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Electroconvulsive shock increases tachykinin NK_1 receptors, but not the encoding mRNA, in rat cortex

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Abstract

Recent studies have suggested that the substance P (tachykinin NK_1) receptor may be a pharmacological target for the treatment of mood disorders. Here, the effects of electroconvulsive shock on tachykinin NK_1 receptor gene expression in the rat brain was investigated. Rats received either a single electroconvulsive shock or five shocks on alternate days. Quantitative autoradiography with [^{125}I]Bolton Hunter-substance P, and in situ hybridisation histochemistry, were used to measure tachykinin NK_1 receptor-binding site densities and mRNA abundance, respectively. Densities of tachykinin NK_1 receptor-binding sites were significantly increased in the cerebral cortex following repeated electroconvulsive shock compared to sham treated animals. Densities remained unchanged in the hippocampus, striatum and amygdala. Neither single nor repeated electroconvulsive shock altered tachykinin NK_1 receptor mRNA in the brain regions examined. Hence, repeated electroconvulsive shock increases tachykinin NK_1 receptors in the rat brain in a regionally specific way. Upregulation of receptor-binding sites without a change in mRNA indicates that translational or post-translational mechanisms underlie this process. © 2001 Published by Elsevier Science B.V.

Keywords: Neurokinin; Brain, rat; Autoradiography; Gene expression; Hybridisation, in situ

1. Introduction

Compounds that attenuate the actions of the neuropeptide substance P by blocking the tachykinin NK_1 receptor have been thought to have their main potential in the management of pain. Recent evidence, however, suggests that this receptor may have a more substantive role in affect rather than in the perception of pain. For instance, mice devoid of the tachykinin NK_1 receptor gene responded normally to acute nociceptive stimuli, but lacked adaptive responses to stress (De Felipe et al., 1998). Furthermore, the distinctive reward behaviours elicited by opiates are absent in these mice (Murtra et al., 2000). The importance of the tachykinin NK_1 receptor in mood has also been implicated by studies demonstrating the anxiolytic effects of tachykinin NK_1 receptor antagonists in the rat (File, 2000), which is consistent with the anxiogenic profile of centrally administered substance P (Gavioli et al., 1999). Perhaps more notably, data from a randomised clinical trial suggested that a new generation of tachykinin NK_1 receptor anatgonists, such as MK869, may be effective in the treatment of depression and anxiety (Kramer et al., 1998). This finding led us to hypothesise that the levels of brain tachykinin NK_1 receptors may be altered in mood disorders. In a post mortem study, we demonstrated that in unipolar mood disorder, although the total density of tachykinin NK_1 receptor-binding sites in the cingulate cortex were unchanged, the ratio of receptor densities in the superficial compared to the deep laminar compartments were decreased (Burnet and Harrison, 2000).

Preclinical studies relevant to mood disorders and their treatment have included the examination of receptor gene expression in the rodent brain following the administration of electroconvulsive shock or antidepressant drugs. Al-

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though the levels of substance P appear to be influenced by some antidepressant agents (Shirayama et al., 1996), data on the effects of electroconvulsive shock are equivocal (Brodin et al., 1989; Stenfors et al., 1995). One investigation demonstrated that repeated electroconvulsive shock decreased the response of rat cingulate neurons to iontophoretically applied substance P (Jones et al., 1985). This suggests that the expression and/or function of the tachykinin NK_1 receptor is affected by electroconvulsive shock. Indeed, a preliminary report has shown decreased tachykinin NK_1 receptor mRNA abundance in the rat striatum following repeated electroconvulsive shock (Zachrisson et al., 1997). To date, tachykinin NK_1 receptor gene expression in other brain areas, which might be involved in the neurobiology or pharmacotherapy of depression, have not been determined in this paradigm.

Here, we report a study of the effects of electroconvulsive shock on tachykinin NK_1 receptor gene expression in several brain regions implicated in mood disorder (Bremner et al., 2000; Drevets et al., 1998; Mervaala et al., 2000), and by measuring the two key parameters of gene expression, i.e. receptor-binding sites and mRNA. Both single and repeated electroconvulsive shock were used in order to estimate the timecourse of any changes.

2. Materials and methods

2.1. Animals and electroconvulsive shock administration

Male Sprague–Dawley rats (250–270 g, Harlan, Olac, Bicester, UK) were used for all experiments. Animals were housed with a 12 h light–dark cycle and fed ad libitum.

