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Stress-induced increase of cortical dopamine metabolism: attenuation by a tachykinin NK_1 receptor antagonist

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Abstract

The present study examined the potential role of tachykinin NK_1 receptors in modulating immobilisation stress-induced increase of dopamine metabolism in rat medial prefrontal cortex. In agreement with previous studies, 20 min immobilisation stress significantly increased medial prefrontal cortex dopamine metabolism as reflected by the concentration of the dopamine metabolite dihydroxyphenylacetic acid (DOPAC). Pretreatment with the high affinity, selective, tachykinin NK_1 receptor antagonist (3(*S*)-(2-methoxy-5-(5-trifluoromethyltetrazol-1-yl)-phenylmethyl amino)-2(*S*)-phenylpiperidine) ((*S*)-GR205171, 10 mg/kg, s.c.), a dose that in ex vivo binding studies extensively occupied rat brain tachykinin NK_1 receptors for approximately 60 min, significantly attenuated the stress-induced increase of mesocortical DOPAC concentration without affecting cortical DOPAC levels per se. In contrast, pretreatment of animals with the less active enantiomer (*R*)-GR205171 (10 mg/kg, s.c.), which demonstrated negligible tachykinin NK_1 receptor occupancy ex vivo, failed to affect either basal or stress-induced DOPAC concentration in medial prefrontal cortex. Furthermore, pretreatment of animals with the benzodiazepine/$GABA_A$ receptor antagonist, flumazenil (15 mg/kg, i.p.), did not affect the ability of (*S*)-GR205171 to attenuate the increase of medial prefrontal cortex DOPAC concentration by acute stress. Results demonstrate that the selective tachykinin NK_1 receptor antagonist, (*S*)-GR205171, attenuated the stress-induced activation of mesocortical dopamine neurones by a mechanism independent of the benzodiazepine modulatory site of the $GABA_A$ receptor.

Keywords: Tachykinin NK_1 receptor antagonist; GR205171; Immobilisation stress; Cortex; Dopamine metabolism; Tachykinin NK_1 receptor occupancy; [^{125}I]Tyrosine–substance P ex vivo binding

1. Introduction

Dopamine neurones arising from cell bodies in the A10, ventral tegmental area ascend via the medial forebrain bundle to predominantly innervate cortical and limbic (e.g. nucleus accumbens, amygdala, septum) structures (Thierry et al., 1990). It has been known for many years that mesocortical dopamine neurones are particularly sensitive to noxious and stressful stimuli. Thus, exposing animals to acute footshock, immobilisation or psychological stress increased dopamine metabolism, synthesis or release in the medial prefrontal cortex (Thierry et al., 1976; Lavielle et al., 1978; Blanc et al., 1980; Reinhard et al., 1982; Deutch et al., 1985a; Claustre et al., 1986; Bradberry et al., 1991; Sorg and Kalivas, 1993; Giorgi et al., 1987; Feenstra et al., 1995; Kaneyuki et al., 1991; Morrow et al., 1993; Hutson and Barton, 1997). This neurochemical response to acute stress has also been found to occur in the ventral tegmental area, nucleus accumbens and amygdala (Thierry et al., 1976; Morrow et al., 1993; Herman et al., 1982), although in contrast to the robust effects observed in cortex, stress-induced activation of dopamine metabolism in limbic regions is less consistent (Lavielle et al., 1978; Deutch et al., 1985b; Kaneyuki et al., 1991; Hutson and Barton, 1997).

Mesocortical dopamine neuronal function is also known to be markedly influenced by the activity of γ-amino-butyric acid (GABA) containing neurones and anxiolytic benzodiazepine/$GABA_A$ receptor agonists including diazepam, zolipidem and the β-carboline, ethyl-6-benzyloxy-4-methoxymethyl-β-carboline-3-carboxylate (ZK 93423) markedly attenuated the stress-induced increase of medial prefrontal cortex dopamine release or dihydroxyphenylacetic acid (DOPAC) concentration without affecting mes-

