(4,30)=4.515$, $P<0.05$) (Fig. 2B). In time course representation, a significant inhibition was obtained from 60 to 150 min for 10 mg/kg, i.p. of SSR125543 ($F(1,13)=19.762$, $P<0.05$) (Fig. 2A). In similar experimental conditions, an acute administration of diazepam (1 mg/kg, i.p., 30 min before FG 7142) completely inhibited the FG 7142-induced increase in hippocampal ACh release (area under the curve up to 90 min after FG 7142: 293±353 vs. 4231±1111, respectively, results not shown).

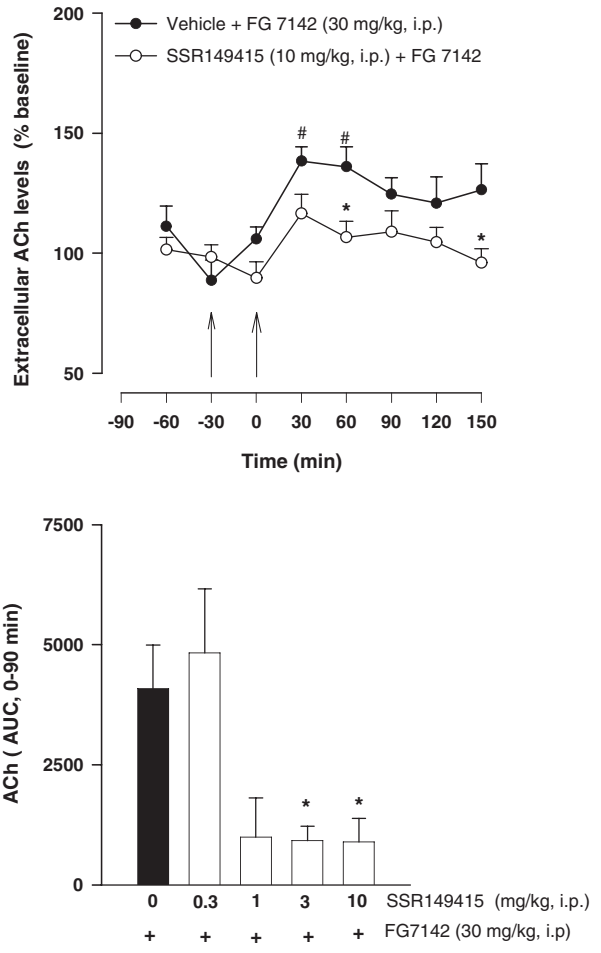
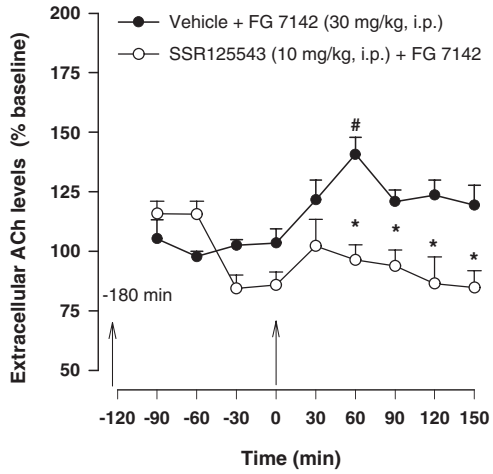


Fig. 1. Effect of an acute administration of SSR149415 on FG 7142-induced increase in hippocampal ACh release. Rats were injected with SSR149415 (○) or with vehicle (●) 30 min before injection of FG 7142. Results are mean±S.E.M. of five to six rats and are expressed (A) as a percentage of basal value obtained on two fractions before SSR149415 (# $P<0.05$ vs. basal value, one-way ANOVA followed by Dunnett's test; * $P<0.05$ vs. the corresponding value for vehicle control, two-way ANOVA followed by Dunnett's test) and (B) as area under the curve between 0 and 90 min after FG 7142 administration (* $P<0.05$ vs. the vehicle control, ANOVA followed by Dunnett's test).