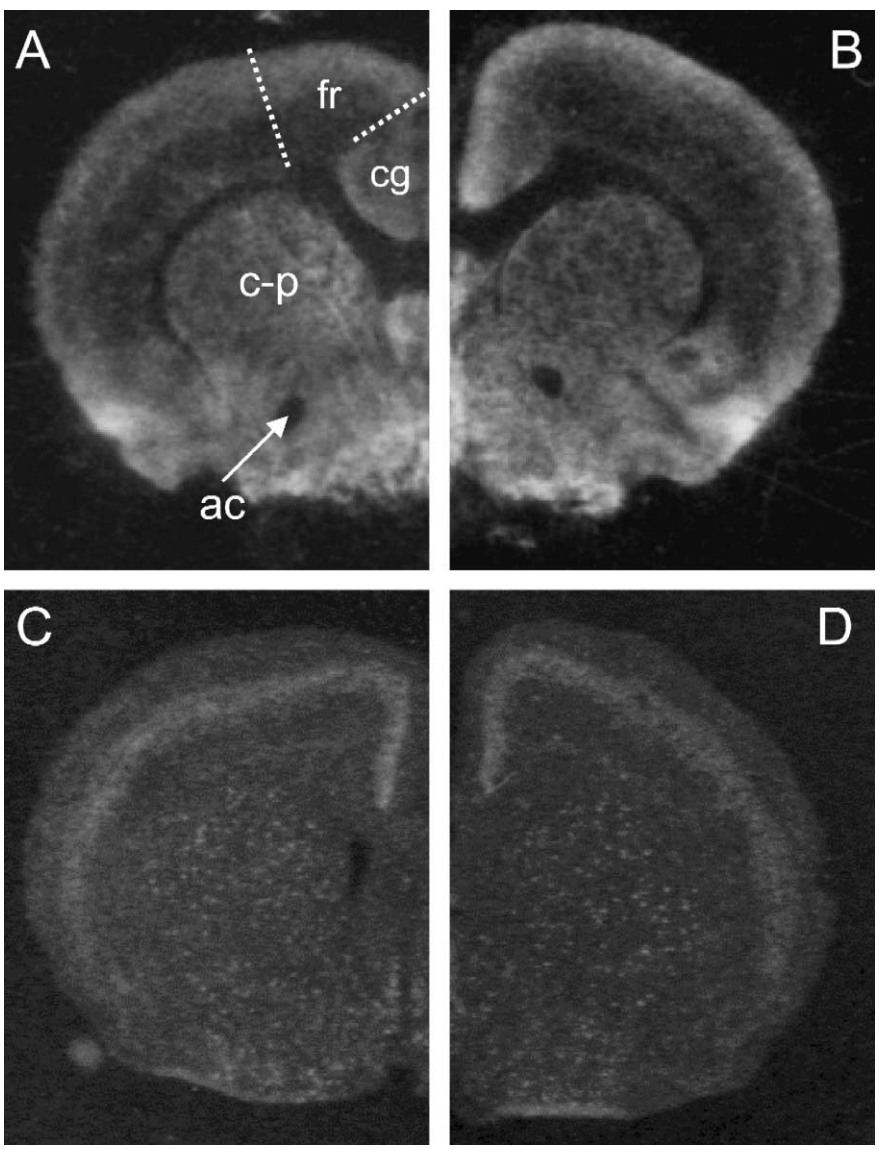

Fig. 1. Representative autoradiograms showing the distribution of tachykinin NK_1 receptors binding sites (A and B) and encoding mRNA (C and D) in coronal sections of the rat brain. The density of binding sites in cortical regions are increased after repeated ECS (B) compared with control animals (A). ac = anterior commissure; cg = cingulate cortex; c-p = caudate putamen; fr = frontal cortex. Broken lines delineate cingulate and frontal cortex.

Electroconvulsive shock was administered to halothane anaesthetised rats (150 V, 50 Hz, 1 s) via ear-clip electrodes. Animals received either a single shock or a total of five shocks on every other day for 10 days. Control ('sham') animals were anaesthetised and had electrodes placed, but no current delivered. Rats receiving electroconvulsive shock demonstrated typical tonic–clonic seizure lasting approximately 20 s. Animals were sacrificed 24 h after the last shock, brains were removed, frozen and stored at −70°C. This protocol has been successfully used to demonstrate changes in the expression of several genes including 5-HT receptors (Burnet et al., 1999) and microtubule associated proteins (Pei et al., 1998).