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ocortical dopamine metabolism or release per se (Lavielle et al., 1978; Feenstra et al., 1995; Kaneyuki et al., 1991; Ida et al., 1989; Reinhard et al., 1982; Giorgi et al., 1987; Claustre et al., 1986; Hutson and Barton, 1997). Conversely, benzodiazepine/$GABA_A$ receptor inverse agonists, e.g. methyl-β-carboline-3-carboxylate (β-CCM), ethyl-β-carboline-3-carboxylate (β-CCE) and *N*′ -methyl-β-carboline-3-carboxymide (FG 7142), are behaviourally anxiogenic (Corda et al., 1983; File et al., 1985) and, in the absence of stress, significantly increased mesocortical dopamine metabolism and release (Bradberry et al., 1991; Deutch and Roth, 1990; Claustre et al., 1986; Giorgi et al., 1987). However, it is also apparent that basal and stress-induced mesocortical dopamine neuronal function is modulated by other neurotransmitter containing pathways including serotonin via activation of $5\text{-}HT_{1A}$ receptors (Saphier and Welch, 1995; Rasmusson et al., 1994), glutamate via blockade of the glycine modulatory site of the NMDA receptor (Morrow et al., 1993; Hutson and Barton, 1997) and also by substance P (Bannon et al., 1983).

Substance P belongs to family of peptides known as the neurokinins which are widely distributed throughout the mammalian central nervous system (CNS). The biological activity of substance P is thought to be mediated through the tachykinin receptors of which three subtypes have been identified and cloned (Yokota et al., 1989; Hershey and Krause, 1990). Tachykinin NK_1 receptors are widely expressed in the mammalian CNS where they appear to be phylogenetically regulated, being more abundant in higher than lower species (Dietl and Palacios, 1991). Conversely, the expression of tachykinin NK_2 and NK_3 receptors appears to mirror that observed for tachykinin NK_1 as they are less abundant than tachykinin NK_1 receptors in the CNS of higher species including man (Pernow, 1983; Maggi, 1995; Regoli et al., 1994). It seems likely that substance P, acting via NK_1 receptors, is involved in the response to stress. Thus, exposure to acute stress increased the release of substance P in the ventral tegmental area (Lisoprawski et al., 1981) and, conversely, tachykinin NK_1 receptor antagonists displayed anxiolytic activity in the elevated plus maze (Teixeira et al., 1996), and prevented stress-induced analgesia (Altier and Stewart, 1999) and c-fos expression (Hahn and Bannon, 1999).

It is also apparent that substance P is involved in the modulation of mesocorticolimbic dopamine neuronal function. Thus, autoradiographic studies demonstrated the widespread distribution of tachykinin NK_1 receptors throughout the rodent CNS including structures associated with the mesocorticolimbic dopamine system, i.e. amygdala, ventral tegmental area, hippocampus, and medial prefrontal cortex (Mantyh et al., 1984, 1989, Maggi et al., 1993). Electrophysiological and neurochemical studies have shown that substance P or structurally related analogues directly modulate midbrain mesocorticolimbic dopamine neuronal function (Maggi et al., 1993; West and Michael, 1991; Seabrook et al., 1995; Stinus et al., 1978; Elliott et al., 1986a,b; Boix et al., 1992a,b; Cador et al., 1989). Direct evidence that stress-induced activation of mesocortical dopamine neuronal function is modulated by substance P was provided by Bannon et al. (1983). This group showed that infusion of a substance P monoclonal antibody into the rat ventral tegmental area attenuated footshock-induced dopamine metabolism in rat prefrontal cortex. However, the lack of potent, selective tachykinin NK receptor antagonists prevented the identification of which tachykinin NK receptor subtypes were responsible for this activity. Therefore, the aim of the present study was to determine the effects of the high affinity, selective tachykinin NK_1 receptor antagonist (3(*S*)-(2-methoxy-5-(5-trifluoromethyltetrazol-1-yl)-phenylmethyl amino)-2(*S*)-phenylpiperidine) (*S*-GR205171) (Gardner et al., 1996) at a dose and time known to extensively occupy central tachykinin NK_1 receptors on the stress-induced increase of medial prefrontal cortex dopamine metabolism. Part of this study has been previously published in abstract form (Barton et al., 1999).

