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L-701,324, a glycine/NMDA receptor antagonist, blocks the increase of cortical dopamine metabolism by stress and DMCM

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Abstract

Dopamine metabolism, as reflected by the concentration of dihydroxyphenylacetic acid (DOPAC), in the medial prefrontal cortex was significantly increased following 30 min immobilisation stress or systemic administration of the benzodiazepine/$GABA_A$ receptor inverse agonist methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM). The response to stress was attenuated by pretreatment of rats with the benzodiazepine/$GABA_A$ receptor agonists diazepam and zolpidem. Furthermore, pretreatment with *R*-(+)-3-amino-1-hydroxypyrrolid-2-one (*R*-(+)-HA-966), a low efficacy partial agonist, and 7-chloro-4-hydroxy-3(3-phenoxy) phenylquinolin-2-(*H*)-one (L-701,324) a novel, high affinity, full antagonist at the glycine/NMDA receptor attenuated the response to both stress and DMCM. These results demonstrate that antagonists at the glycine/NMDA receptor complex are comparable with benzodiazepine/$GABA_A$ receptor agonists in their ability to prevent activation of the mesocortical dopamine system by stress and $GABA_A$ receptor inverse agonists. Results are discussed in relation to the interaction between glycine/NMDA receptor antagonists, the mesocorticolimbic dopamine system and stress related disorders.

Keywords: Mesocortical dopamine neuron; Stress; Benzodiazepine/$GABA_A$ receptor agonist; Agonist, inverse; Glycine/NMDA receptor antagonist

1. Introduction

Dopamine neurones arising from cell bodies in the A10 ventral tegmental area innervate both limbic (nucleus accumbens, amygdala, olfactory tubercules, septum, etc.) and cortical structures. Mesocortical dopamine neurones are particularly sensitive to the effects of stress and several studies have shown that acute footshock, immobilisation or psychological stress will preferentially increase dopamine release and metabolism as indicated by the increase of dihydroxyphenylacetic acid (DOPAC) in the medial prefrontal cortex (Bradberry et al., 1991; Sorg and Kalivas, 1993; Feenstra et al., 1995; Thierry et al., 1976). Dopamine metabolism has also been shown in some, but not all, studies to be enhanced by stress in limbic regions such as the nucleus accumbens (Morrow et al., 1993) and amygdala (Herman et al., 1982).

Mesocortical dopamine neurones are also modulated by benzodiazepine/$GABA_A$ receptor ligands. Thus, benzodiazepine/$GABA_A$ receptor inverse agonists, e.g., *N*-methyl-β-carboline-3-carboxamide (FG 7142) and ethyl β-carboline-3-carboxylate (βCCE) which are anxiogenic (Corda et al., 1983; Crawley et al., 1985; File et al., 1985; Morrow et al., 1993), increase mesocortical dopamine metabolism and release comparable to that observed following acute stress (Claustre et al., 1986; Giorgio et al., 1987; Bradberry et al., 1991; Tam and Roth, 1985). Conversely, benzodiazepine/$GABA_A$ receptor agonists including diazepam, chlordiazepoxide and zolpidem do not appear to influence mesocortical dopamine metabolism per se but attenuate the stress induced increase of mesocortical dopamine metabolism (Kaneyuki et al., 1991; Fadda et al., 1978; Ida et al., 1989; Reinhard et al., 1982) at anxiolytic doses.

The *N*-methyl-D-aspartic acid (NMDA) receptor is a hetero-oligomeric, ligand gated ion channel and has at least four distinct sites which modulate its function including the glutamate agonist recognition site, the strychnine insensitive glycine co-agonist site, the polyamine site and the phencyclidine binding site of the associated ion channel. *R*-(+)-HA-966 (*R*-(+)-3-amino-1-hydroxypyrrolid-2-one) is a low efficacy partial agonist at the strychnine insensitive glycine site (Singh et al., 1990) and in contrast