2.2. Quantitative receptor autoradiography and in situ hybridisation histochemistry (ISHH)

In vitro receptor autoradiography was performed as described (Beaujouan et al., 1986) using [^{125}I] Bolton Hunter-substance P (New England Nuclear). Briefly, sections were thawed, dried and preincubated in Tris–HCl, (50 mM, pH = 7.4) containing bovine serum albumin (0.02%) for 20 min at room temperature. Slides were then incubated in Tris–HCl (50 mM, pH = 7.4) containing bovine serum albumin (0.02%), $MnCl_2$ (3 mM), bacitracin (40 μM), chymostatin (2 μg/ml), leupeptin (4 μg/ml) and 65 pM [^{125}I] Bolton Hunter substance P at room temperature for 30 min. Post-incubation washes included 4 × 20 s immersions in ice-cold Tris buffer followed by a 5 s dip in water. Nonspecific binding was determined by the addition of 1 μM substance P. Sections were apposed to [^{3}H]-sensitive Hyperfilm (Amersham) at 4°C for 4 days.

In situ hybridisation hystochemistry was performed using an oligodeoxyribonucleotide complementary to base pairs 301–335 of the rat tachykinin NK_1 receptor, exon 4 cDNA clone (Hershey et al., 1991). This region is not subject to alternate splicing (Fong et al., 1992) and corresponds to amino acids 298–309 of the tachykinin NK_1 receptor protein (Yokota et al., 1989). No cross-homology of the oligodeoxyribenucleotide with other receptor genes was detected on a GenBank search. The probes were 3′-end labelled with [α^{35}S]-dATP (1500 Ci/mmol) in a 1:10 molar ratio, using terminal deoxynucleotidyl transferase and standard labelling buffer (Promega, UK). The labelled probe was diluted in hybridisation buffer and 8×10^5 cpm were added to each section. Nonspecific hybridisation was determined using a twenty-fold excess of unlabelled probe. After hybridisation slides were washed in 1 × saline sodium citrate (SSC) at 55°C for 20 min (×3) followed by 1 × SSC at room temperature for 45

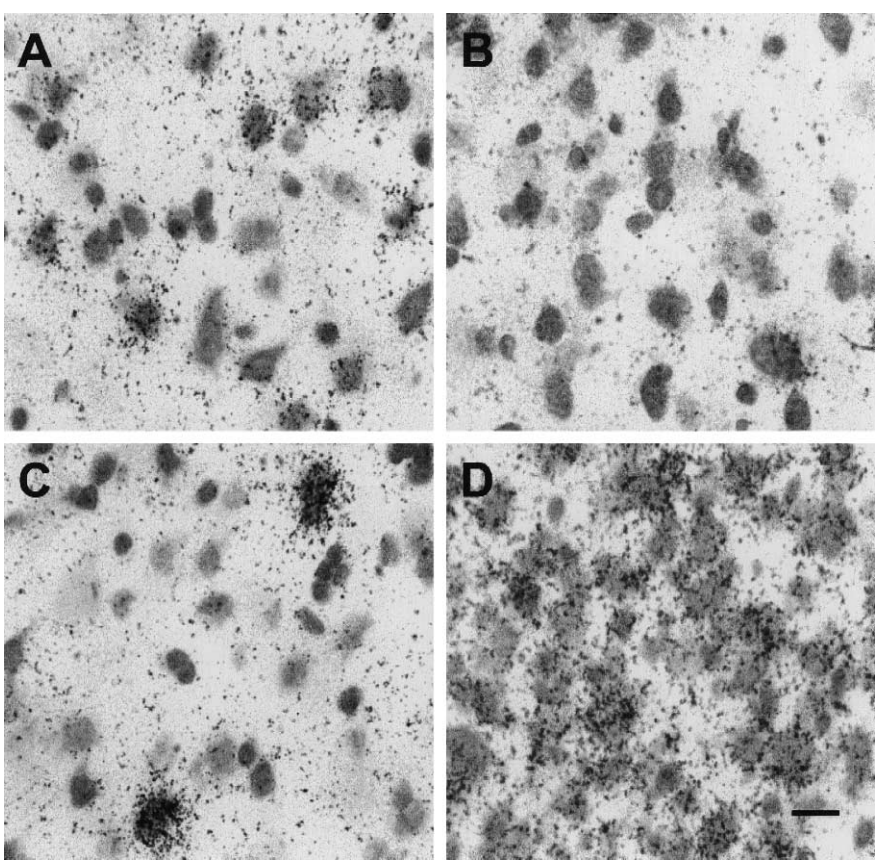

Fig. 2. The cellular distribution of tachykinin NK_1 receptor mRNA in the rat brain. A, middle laminae of the cingulate cortex; B, deep laminae of the cingulate cortex; C, caudate-putamen; D, amygdala. Scale bar = 20 μm.