2. Methods

2.1. Animals

Male Sprague–Dawley rats (B&K, UK, weight range 250–300 or 160–200 g for the ex vivo binding studies) were housed five per cage on a 12-h light/dark cycle (lights on 07:00, off 19:00 h) for a minimum period of 5 days before the experiment. Food and water were available ad libitum. All animal experimentation was carried out in accordance with the UK Animals (Scientific Procedures) Act 1986 and associated guidelines.

2.2. Tachykinin NK_1 receptor binding in vitro

Animals were humanely killed, the cerebral cortex removed and a P_2 membrane pellet prepared using 10 volumes of 5 mM Tris–acetate (0.1 mM sodium ethylenediaminetetraacetic acid (Na-EDTA), 0.32 M sucrose, pH 7.4 at 4 °C), homogenised for 10 in glass-Teflon homogeniser (speed 200). The homogenate was centrifuged at 3000 rpm for 10 min at 4 °C and the supernatant collected on ice. The pellet was resuspended as above and again centrifuged at 3000 rpm for 10 min at 4 °C. This pellet was discarded and supernatants combined and centrifuged at 12,500 rpm for 20 min at 4 °C. The resulting pellet was resuspended in 20 volumes of 5 mM Tris–acetate, pH 8.0 at 4 °C. The homogenate was then stirred on ice for 1 h, centrifuged at 20,000 rpm for 30 min at 4 °C before being resuspended at 1 mg/ml in 5 mM Tris–acetate, pH 7.4 at 4 °C. The radioligand binding method was essentially as described by Pernow (1983): briefly, pellets were resuspended in 50 mM Tris–acetate buffer (5 mM $MnCl_2$, leupeptin (4 μg/ml),

chymostatin (2 μg/ml), bacitracin (40 μg/ml), pH 7.7). Incubations were initiated using 150 μl aliquots of P_2 membranes in a final assay volume of 200 μl. Displacement studies were performed using [^{125}I]tyrosine–substance P (0.5 nM) in the presence and absence of (*S*)-GR205171 or (*R*)-GR205171 (0.1 nM–1000 μM in a volume of 20 μl) using 5 μM substance P (Penninsula) to define nonspecific binding. After a 30-min incubation at room temperature, the assay was terminated by rapid filtration over GF/C filters (presoaked for 1 h in 0.5% PEI) with 3 × 4 ml wash 50 mM Tris–acetate (5 mM $MnCl_2$, pH 7.7).

2.3. Tachykinin NK_1 receptor occupancy ex vivo

Animals were injected subcutaneously (s.c.) with either (*S*)-GR205171 (0.01–10 mg/kg), (*R*)-GR205171 (0.1–50 mg/kg), or vehicle (water, 1 ml/kg) and were humanely killed 30 min later. In a second study, rats were injected with either vehicle (water, 1 ml/kg) or (*S*)-GR205171 (10 mg/kg) and humanely killed at various times (10, 20, 30, 60, 90, 240 and 420 min) after injection. In both studies, the cortex and striatum were rapidly dissected, pooled and homogenised in 20 volumes of assay buffer (50 mM Tris–HCl, pH 7.7, containing 5 mM $MnCl_2$, leupeptin (4 μg/ml), chymostatin (2 μg/ml) and bacitracin (40 μg/ml) at 4 °C). Homogenates were stored on ice prior to [^{125}I] tyrosine–substance P binding, which was carried out as described above, using 150 μl of homogenate per tube.

2.4. Radioligand binding analyses

Inhibition curves were analysed, using Grafit, for a one-site model using the equation $\%I=\%I_{max}/1+(IC_{50}/[L])^{nH}$, where I is inhibition of binding, IC_{50} is the concentration of ligand giving 50% inhibition of specific binding, [L] is the ligand (inhibitor) concentration and nH is the Hill coefficient. For the ex vivo [^{125}I]tyrosine–substance P studies, results are expressed as ED_{50} (mg/kg, s.c.), i.e. the effective dose of compound to inhibit 50% of specific binding, where specific binding is defined as total binding, i.e. (dpm obtained by dosing with vehicle) minus nonspecific binding (dpm in the presence of 5 μM substance P).