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to the non-competitive NMDA ion channel blockers phencyclidine (PCP) and dizocilpine (MK801) does not stimulate locomotor activity or increase mesocorticolimbic dopamine metabolism per se (Hutson et al., 1991; Bristow et al., 1993). *R*-(+)-HA-966 has been shown to have anxiolytic activity in behavioural studies (Dunn et al., 1992; Corbett and Dunn, 1991) and was subsequently found to attenuate the activation of dopamine metabolism in medial prefrontal cortex following 30 min restraint stress (Morrow et al., 1993). However, this compound is a weak, partial agonist at the glycine/NMDA receptor and it is unclear whether its ability to block stress induced cortical dopamine metabolism is related to its partial agonist properties. Recently we have described a novel series of glycine/NMDA receptor antagonists which are exemplified by L-701,324 (7-chloro-4-hydroxy-3(3-phenoxy) phenylquinolin-2-(1*H*)-one, Kulagowski et al., 1994). L-701,324 binds with high affinity to rat brain membranes ($IC_{50} = 2$ nM) and is highly selective not only with respect to other excitatory amino acid receptors (AMPA and kainate) but also against many other neurotransmitter receptors including $GABA_A$ ($IC_{50} > 10$ μM to human cloned receptor subtypes and rat brain membranes). Functionally, L-701,324 is a full antagonist at the glycine site and unlike *R*-(+)-HA-966 shows no efficacy in the rat cortical slice preparation (Priestley et al., 1994). In vivo, L-701,324 is anticonvulsant against *N*-methyl-D,L-aspartic acid (NMDA) and audiogenic induced seizures (Bristow et al., 1996b) and significantly attenuated the activation of mesolimbic dopamine neurones following amphetamine (Bristow et al., 1996a) and PCP (Hutson et al., 1995) without affecting dopamine neuronal function per se. Therefore in the present study we have compared the effects of the benzodiazepine/$GABA_A$ receptor agonists diazepam and zolpidem with *R*-(+)-HA-966 and L-701,324 on the increase of dopamine metabolism in medial prefrontal cortex induced by immobilisation stress and the benzodiazepine/$GABA_A$ receptor inverse agonist DMCM.

2. Materials and methods

2.1. Drug treatment and biochemical analysis

All studies were performed in accordance with the U.K. Animals (Scientific Procedures) Act, 1986. Male Sprague-Dawley rats (weight range 200–250 g, B&K, UK) were housed five per cage on a 12 h light/dark cycle (lights on 07:00 h, off 19:00 h) for a minimum period of 5 days before the experiment. Food and water were available ad libitum. In a preliminary experiment, groups of rats were either left in the home cage or immobilised by taping to a wire grid for 30 min and then immediately killed. Brains were removed, dissected on ice into medial prefrontal cortex, amygdala, striatum, nucleus accumbens and ventral tegmental area, rapidly frozen on solid CO_2 and stored at −70°C. In subsequent experiments groups of rats were pre-treated with either diazepam (5 mg/kg, i.p.), zolpidem (5 mg/kg, i.p.), the glycine receptor antagonists *R*-(+)-HA-966 (20 mg/kg, i.p.), L-701,324 (1 and 5 mg/kg, i.p.), L-701,357 (10 mg/kg, i.p.) or vehicle (0.5% carboxy methylcellulose in 0.9% saline, 1 ml/kg, i.p.). Thirty minutes later rats were either injected with DMCM (5 mg/kg, i.p.) or vehicle (1 ml/kg, i.p.) and killed 30 min later or in the stress studies either left in the home cage or immobilised for 30 min and immediately killed. Brains were removed, the medial prefrontal cortex dissected, frozen on solid CO_2, and stored at −70°C. All 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 vols. of 0.4 M perchloric acid containing 0.1% cysteine, 0.01% sodium metabisulphite and 0.01% sodium ethylene diaminetetraacetic acid (NaEDTA) and centrifuged at $3000 \times g/10$ min. The HPLC system comprised an HPLC Technology Techsphere 3μ ODS column (4.6 mm × 7.5 cm). The mobile phase consisted of 0.07 M KH_2PO_4, 0.0035% NaEDTA, 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.

2.2. Statistical analysis

Data were subjected to analysis of variance followed where appropriate by Tukey's studentised range test. A value of $P < 0.05$ was considered significant.