min (×2). Dry sections were apposed to Hyperfilm, Betamax (Amersham, UK) for 3 weeks at room temperature.

2.3. Image analysis

The densities of tachykinin NK_1 receptor-binding sites and the abundance of encoding mRNA in cortical areas, hippocampus, amygdala and striatum were determined using densitometric quantification of autoradiograms, corrected for nonspecific signal as previously described (Burnet et al., 1996). Cortical readings were taken over the full depth of the grey matter as well as over the superficial and deep laminae separately. Measurements in the hippocampus were performed separately on CA1, CA3, hilus and dentate gyrus, and in the striatum over the dorso-lateral and ventro-medial caudate-putamen and the core and shell of the nucleus accumbens.

Average optical density values for receptor autoradiography were calibrated using [^{125}I] standards prepared in the laboratory (Beaujouan et al., 1986). Briefly, increasing concentrations of [^{125}I]Bolton Hunter substance P were added to 4 mm × 2 mm × 15 μm sections of frozen homogenised rat brain, which had been thaw mounted onto gelatine-coated slides. The radioactivity and protein in the standard samples were measured to calculate the standard values in fmol/mg protein. For in situ hybridisation histochemistry, grey density values were calibrated to ^{14}C tissue equivalents (Amersham).

2.4. Data analysis

The effects of each electroconvulsive shock treatment on tachykinin NK_1 receptor-binding site densities and NK_1R mRNA abundance in all brain areas were tested using one-way analysis of variance (ANOVA).

3. Results

3.1. Distribution of tachykinin NK_1 receptor-binding sites and encoding mRNA

In all cortical regions, [^{125}I] Bolton Hunter substance P binding in the superficial laminae was greater than in the deep laminae. High densities of binding sites were also observed in the caudate-putamen and nucleus accumbens (Fig. 1A), and in the amygdala and hilar region of the hippocampus (not shown). This distribution corresponds to that previously described (Beaujouan et al., 1986). Nonspecific binding represented < 10% of total [^{125}I] Bolton Hunter substance P binding (data not shown).

Moderate signals for tachykinin NK_1 receptor mRNA were detected in all cortical regions with lower levels in the striatum (Fig. 1C) as previously reported (Maeno et al., 1993). The expression of tachykinin NK_1 receptor mRNA was also significant in the amygdala (not presented). Nonspecific hybridisation was < 15% of the total signal generated by the probe (data not shown).

Sections dipped in photographic emulsion (Fig. 2) revealed that tachykinin NK_1 receptor mRNA in all cortical areas appeared to be expressed mainly by non-pyramidal cells; this labelling was greater over cells in the middle laminae of the cortex (Fig. 2A) compared with the more superficial and deep laminae (Fig. 2B) consistent with the film images (see Fig. 1C, D). In the caudate-putamen, tachykinin NK_1 receptor mRNA was expressed by a population of large cells (Fig. 2C). Most cells in the amygdala appear to express tachykinin NK_1 receptor mRNA (Fig. 2D). Our observations are concordant with earlier reports (Maeno et al., 1993).

3.2. Effect of electroconvulsive shock on tachykinin NK_1 receptor-binding site densities and mRNA abundance

A single electroconvulsive shock did not change the densities of tachykinin NK_1 receptor-binding sites in any brain region examined (Table 1). An increase of tachykinin NK_1 receptor-binding sites densities after repeated electroconvulsive shock was observed in several cortical areas (e.g. Fig. 1B), (frontal: +53%, $F = 7.91$, $P = 0.023$; cin-

Table 1
The effect of single and repeated ECS on tachykinin NK_1 receptors binding site densities in the rat brain