2.5. Neurochemical studies

Groups of rats were injected with either (*S*)-GR205171 (5 or 10 mg/kg, s.c.), (*R*)-GR205171 (10 mg/kg, s.c.) or vehicle (water, 1 ml/kg, s.c.). Thirty minutes later, rats were either left in the home cage or immobilised for 20 min as previously described (Hutson and Barton, 1997). In a second study, rats were pretreated with either the benzodiazepine/$GABA_A$ receptor antagonist, flumazenil (15 mg/kg, i.p.), 15 min before administration of (*S*)-GR205171 (10 mg/kg, s.c.) or vehicle (water, 1 ml/kg, s.c.). Animals were either left in the home cage or subjected to 20 min immobilisation stress. In both studies, rats were humanely killed immediately following the stress period, brains removed and the medial prefrontal cortex dissected, frozen and stored at −70 °C until ready for analysis. Brain samples were analysed for dopamine and the acidic metabolite dihydroxyphenylacetic acid (DOPAC) by high-pressure liquid chromatography (HPLC) with electrochemical detection (Hutson et al., 1991). Briefly, tissue samples were homogenised in 10 volumes of homogenising buffer (0.4 M perchloric acid, 0.1% cysteine, 0.1% sodium metabisulphite, 0.01% Na-EDTA) and centrifuged at 3000 × *g* for 10 min. The HPLC system comprised an HPLC Technology 3 μm ODS column (4.6 mm × 10 cm), the mobile phase consisted of 0.07 M KH_2PO_4, 0.0035% Na-EDTA, 0.023% octyl sodium sulphate and 12.5% methanol, pH 2.75 at a flow rate of 1 ml/min. Dopamine and metabolites were detected using an Antec electrochemical detector (Presearch) with the working electrode set at +0.65 V relative to a silver/silver chloride reference electrode. Under these conditions, DOPAC and dopamine exhibited retention times of 3.2 and 6.5 min, respectively.

2.6. Statistical analysis

Data were subjected to either one- or two-way analysis of variance followed, where appropriate, by Tukey's studentised range test. A value of $P<0.05$ was considered significant.

3. Results

Radioligand binding studies in rat cortical membranes in vitro demonstrated that (*S*)-GR205171 displaced [^{125}I]tyrosine–substance P binding with high affinity ($IC_{50}=1.37\pm0.52$ nM; mean ± S.E.M., $n=3$) while (*R*)-GR205171 was approximately 7500-fold weaker ($IC_{50}=10.2\pm4.8$ μM; mean ± S.E.M., $n=3$). Thirty minutes after systemic administration, (*S*)-GR205171 (1–10 mg/kg, s.c) dose-dependently inhibited the binding of [^{125}I]tyrosine–substance P to rat brain membranes determined ex vivo with an ED_{50} value (geometric mean, high and low errors in parenthesis) of 1.0 (0.63:1.60) mg/kg, s.c. ($n=3$) and a maximal inhibition at 10 mg/kg of approximately 90% (Fig. 1A), indicating brain penetration and dose-related receptor occupancy. In contrast, systemic administration of (*R*)-GR205171, the less active enantiomer, weakly displaced [^{125}I]tyrosine–substance P from rat brain membranes determined ex vivo with an estimated ED_{50} value of >50 mg/kg, s.c. ($n=2$) and a maximal inhibition of approximately 17% at a dose of 10 mg/kg. (Fig. 1A). However, it must be recognised that the values for (*R*)-GR205171 are only projected estimates based on relatively weak binding data that suggest either poor brain penetration and/or poor NK_1 receptor occupancy consistent