Effect of chronic administration of SSR149415 and SSR125543 on FG 7142-induced increase in hippocampal ACh release

Twenty-four hours after the last administration of a chronic treatment with SSR149415 (10 mg/kg, i.p., once a day (q.d.) for 21 days) the FG 7142-induced increase of ACh release in the hippocampus of anesthetized rats was reduced as compared with its effect on a control group receiving chronically vehicle (688±734 vs. 4417±960, respectively) ($F(1,22)=8.249$, $P<0.05$) (Fig. 3B). The time course representation



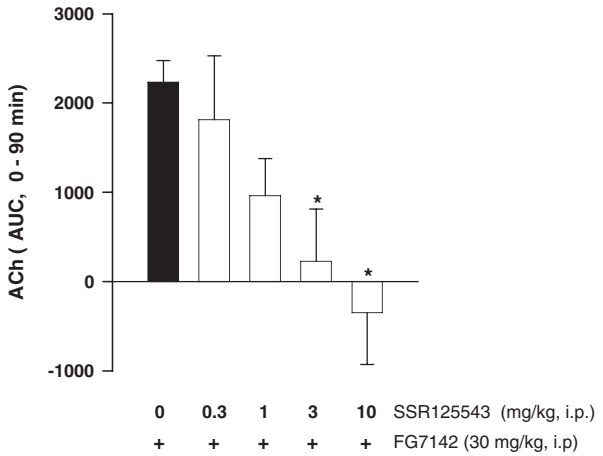


Fig. 2. Effect of an acute administration of SSR125543 on FG 7142-induced increase in hippocampal ACh release. Rats were injected with SSR125543 (○) or with vehicle (●) 180 min before injection of FG 7142. Results are mean±S.E.M. of 4 to 11 rats and are expressed (A) as a percentage of basal value obtained on two fractions before SSR125543 (# $P<0.05$ vs. basal value, one-way ANOVA followed by Dunnett's test; * $P<0.05$ vs. the corresponding value for vehicle control, two-way ANOVA followed by Dunnett's test) and (B) as area under the curve between 0 and 90 min after FG 7142 administration (* $P<0.05$ vs. the vehicle control, ANOVA followed by Dunnett's test).

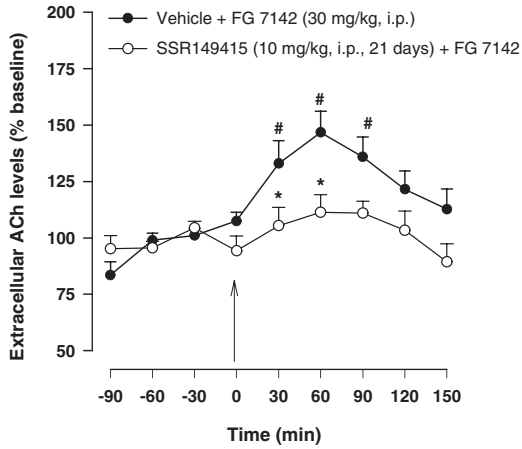
showed a significant inhibition for times 30 and 60 min ($F(1,18)=6.646$, $P<0.05$) (Fig. 3A). In similar experimental conditions, a chronic treatment with SSR125543 partially inhibited the FG 7142-induced increase of hippocampal ACh release (786±372 vs. 1943±325, respectively) ($F(1,20)=5.484$, $P<0.05$) (Fig. 4B). The time course representation showed a significant inhibition for times 60, 90 and 150 min ($F(1,20)=6.113$, $P<0.05$) (Fig. 4A).

Effect of a chronic administration of imipramine on FG 7142-induced increase in hippocampal ACh release

Twenty-four hours after the last administration of a chronic treatment with imipramine (10 mg/kg, i.p., q.d. for 21 days) the FG 7142-induced increase of ACh release in the hippocampus of anesthetized rats was significantly reduced as compared with its effect on a control group receiving chronic vehicle (157±530 vs. 3330±712, respectively) ($F(1,10)=12.762$, $P<0.05$) (Fig. 5B). The time course representation showed a significant inhibition for times 60, 90, 120 and 150 min ($F(1,10)=16.807$, $P<0.05$) (Fig. 5A).