Tachykinin NK_1 receptor binding site densities (fmol/mg tissue)				
Brain area	Single		Repeated	
	Sham	ECS	Sham	ECS
Cortex				
Frontal	6.1 ± 0.1(5)	6.9 ± 0.3(5)	5.4 ± 0.7(5)	8.3 ± 0.5(5)[a]
Cingulate	6.0 ± 0.3(5)	6.4 ± 0.2(5)	6.6 ± 0.5(5)	8.9 ± 0.4(6)[a]
Parietal	5.8 ± 0.4(5)	6.1 ± 0.3(5)	6.6 ± 0.3(5)	7.2 ± 0.4(6)
Occipital	7.9 ± 0.6(4)	8.5 ± 0.9(5)	7.5 ± 0.4(4)	9.3 ± 0.5(6)[a]
Hippocampus				
Dentate gyrus	10.7 ± 0.6(4)	10.3 ± 0.7(5)	9.4 ± 0.6(5)	10.4 ± 0.5(6)
Hilus	19.1 ± 1.1(5)	20.4 ± 1.5(5)	18.4 ± 1.4(5)	20.5 ± 1.1(6)
CA3	11.1 ± 0.7(4)	10.6 ± 0.4(5)	10.3 ± 0.6(5)	10.7 ± 0.9(6)
CA1	8.3 ± 0.4(4)	8.8 ± 0.2(5)	8.8 ± 0.5(5)	9.4 ± 0.5(6)
Amygdala	18.9 ± 1.5(4)	17.4 ± 1.2(5)	14.9 ± 1.7(5)	17.9 ± 2.0(5)
Caudate-putamen				
Dorso-Lateral	7.3 ± 0.6(5)	6.9 ± 0.7(5)	6.8 ± 0.6(5)	6.7 ± 0.5(6)
Ventro-Medial	8.1 ± 0.7(5)	7.9 ± 0.7(5)	7.8 ± 0.7(5)	7.9 ± 0.7(6)
Nucleus accumbens				
Shell	9.6 ± 0.8(5)	9.7 ± 0.7(5)	9.0 ± 0.4(5)	9.1 ± 0.5(6)
Core	9.3 ± 0.8(5)	9.4 ± 0.9(5)	10.0 ± 0.6(5)	9.9 ± 0.6(6)

Coronal sections of rat brain from each treatment group were incubated with [^{125}I]Bolton Hunter substance P and exposed to X-ray film. The density of [^{125}I]Bolton Hunter substance P binding sites were measured from autoradiographic images using computerised densitometry, precalibrated with [^{125}I] standards prepared 'in house'. Values are mean ± SEM. The number of rats in each group are indicated in parentheses.

[a] $p < 0.05$.

Table 2
Tachykinin NK_1 receptor mRNA abundance in the cortex and amygdala of the rat after single and repeated ECS

Tachykinin NK_1 receptor mRNA (nCi/g tissue)				
Brain area	Single		Repeated	
	Sham	ECS	Sham	ECS
Cortex				
Frontal	63±3(5)	60±4(5)	64±6(5)	57±5(6)
Cingulate	59±4(5)	61±3(5)	70±4(5)	57±4(6)
Parietal	51±3(5)	54±3(5)	51±2(5)	53±6(6)
Occipital	55±4(5)	52±5(5)	49±4(5)	50±6(6)
Amygdala	129±9(5)	115±11(5)	116±10(5)	120±12(5)

Coronal sections of rat brain from each treatment group were hybridized with a [^{35}S]-labelled oligodeoxyribonucleotide probe, complementary to a small region of the rat NK1R mRNA. Sections were exposed to X-ray film and autoradiographic images of hybridized probe in several cortical regions and the amygdala were quantified using computerised densitometry, precalibrated with [^{14}C] standard microscales. Values are mean± S.E.M. The number of rats in each group are indicated in parentheses.

gulate: +35%, $F = 9.74$, $P = 0.012$ and occipital: +24%, $F = 6.06$, $P = 0.039$, Table 1) and occurred in both deep and superficial laminae of the cortex (not shown). Tachykinin NK_1 receptor-binding sites densities in the hippocampus, caudate-putamen, nucleus accumbens and amygdala remained unaltered after electroconvulsive shock (Table 1).

The abundance of tachykinin NK_1 receptor mRNA could only be reliably measured in the cortex and the amygdala. In these brain regions, neither single nor repeated electroconvulsive shock affected tachykinin NK_1 receptor mRNA abundance (Table 2, see also Fig. 1C, D).