2.3. Drugs

Diazepam (7-chloro-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one), DMCM (methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate), zolpidem (*N*,*N*,6-trimethyl-2-(4-methylphenyl)-imidazol[1,2-*a*]pyridine-3-acetamide) were purchased from RBI. *R*-(+)-HA-966, (*R*-(+)-3-amino-1-hydroxypyrrolid-2-one), L-701,324 (7-chloro-4-hydroxy-3(3-phenoxy) phenylquinolin-2-(*H*)-one) and L-701,357 (7-chloro-4-hydroxy-3(2-phenoxy) phenylquinolin-2-(1*H*)-one) were synthesised in the Department of Chemistry at MSD (Terlings Park, UK).

3. Results

3.1. Effects of immobilisation stress on regional brain DOPAC concentration

The concentration of DOPAC in medial prefrontal cortex was significantly increased following immobilisation

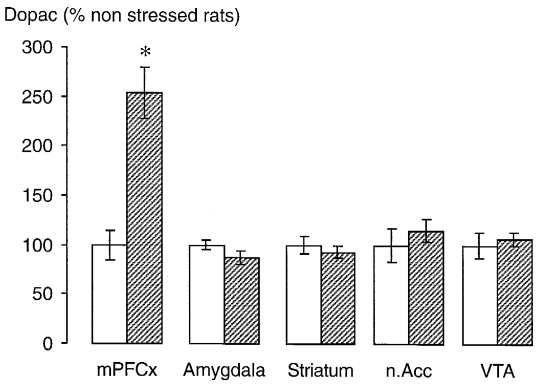

Fig. 1. The effects of (hatched columns) 30 min immobilisation stress on the concentration of DOPAC in medial prefrontal cortex (mPFCx), amygdala, striatum, nucleus accumbens (n.Acc) and ventral tegmental area (VTA). Values are mean ± S.E.M. as a percentage of (open columns) non-stressed control values, $n = 6$ per group. * $P < 0.05$ compared with non-stressed control rats by analysis of variance followed by Tukey's studentised range test. DOPAC concentrations (ng/g) for the above brain regions of non-stressed animals were 9 ± 1; 99 ± 14; 1331 ± 121; 1733 ± 287 and 178 ± 23, respectively.

for 30 min. In contrast, DOPAC concentration in amygdala, striatum, nucleus accumbens and ventral tegmental area was unaffected by this procedure (Fig. 1). The concentration of dopamine was not significantly affected by stress in any brain region (data not shown).

3.2. Effects of benzodiazepine / $GABA_A$ receptor agonists on the stress induced increase of mesocortical DOPAC concentration

Dopamine metabolism as indicated by the concentration of DOPAC in medial prefrontal cortex was significantly increased following 30 min immobilisation stress. Pre-treatment of rats with diazepam (5 mg/kg, i.p.) or zolpidem (5 mg/kg, i.p.) significantly attenuated the stress induced increase of cortical DOPAC concentration without affecting DOPAC concentration per se (Fig. 2a,b). The

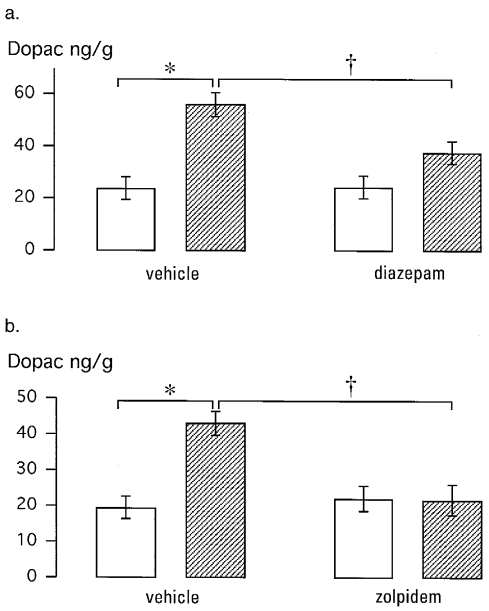

Fig. 2. The effects of (a) diazepam (5 mg/kg, i.p.), (b) zolpidem (5 mg/kg, i.p.) or vehicle (1 ml/kg, i.p.) on medial prefrontal cortex DOPAC concentration in (open columns) non-stressed rats, or (hatched columns) rats immobilised for 30 min. Values are mean ± S.E.M., $n = 6/8$ per group. * $P < 0.05$ compared with non-stressed vehicle treated rats and + $P < 0.05$ compared with stressed vehicle treated rats by analysis of variance followed by Tukey's studentised range test.