Effect of acute and chronic administrations of SSR149415 on cortical NE release

The average baseline outflow of NE (mean±S.E.M.) over all animals ($n=35$), and not corrected for *in vitro* recovery, was 33.34±11.53 fmol/20 min in the prefrontal cortex of naïve freely moving rats. An acute administration of SSR149415 (10 or 20 mg/kg, i.p.) induced a dose-dependent increase in the cortical NE outflow which was only



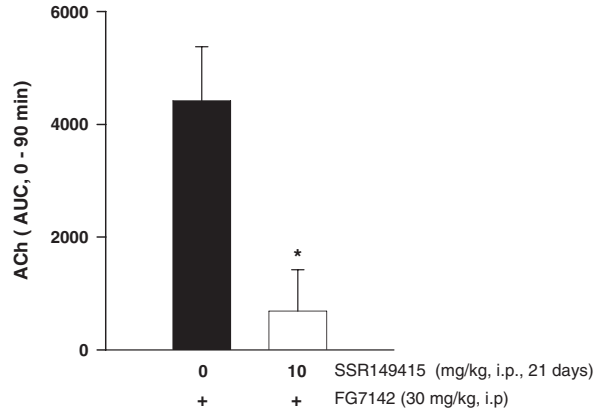
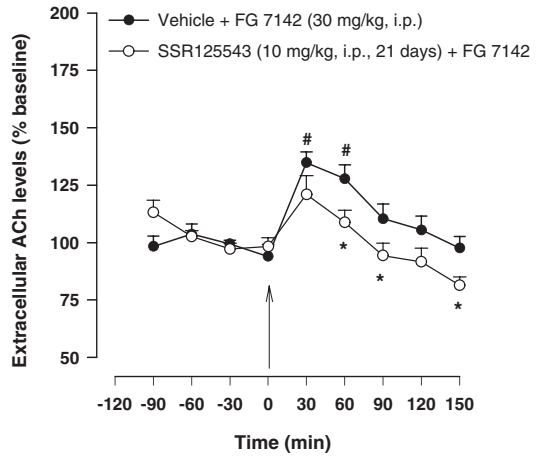


Fig. 3. Effect of a chronic administration of SSR149415 on FG 7142-induced increase in hippocampal ACh release. Rats were injected with SSR149415 (○) or with vehicle (●) for 21 days and with FG 7142 24 h after the last injection of SSR149415 or vehicle. Results are mean±S.E.M. of 10 rats and are expressed (A) as a percentage of basal value obtained on two fractions before FG 7142 (# $P<0.05$ vs. basal value, one-way ANOVA followed by Dunnett's test; * $P<0.05$ vs. the corresponding value for vehicle control, two-way ANOVA followed by Dunnett's test) and (B) as area under the curve between 0 and 90 min after FG 7142 administration (* $P<0.05$ vs. the vehicle control, ANOVA followed by Dunnett's test).



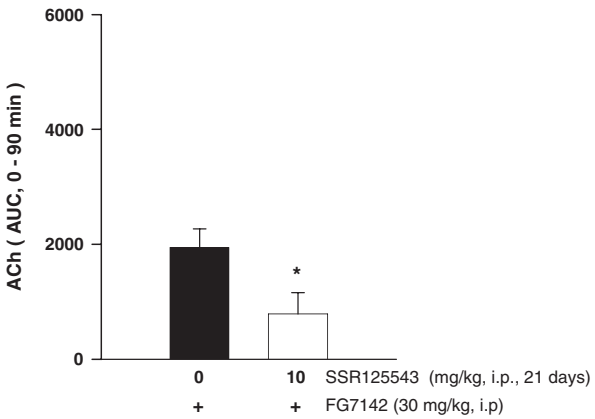


Fig. 4. Effect of a chronic administration of SSR125543 on FG 7142-induced increase in hippocampal ACh release. Rats were injected with SSR125543 (○) or with vehicle (●) for 21 days and with FG 7142 24 h after the last injection of SSR149415 or vehicle. Results are mean±S.E.M. of 10 rats and are expressed (A) as a percentage of basal value obtained on two fractions before FG 7142 (# $P<0.05$ vs. basal value, one-way ANOVA followed by Dunnett's test; * $P<0.05$ vs. the corresponding value for vehicle control, two-way ANOVA followed by Dunnett's test) and (B) as area under the curve between 0 and 90 min after FG 7142 administration (* $P<0.05$ vs. the vehicle control, ANOVA followed by Dunnett's test).