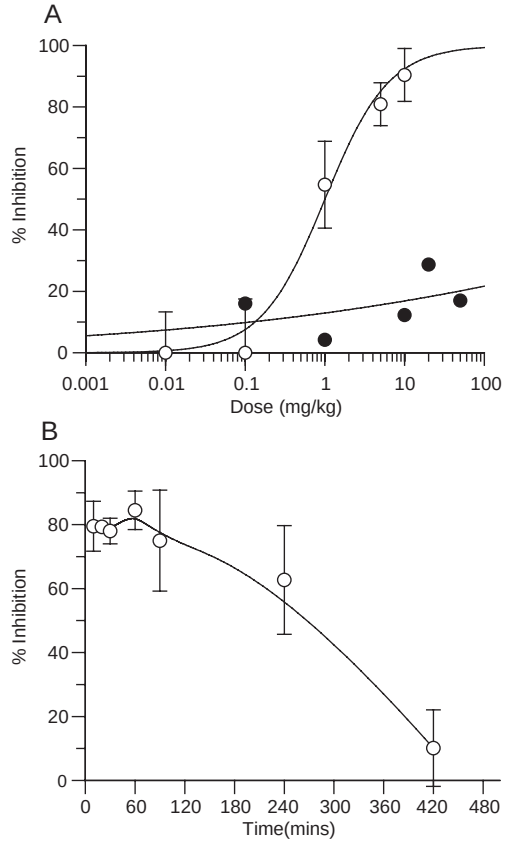

Fig. 1. (A) Ex vivo displacement of [^{125}I]tyrosine–substance P from rat cortex by (*S*)-GR205171 (○) and (*R*)-GR205171 (●) where values are mean ± S.E.M., $n=3$ per group and saturation curves were fitted using a one-site model. (B) Time course for ex vivo displacement of [^{125}I]tyrosine–substance P from rat cortex by (*S*)-GR 205171 (10 mg/kg, s.c.) where values are mean ± S.E.M., $n=3$ per group.

with a compound with weak affinity at the tachykinin NK_1 receptor. From these results, it is apparent that 30 min after a dose of 10 mg/kg, s.c. (*R*)-GR205171 and (*S*)-GR205171 occupied approximately 17% and 90% of the cortical/striatal tachykinin NK_1 receptor population, respectively (Fig. 1A). Time course studies with (*S*)-GR205171 indicated that following a single systemic dose of 10 mg/kg, s.c., approximately 80% tachykinin NK_1 receptor occupancy was achieved within 10 min and was maintained for approximately 60 min, after which time tachykinin NK_1 receptor occupancy slowly declined and was essentially negligible by 7 h (Fig. 1B).

Dopamine metabolism (as indicated by the concentration of DOPAC) in medial prefrontal cortex was significantly ($P<0.05$) increased above basal values following 20 min immobilisation stress (Figs. 2 and 3). Pretreatment of rats with the tachykinin NK_1 receptor antagonist, (*S*)-GR205171 (5 and 10 mg/kg, s.c.), dose-dependently attenuated the stress-induced increase of cortical DOPAC concentration without affecting medial prefrontal cortex DOPAC concentration per se (Fig. 2A and B). The effect of (*S*)-GR205171 on stress-induced cortical dopamine metabolism was significant ($P<0.05$) at 10 but not 5 mg/kg. In contrast, the less active enantiomer (*R*)-GR205171 (10 mg/kg, s.c.) did not significantly affect the neurochemical response to stress or the concentration of cortical DOPAC in nonstressed animals (Fig. 2C). In a second study, the attenuation by (*S*)-GR205171 (10 mg/kg, s.c.) of the stress-induced increase of cortical DOPAC concentration was unaffected by pretreatment with flumazenil (15 mg/kg, i.p.) (Fig. 3B), at a dose that did not affect either basal or the increase of cortical DOPAC concentration induced by stress (Fig. 3A). The concentration of dopamine in the medial prefrontal cortex in all experiments was not significantly affected by stress, drug