concentration of dopamine in the medial prefrontal cortex was not significantly affected by either stress or drug treatment (data not shown).

3.3. Effects of the glycine / NMDA receptor antagonists R-(+)-HA-966 and L-701,324 on the stress induced increase of mesocortical DOPAC concentration

Pre-treatment of rats with either *R*-(+)-HA-966 (20 mg/kg, i.p.) or L-701,324 (5 but not 1 mg/kg, i.p.)

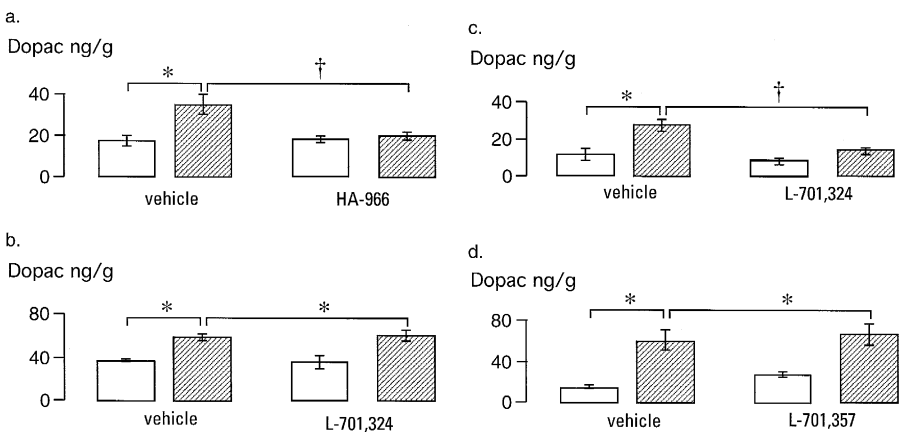

Fig. 3. The effects of (a) *R*-(+)-HA-966 (20 mg/kg, i.p.), (b,c) L-701,324 (1 and 5 mg/kg, i.p.), (d) L-701,357 (10 mg/kg, i.p.) or vehicle (1 ml/kg, i.p.) on medial prefrontal cortex DOPAC concentration in (open columns) non-stressed rats, or (hatched columns) rats immobilised for 30 min. Values are mean ± S.E.M., $n = 6/8$ per group. * $P < 0.05$ compared with non-stressed vehicle treated rats and + $P < 0.05$ compared with stressed vehicle treated rats by analysis of variance followed by Tukey's studentised range test.