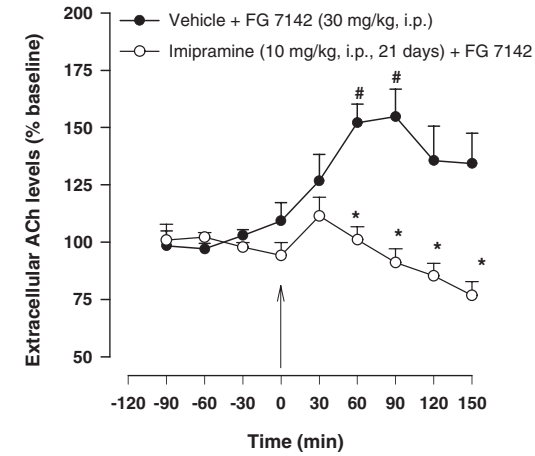
significant at 20 mg/kg i.p. (maximal increase, +73% over basal value ($F(1,14)=6.398$, $P<0.05$)) (results not shown). Twenty-four hours after a chronic treatment of either SSR149415 (10 mg/kg, i.p., q.d. for 21 days) or vehicle, extracellular basal levels of cortical NE were similar (7.33±1.12 fmol/20 min and 7.45±0.59 fmol/20 min, respectively ($F(1,8)=0.0024$)) (results not shown).

Effect of an acute administration of SSR149415 on FG 7142-induced increase in cortical NE release

The administration of FG 7142 (30 mg/kg, i.p.) induced an increase in NE release in the prefrontal cortex of freely moving rats (+105% over basal values, 20 min following injection) which persisted ~60 min. An acute administration of SSR149415 (10 mg/kg, i.p., 30 min before FG 7142) did not affect the FG 7142-induced increase in NE release when comparing either kinetic values ($F(1,9)=0.7548$) (Fig. 6A) or area under the curve (0–80 min) (9432±2068 vs. 7392±1538, respectively, $F(1,9)=0.6295$) (Fig. 6B).

Effect of a chronic administration of SSR149415 on FG 7142-induced increase in cortical NE outflow

Twenty-four hours after the last administration of a chronic treatment of either SSR149415 (10 mg/kg, i.p., q.d. for 21 days) or vehicle, FG 7142 increased NE release in the prefrontal cortex of freely moving rats with a similar amplitude in the two groups when comparing either kinetic values ($F(1,9)=0.8135$) (Fig. 7A) or area under the curve (0–80 min) (5400±1304 vs. 7101±1752, respectively, $F(1,9)=0.4472$) (Fig. 7B).