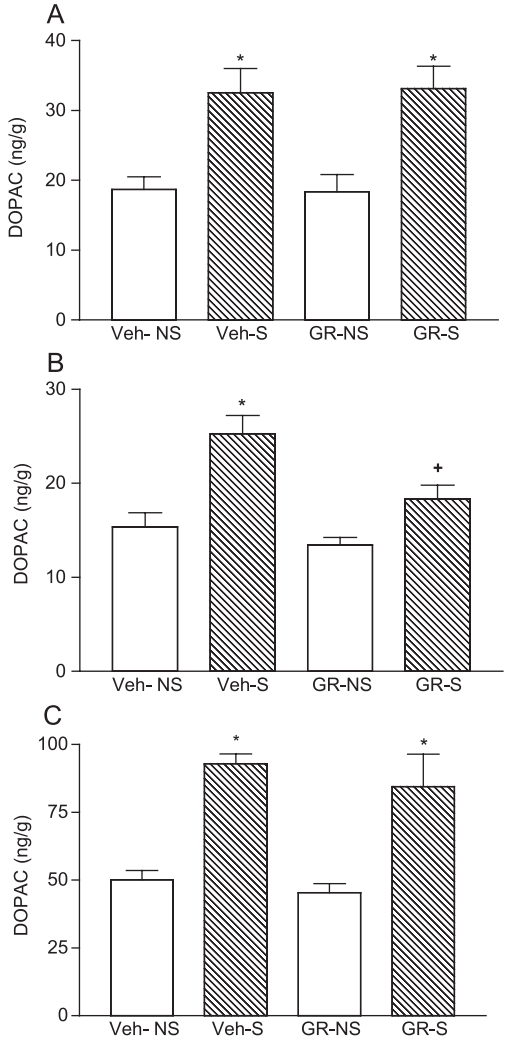

Fig. 2. The effects of (A) (*S*)-GR205171 (5 mg/kg, s.c.), (B) (*S*)-GR205171 (10 mg/kg, s.c.) and (C), (*R*)-GR205171 (10 mg/kg, s.c.) or vehicle (1 ml/kg s.c.) on medial prefrontal cortex DOPAC concentration in nonstressed rats (NS, open columns), or rats immobilised for 20 min (S, cross hatched columns). All values are mean ± S.E.M., $n=6–14$ per group. *$P<0.05$ compared with nonstressed, vehicle-treated rats and $^{+}P<0.05$ compared with stressed, vehicle-treated rats by two-way analysis of variance followed by Tukey's test.

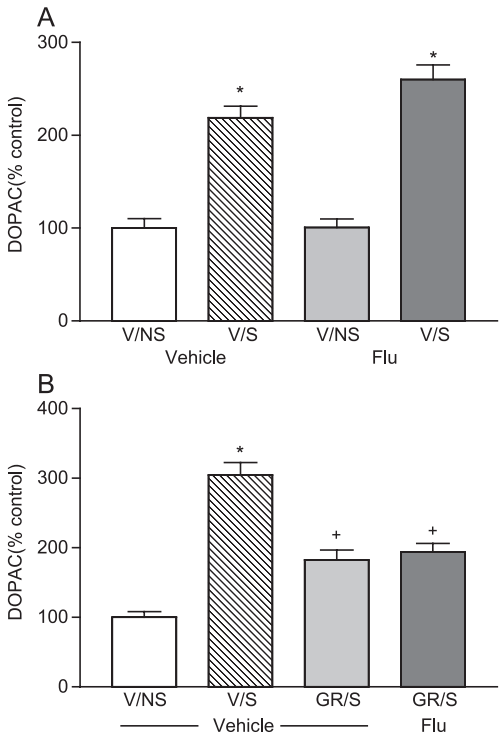

Fig. 3. (A) The effects of flumazenil (15 mg/kg, i.p.) on basal cortical DOPAC concentration and the increase of cortical DOPAC concentration induced by 20 min immobilisation stress (V/S, cross-hatched column). (B) The effects of pretreatment with flumazenil (15 mg/kg, i.p.) (GR/S, dark grey filled column) on the ability of (*S*)-GR205171 (10 mg/kg, s.c.) (GR/S, light grey filled column) to attenuate the increase of cortical DOPAC concentration induced by 20 min immobilisation stress (V/S, cross-hatched column). All values are mean ± S.E.M. expressed as percentage of control (vehicle-treated, nonstressed animals, V/NS), $n=6$ per group. $^{*}P<0.05$ compared with nonstressed, vehicle-treated rats and $^{+}P<0.05$ compared with stressed, vehicle-treated rats by analysis of variance followed by Tukey's test. The concentration of DOPAC in the medial prefrontal cortex of vehicle-treated, nonstressed rats was (A) 15.3 ± 1.96 and (B) 7.50 ± 0.19 ng/g, mean ± S.E.M.

treatment, or the combination of stress and drug treatment (data not shown).