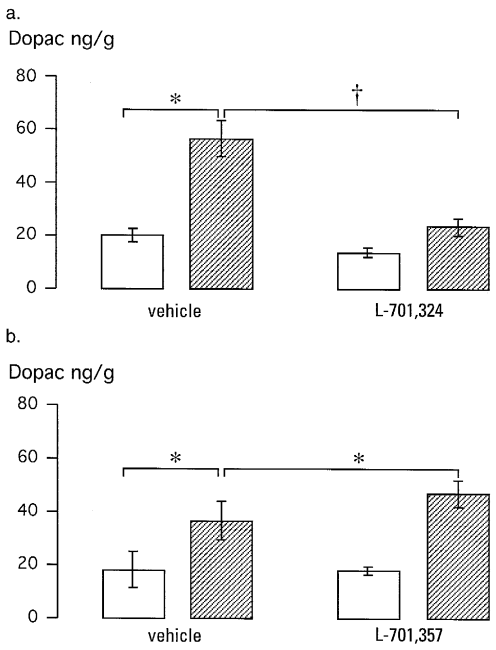

Fig. 4. The effects of (a) L-701,324 (5 mg/kg, i.p.), (b) L-701,357 (10 mg/kg, i.p.) or vehicle (1 ml/kg, i.p.) on medial prefrontal cortex DOPAC concentration in (open columns) vehicle (0.5% carboxy methylcellulose, 1 ml/kg, i.p.) or (hatched columns) DMCM (5 mg/kg, i.p.) treated rats. Values are mean ± S.E.M., $n = 5/8$ per group. * $P < 0.05$ compared with vehicle/vehicle treated rats and $^{+}$ $P < 0.05$ compared with vehicle/DMCM treated rats by analysis of variance followed by Tukey's studentised range test.

significantly attenuated the increase of DOPAC concentration in medial prefrontal cortex following 30 min immobilisation stress without affecting DOPAC concentration per se (Fig. 3a–c). In contrast, pre-treatment with L-701,357 (10 mg/kg, i.p.), the inactive enantiomer of L-701,324, did not significantly affect either basal or stress induced increase of DOPAC concentration in the medial prefrontal cortex (Fig. 3d). The concentration of dopamine in the medial prefrontal cortex was unaffected by stress or drug treatment (data not shown).

3.4. Effects of the glycine/NMDA receptor antagonist L-701,324 on the increase of mesocortical DOPAC concentration induced by the benzodiazepine/GABA$_A$ receptor inverse agonist DMCM

Pre-treatment of rats with L-701,324 (5 mg/kg, i.p.) significantly attenuated the increase of DOPAC concentration in medial prefrontal cortex following administration of the GABA$_A$ receptor inverse agonist DMCM (5 mg/kg, i.p.). In contrast, pre-treatment with L-701,357 (10 mg/kg, i.p.), the inactive regioisomer of L-701,324, did not affect the increase of medial prefrontal cortex DOPAC concentration by DMCM (Fig. 4). The concentration of dopamine in medial prefrontal cortex was unaffected by DMCM or L-701,324 (data not shown).

4. Discussion

Results in the present study confirm previous reports that acute stress significantly and preferentially increased dopamine metabolism, as reflected by the increase of tissue DOPAC concentration, in medial prefrontal cortex and that benzodiazepine/GABA$_A$ receptor agonists such as diazepam and zolpidem attenuate this response (Ida et al., 1989; Kaneyuki et al., 1991; Lavielle et al., 1978; Feenstra et al., 1995; Giorgio et al., 1987; Claustre et al., 1986; Reinhard et al., 1982). Under these conditions, L-701,324, a potent and selective full antagonist (Kulagowski et al., 1994), and *R*-(+)-HA-966, a low efficacy partial agonist (Singh et al., 1990) at the strychnine insensitive glycine site of the NMDA receptor complex significantly attenuated the increase of mesocortical dopamine metabolism following acute immobilisation stress. This effect, which occurred in the absence of any significant alteration by L-701,324 or *R*-(+)-HA-966 on basal dopamine metabolism in medial prefrontal cortex was comparable to that observed following pre-treatment with anxiolytic doses of the benzodiazepine/GABA$_A$ receptor agonists diazepam and zolpidem. In contrast, and as a negative control L-701,357 the regioisomer of L-701,324 which is inactive ($IC_{50} > 10$ μM) at the glycine/NMDA receptor (Bristow et al., 1996a) failed to affect either basal or stress induced changes of mesocortical dopamine metabolism. These results confirm the finding that *R*-(+)-HA-966, a partial agonist at the glycine/NMDA receptor, attenuated the increase of mesocortical dopamine metabolism following immobilisation stress (Morrow et al., 1993) and further extend this observation by demonstrating that this effect is mediated by a full antagonist at the glycine/NMDA receptor which has negligible affinity for numerous other receptors including dopamine and GABA$_A$ subtypes. In the present study, immobilisation stress for 30 min increased dopamine metabolism in medial prefrontal cortex but not other brain regions including the nucleus accumbens and therefore we were unable to confirm the finding by Morrow et al. (1993) that *R*-(+)-HA-966 attenuated stress induced changes of dopamine metabolism in the cortex but not the nucleus accumbens. Interestingly, L-701,324 but not the inactive regioisomer L-701,357 also attenuated the increase of cortical dopamine metabolism induced by the anxiogenic benzodiazepine/GABA$_A$ receptor inverse agonist DMCM.