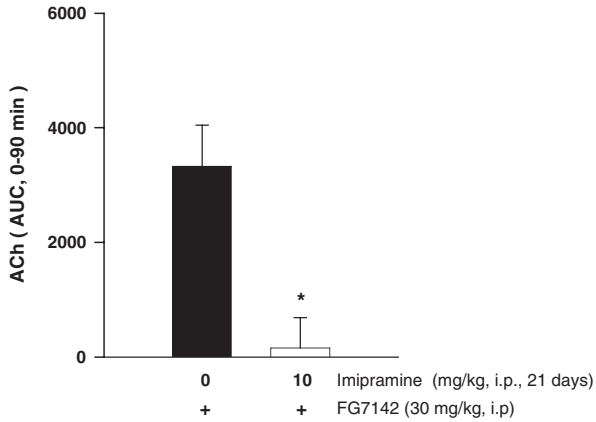


Fig. 5. Effect of a chronic administration of imipramine on FG 7142-induced increase in hippocampal ACh release. Rats were injected with imipramine (○) or with vehicle (●) for 21 days and with FG 7142 24 h after the last injection of SSR149415 or vehicle. Results are mean±S.E.M. of 10 rats and are expressed (A) as a percentage of basal value obtained on two fractions before FG 7142 (# $P<0.05$ vs. basal value, one-way ANOVA followed by Dunnett's test; * $P<0.05$ vs. the corresponding value for vehicle control, two-way ANOVA followed by Dunnett's test) and (B) as area under the curve between 0 and 90 min after FG 7142 administration (* $P<0.05$ vs. the vehicle control, ANOVA followed by Dunnett's test).

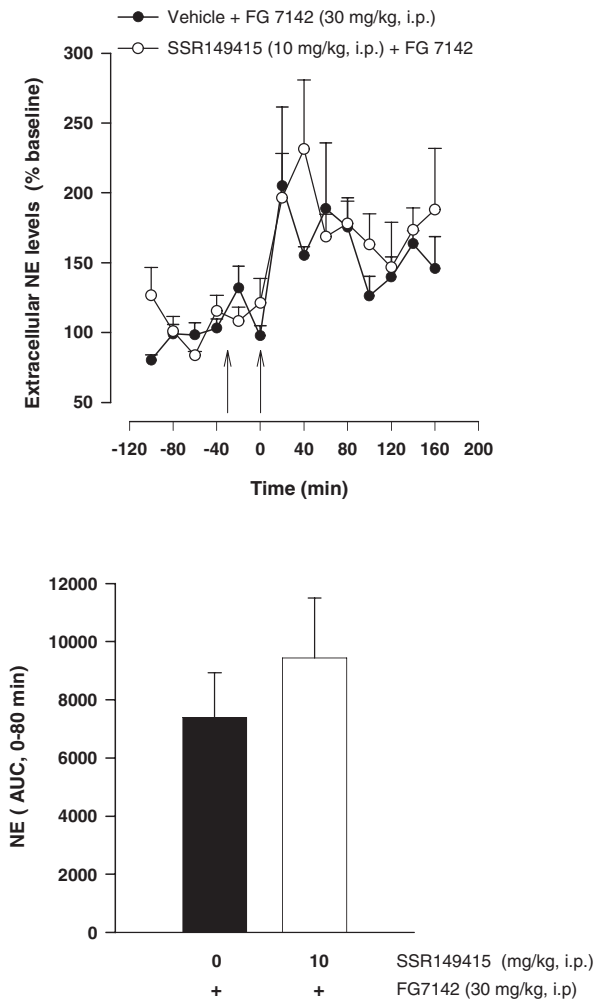


Fig. 6. Effect of an acute administration of SSR149415 on FG 7142-induced increase in cortical NE release. Rats were injected with SSR149415 (○) or with vehicle (●) 30 min before injection of FG 7142. Results are mean±S.E.M. of 4 to 7 rats and are expressed (A) as a percentage of basal value obtained on two fractions before SSR149415 and (B) as area under the curve between 0 and 90 min after FG 7142 administration.

DISCUSSION

The present study demonstrates that the excitatory effect of the anxiogenic drug FG 7142 on ACh release in the rat hippocampus is reversed by chronic administration of the V_{1b} receptors antagonist SSR149415, the CRF_1 receptor antagonist SSR125543 and the reference antidepressant imipramine. These results corroborate with the involvement of AVP and CRF in neurochemical responses to stress and are consistent with the antidepressant and anxiolytic-like properties of the V_{1b} and CRF_1 receptors antagonists (Griebel et al., 2002a,b; Gully et al., 2002). Whereas FG 7142 has been shown, in both animals and humans, to reproduce the behavioral and biochemical changes induced by stressful stimuli, including the activation of the cholinergic, noradrenergic and dopaminergic (DA) systems (Ida et al., 1991; Bradberry et al.,1991; Moore et al., 1995b; Sarter et al., 2001; Dazzi et al., 2001), the present study demonstrates that FG 7142 also induces an increase in the activity of the hippocampal cholinergic system. The magnitude of the excitatory effects of FG 7142 on the release of hippocampal ACh is lower than that described in the cortex (Dazzi et al., 2001). However, the stimulatory effect of FG 7142 in the hippocampus appears to be similar to that obtained under exposure to a stressful, sensory stimulation or central CRF application (Inglis and Fibiger, 1995; Day et al., 1998).