4. Discussion

In agreement with Gardner et al. (1996), the selective tachykinin NK_1 receptor antagonist (*S*)-GR205171 was found to display high affinity (IC_{50} = approximately 1 nM) for the rat NK_1 receptor in vitro. Furthermore, when injected subcutaneously in rats, (*S*)-GR205171 demonstrated rapid brain penetration and high receptor occupancy (approximately 95% of cortical/striatal tachykinin NK_1 receptors, 30 min after a dose of 10 mg/kg, s.c.) as determined by ex vivo radioligand binding studies. Time course studies revealed that following systemic administration of (*S*)-GR 205171 (10 mg/kg, s.c.), maximal rat brain tachykinin NK_1 receptor occupancy was rapidly achieved and maintained at approximately 80% for approximately 1 h providing an adequate time window to perform subsequent stress studies. Conversely, the affinity of (*R*)-GR205171 for rat brain tachykinin NK_1 receptors in vitro was approximately 7500-fold weaker than the (*S*)-enantiomer and following systemic administration displayed a low level of tachykinin NK_1 receptor occupancy in rat brain (approximately 17% at a dose of 10 mg/kg, s.c.). Despite the potential pitfalls of underestimating receptor occupancy due to excessive tissue dilution causing receptor–ligand dissociation, the current ex vivo receptor binding studies validate the utility of (*S*)-GR205171 for in vivo studies in the rat within a 1-h period following systemic administration. Similarly, because the (*R*)-enantiomer has much weaker affinity at the rat tachykinin NK_1 receptor in vitro and consequently occupies only a small proportion of rat brain tachykinin NK_1 receptors, it is of considerable value to demonstrate the enantiomeric selectivity of any effects observed with (*S*)-GR205171.

Results from the present neurochemical studies confirmed a wealth of previous findings that acute stress significantly increased medial prefrontal cortex dopamine metabolism as determined by the concentration of DOPAC (Lavielle et al., 1978; Blanc et al., 1980; Herman et al., 1982; Kaneyuki et al., 1991; Morrow et al., 1993; Hutson and Barton, 1997). In agreement with Bannon et al. (1983) and Kaneyuki et al. (1991) but in contrast to other reports (Lavielle et al., 1978; Reinhard et al., 1982), we did not observe an effect of stress on cortical dopamine concentration. The reason(s) for this discrepancy remains obscure but may relate to the different stressors used. Under these conditions, pretreatment of animals with the selective, high affinity tachykinin NK_1 receptor antagonist (*S*)-GR205171, at a dose and time shown in ex vivo receptor binding studies to occupy a high proportion of rat brain tachykinin NK_1 receptors, significantly attenuated the stress-induced increase of cortical DOPAC concentration. This effect, which occurred in the absence of any significant alteration by (*S*)-GR205171 of basal cortical dopamine metabolism, appeared to be a selective tachykinin NK_1 receptor-mediated effect as the less active enantiomer (*R*)-GR205171 failed to affect either basal or stress-induced changes in mesocortical dopamine metabolism. The lack of effect of *S*-GR205171 on cortical dopamine metabolism per se is consistent with the study by Bannon et al. (1983) which found no effect of a substance P antibody on basal DOPAC concentration. However, these results contrast with recent findings demonstrating that GR205171 significantly increased extracellular dopamine concentration in the medial prefrontal cortex and dopaminergic cell body firing rate in an enantiomerically selective manner (Lejeune et al., 2002). However, apart from the obvious methodological differences between the two studies, i.e. doses, route of administration and neurochemical parameters measured, there is no obvious explanation for this apparent discrepancy, although it is possible that the determination of cortical DOPAC concentration is not sensitive enough to detect such modulatory effects. Interestingly, the neurochemical and electrophysio-

logical profile of (*S*)-GR 205171, as observed by Lejeune et al. (2002), in the absence of stress is reminiscent of anxiogenic benzodiazepine/$GABA_A$ receptor inverse agonists. Results in the present study confirm the finding by Bannon et al. (1983) that blockade of substance P activity is involved in modulating the stress-induced increase of cortical dopamine metabolism and extend this observation by demonstrating that this effect is probably mediated by the tachykinin NK_1 receptor subtype.