The anatomical site at which benzodiazepine/GABA$_A$ receptor ligands and NMDA receptor antagonists interact with mesocortical dopamine neurones to modulate stress induced changes is not entirely clear. Thus, Claustre et al. (1986) found that the activation of cortical dopamine neurones by stress was not affected by infusion of zolpidem into the medial prefrontal cortex or the ventral tegmental area. However, Deutch and Roth (1990) found that infusion of chlordiazepoxide into the ventral tegmental area blocked the stress induced increase of cortical

dopamine metabolism and conversely infusion of DMCM into the ventral tegmental area increased cortical dopamine to an extent similar to that found with its systemic administration. The reason for the discrepancy in these two studies is unclear but benzodiazepine receptor ligands are renowned for their poor aqueous solubility which is a problem with focal infusions of high concentrations of such ligands. The ventral tegmental area may also be one of the sites at which NMDA receptor ligands interact with mesocortical dopamine neurones. Thus, infusion of glutamate or NMDA into the ventral tegmental area increased dopamine release and utilisation in the medial prefrontal cortex and nucleus accumbens. Furthermore, infusion of the competitive glutamate receptor antagonist CPP (3-[9 − / + -2-carboxypiperazin-4-yl]-propyl-1-phosphonic acid) and the glycine/NMDA receptor antagonist *R*-(+)-HA-966 into the ventral tegmental area attenuated the stress induced activation of prefrontal cortex dopamine utilisation (Kalivas et al., 1989; Morrow et al., 1993). Interestingly, in the latter study *R*-(+)-HA-966 failed to block the stress induced increase of dopamine metabolism in nucleus accumbens which is also innervated by dopamine neurones arising in the ventral tegmental area. It is apparent from lesion studies, however, that other anatomical sites, e.g., the amygdala, may also be of importance in the modulation of mesocortical dopamine metabolism by stress (Davies et al., 1994).

The present findings that L-701,324, a full antagonist at the glycine/NMDA receptor, attenuated both stress and benzodiazepine/$GABA_A$ receptor inverse agonist induced changes of cortical dopamine metabolism are consistent with evidence implicating glutamate and, in particular, the NMDA receptor complex in the response to stress and anxiety. Thus, behavioural studies have demonstrated that antagonists at the glutamate, glycine and ion channel sites of the NMDA receptor complex demonstrate anxiolytic activity in both conditioned and non-conditioned tests in rodents (Trullas et al., 1989; Corbett and Dunn, 1991; Dunn et al., 1992; Kehne et al., 1991; Faiman et al., 1994).

Results in the present study also support the previous finding that antagonists at the strychnine insensitive glycine site of the NMDA receptor interact with mesocorticolimbic dopamine neurones but only when those neurones are activated. Thus, both *R*-(+)-HA-966 and L-701,324 have been shown not only to lack the ability of the non-competitive ion channel blockers phencyclidine and MK801 to increase mesocorticolimbic dopamine metabolism and induce hyperlocomotion but in addition to markedly attenuate these effects of PCP and MK801 (Bristow et al., 1993, 1996a; Hutson et al., 1991, 1995). Based on these and other studies it has been suggested that glycine/NMDA receptor antagonists display an atypical neuroleptic-like profile in rodents. Given the importance of the frontal cortex in the pathophysiology of schizophrenia and that stress may be a mitigating factor in the relapse of schizophrenic patients (Dohrenwend and Egri, 1981) and the exacerbation of schizophrenic symptoms (Bebbington et al., 1993), the present findings emphasize the previous suggestion that glutamate systems may be important in schizophrenia and that this class of compound may be a novel approach for the treatment of schizophrenia. Whether such compounds would be clinically effective in the treatment of schizophrenia or anxiety related disorders remains to be determined.

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