The present data show that acute administration of either SSR149415 or SSR125543 dose dependently and markedly inhibits the FG 7142-induced increase in hippocampal ACh output at doses at which they behave as selective V_{1b} and CRF_1 receptors antagonists in relevant models. Their efficacy to block hippocampal ACh release induced by FG 7142 is similar to that observed following acute administration of the classical benzodiazepine anxiolytic drug diazepam (present data) or of other positive allosteric modulators of GABAA receptors on cortical ACh release (Dazzi et al., 1995, 1996). The blockade of V_{1b} or CRF_1 receptors with SSR149415 or SSR125543, respectively, did not affect basal levels of ACh in the hippocampus suggesting a lack of tonically active AVP or CRF

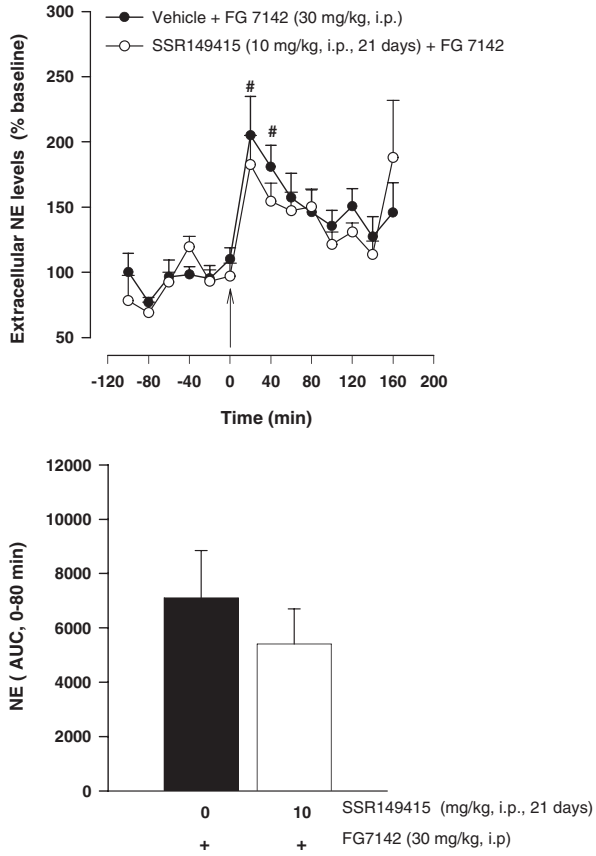


Fig. 7. Effect of a chronic administration of SSR149415 on FG 7142-nduced increase in cortical NE release. Rats were injected with SSR149415 (○) or with vehicle (●) for 21 days and with FG 7142 24 h after the last injection of SSR149415 or vehicle. Results are mean±S.E.M. of 5 to 6 rats and are expressed (A) as a percentage of basal value obtained on two fractions before FG 7142 ($^{\#}$ $P<0.05$ vs. basal value, one-way ANOVA followed by Dunnett's test) and (B) as area under the curve between 0 and 90 min after FG 7142 administration.

control on ACh release in anesthetized rats. The absence of tonic control of the activity of the cholinergic system is a property shared by other neuropeptides involved in stress responses (Steinberg et al., 1998) and may explain that these antagonists do not exert deleterious effect on cognition and attentional functions. In contrast the benzodiazepine receptor partial inverse agonist FG 7142 stimulates central ACh efflux by reducing the tonic inhibition of this efflux exerted by endogenous activation of GABA/BZ receptors on cholinergic neurons (Moore et al., 1995a) and conversely, agonists of benzodiazepine receptors reduce the output of ACh in the hippocampus and cerebral cortex (Imperato et al., 1993, 1994).