Benzodiazepine/$GABA_A$ ligands are recognised to exert a powerful modulatory influence on mesocortical dopamine neuronal function whereby inverse agonists are anxiogenic (Crawley et al., 1985; File et al., 1985) and enhance cortical dopamine metabolism (Bradberry et al., 1991; Tam and Roth, 1985) whilst agonists are anxiolytic and attenuate stress-induced dopamine metabolism (Lavielle et al., 1978; Feenstra et al., 1995; Kaneyuki et al., 1991; Ida et al., 1989; Reinhard et al., 1982; Giorgi et al., 1987; Claustre et al., 1986; Hutson and Barton, 1997). It is conceivable therefore that the observed effect of (*S*)-GR 205171 in attenuating the increase of cortical dopamine metabolism by stress is mediated directly or indirectly by an interaction of (*S*)-GR205171 with benzodiazepine/$GABA_A$ receptors. Flumazenil is a benzodiazepine/$GABA_A$ receptor antagonist which at a dose of 15 mg/kg was shown to reverse the effects of diazepam on stress-induced cortical DOPAC concentration (Kaneyuki et al., 1981). However, in the present study, pretreatment of animals with flumazenil failed to affect basal or the increase of cortical DOPAC concentration due to stress. Neither did flumazenil affect the ability of (*S*)-GR205171 to attenuate the stress-induced increase of cortical DOPAC concentration. This suggests that the attenuation of stress-induced cortical dopamine metabolism by the tachykinin NK_1 receptor antagonist is not mediated by an interaction with benzodiazepine modulatory site of the $GABA_A$ receptor.

The ability of (*S*)-GR205171 to attenuate stress-induced mesocortical dopamine metabolism suggests that tachykinin NK_1 receptor antagonists along with benzodiazepine/$GABA_A$ receptor agonists, 5-HT_{1A} receptor agonists and NMDA receptor antagonists may have anxiolytic activity, a view supported by both behavioural and biochemical studies (Teixeira et al., 1996; Altier and Stewart, 1999; Hahn and Bannon, 1999). Interestingly, a recent study also demonstrated that chronic but not acute administration of the antidepressant agents imipramine and mirtazapine prevented the increase of cortical dopamine efflux by footshock stress (Dazzi et al., 2001). The tachykinin NK_1 receptor antagonist MK869 has shown antidepressant efficacy in a clinical study (Kramer et al., 1998) via a novel mechanism that may involve subtle modulation of brain monoamine function (Maubach et al., 2002). The efficacy of such compounds following a single dose in the current preclinical studies may differentiate tachykinin NK_1 receptor antagonists from other antidepressants that require repeated administration to observe a similar effect. However, robust antidepressant effects were observed with MK869 over a similar time course to that of paroxetine in patients with moderate to severe depression (Kramer et al., 1998).

Further studies are required in order to elucidate the anatomical site(s) at which tachykinin NK_1 receptor antagonists interact with mesocortical dopamine neurones to modulate the effects of stress. It is possible that tachykinin NK_1 receptors located within the ventral tegmental area are involved. Thus infusion of substance P or stable substance P analogues into the ventral tegmental area increased mesocortical dopamine release and metabolism (Elliott et al., 1986b) and induced anxiogenic-like behaviour in the elevated plus maze (Teixeira et al., 1996), acoustic startle potentiation (Krase et al., 1994). However, other brain regions, e.g. the amygdala, are also involved in regulating the activity of cortical dopamine neurones to stress and are influenced by substance P (Davis et al., 1994; Mantyh et al., 1984; 1989; Mitrovic and Napier, 1998). It must be remembered however that the anatomical distribution of tachykinin NK_1 receptors displays considerable species differences (Dietl and Palacios, 1991), suggesting that tachykinin NK_1 receptor-mediated neurochemical or behavioural responses that are observed in the rat may not be recapitulated in other species including man.

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