From binding studies we suggest that SSR149415 and SSR125543 reverse FG 7142-induced hippocampal ACh release without directly affecting GABA A receptor-mediated neurotransmission. These results point for the first time to the possibility that FG 7142 activates the hippocampal cholinergic system by stimulating AVP and CRF release thereby causing activation of V_{1b} and CRF_1 receptors. As previously described, central CRF administration stimulates ACh release in the hippocampus and this excitatory effect of exogenous application of CRF was sensitive to SSR125543 but not to SSR149415 (R. Steinberg, personal communication). This suggests that no interplay exists between CRF and AVP as it may be postulated for the HPA regulation and that FG 7142-evoked ACh release is not a consequence of sequential release of CRF and AVP, although the opposite order of sequential release AVP/CRF may not be ruled out.

The neurochemical mechanisms underlying the acute action of these compounds on hippocampal reactivity to anxiogenic drug remains to be fully elucidated. A DA regulation of septohippocampal cholinergic neurons has been demonstrated by Day and Fibiger (1994) and it could be speculated that besides disinhibition of cholinergic projection neurons, FG 7142-stimulated hippocampal ACh outflow could be a consequence of stimulation of DA release as it has been proposed for cortical ACh outflow (McCullough and Salamone, 1992). However evidence for this is not compelling and furthermore the lack of effect of SSR149415 or SSR125543 on DA release, as demonstrated by Ring et al. (2004) suggests that their inhibition of FG 7142-induced increase in hippocampal ACh release is not mediated by an action on DA system. Moreover the lack of effect of the compounds on other classical monoaminergic and acid aminergic (serotonergic, GABAergic, NE, glutamatergic) systems (Steinberg et al., unpublished observations, Ring et al. (2004) and see below) suggests that more complex interactions are involved.

From this and previously published studies (Dazzi et al., 2001), two groups of compounds can be identified following their acute administration, one exemplified by SSR149415, SSR125543, lacking a direct effect on hippocampal ACh release but preventing FG 7142-induced increase in hippocampal ACh release and another class including the antidepressants imipramine and mirtazapine, which increase cortical ACh release but do not inhibit FG 7142-induced increases. In contrast after long-term administration SSR149415, SSR125543 and imipramine markedly inhibited the effect of FG 7142 on hippocampal ACh release as imipramine and mirtazapine did on FG 7142-induced cortical ACh release. This common efficacy suggests that SSR149415 and SSR125543 would behave as putative antistress, anxiolytic and antidepressant drugs.

The results of the present study also indicate that an acute administration of SSR149415 did not reduce the FG 7142-induced increase in cortical NE output in freely moving rats. In similar experimental conditions SSR125543 was also devoid of effect on this FG 7142-induced activation (data not shown). It is worth noting that several antidepressant drugs (imipramine, mirtazapine, venlafaxine, reboxetine) (Dazzi et al., 2002a,b, 2003) did not reduce this FG 7142-induced increase in cortical NE output. But these antidepressant drugs by themselves alter the noradrenergic system and after acute treatment increase extracellular levels of NE due to their property of inhibiting NE uptake, while SSR149415 only increased NE release in the prefrontal cortex of the freely moving rat after an acute administration at 20 mg/kg, i.p. whereas after chronic administration at pharmacologically active dose (10 mg/kg, i.p.) it did not affect extracellular cortical NE levels. In contrast to these antidepressant drugs, which when chronically administered partially or completely block the FG 7142-induced increase in cortical NE outflow, long-term administration of SSR149415 still failed to modulate the FG 7142 effect although its antidepressant potential has been confirmed when given repeatedly in the chronic mild stress paradigm (Griebel et al., 2002b).

CONCLUSION

In conclusion, the present series of experiments demonstrates that acute or chronic administration of V_{1b} and CRF_1 receptor antagonists reverses FG 7142-induced increase in hippocampal ACh release, an antagonistic action similar to that exerted by an acute administration of benzodiazepine or chronic administration of classical antidepressant drugs. These results suggest that such a mechanism may play a critical role in the anxiolytic and antidepressant-like properties of SSR149415 and SSR125543.

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