4. Discussion

The present study has demonstrated that tachykinin NK_1 receptor densities are increased by ECS in the rat cortex, but not other brain regions examined. Tachykinin NK_1 receptor mRNA abundance was unaffected by electroconvulsive shock. This study provides first evidence for an involvement of the tachykinin NK_1 receptor in response to a conventional antidepressant treatment, thereby supporting current notions that this receptor may be a novel target for the pharmacotherapy of mood disorders (Kramer et al., 1998)

4.1. Tachykinin NK_1 receptor gene expression and electroconvulsive shock

In both the rat and human brain, there are precedents for a change in receptor-binding site densities without alterations in the encoding mRNA as observed here. For example, the chronic administration of imipramine raises hippocampal 5-HT_{1A} receptor-binding site densities without altering the receptor mRNA (Burnet et al., 1994). Antidepressants decrease the levels of 5-HT_{2A} receptors usually without affecting the receptor mRNA (Roth and Ciaranello, 1991). In schizophrenia, the density of 5-HT_{1A} receptor-binding sites are increased in the cortex, but the abundance of receptor mRNA remains unaltered (Burnet et al., 1996). In the absence of a change in encoding mRNA, alterations in receptor densities presumably involve translational and/or post-translational processes. The three most likely of these processes ,which may underlie the current data are as follows.

First, there might be an increase in the rate and/or efficiency of synthesis of the receptor protein from tachykinin NK_1 receptor mRNA templates in the cell. Second, it is possible that cytosolic pools of readily available, or 'spare', tachykinin NK_1 receptor exist in the postsynaptic cell, which after an appropriate stimulus can be mobilised and reinserted into the synaptic membrane. Extensive investigations have demonstrated that tachykinin NK_1 receptors bound to SP are rapidly internalised into cytosolic vesicles, or endosomes (Grady et al., 1995; McConalogue et al., 1999). In these structures, substance P/tachykinin NK_1 receptor complexes are dissociated and the liberated receptors are 'recycled' back into the synaptic membrane. These data, therefore, support the notion that tachykinin NK_1 receptor membrane trafficking rather than receptor synthesis might be altered by electroconvulsive shock. Third, a population of 'spare' tachykinin NK_1 receptor may also be present on the postsynaptic membrane itself. The [^{125}I]Bolton Hunter substance P used in quantitative autoradiography is an agonist and as such only binds to receptors which are coupled to G-proteins (Beaujouan et al., 1986). The increased densities of the tachykinin NK_1 receptor following electroconvulsive shock might, therefore, indicate an increase in the proportion of G-protein coupled receptors, derived from a pre-existing population of uncoupled tachykinin NK_1 receptor already present on the postsynaptic membrane. The differential coupling of membrane receptors to G-proteins is not uncommon. Studies of the 5-HT_{1A} receptor in the rat (Khawaja et al., 1995) and human (Burnet et al., 1997) brain have also revealed G-protein coupled and uncoupled receptor populations. Thus, modulating the process of coupling tachykinin NK_1 receptors to G-proteins might be a realistic posttranslational mechanism by which its signal transduction can be influenced. Whether this process underlies the increase of tachykinin NK_1 receptor densities in the rat cortex following electroconvulsive shock could be tested by concomitantly measuring the total population of tachykinin NK_1 receptors (using an antagonist) and the subpopulation which are G-protein coupled (using an agonist).

Although this study demonstrates that the abundance of tachykinin NK_1 receptor mRNA is unaltered 24 h following electroconvulsive shock, it is conceivable that a short-lived increase in the levels of tachykinin NK_1 receptor

mRNA occurred before that time. For example, dopamine D1 and D2 receptor mRNA abundance are increased 4 h, but not 24 h, after the final administration of electroconvulsive shock (Smith et al., 1995). This might also be the case for the tachykinin NK_1 receptor transcript. A more detailed temporal study of the effects of electroconvulsive shock on tachykinin NK_1 receptor gene expression, with tissue harvested at times shorter than 24 h would test this hypothesis.

4.2. Other considerations

We have previously demonstrated that although the densities of tachykinin NK_1 receptor-binding site densities in the cingulate gyrus are not altered in patients with mood disorders (Burnet and Harrison, 2000), the ratio of tachykinin NK_1 receptors in the superficial compared to deep laminae was decreased in unipolar depression. In the present study, however, laminar ratios of tachykinin NK_1 receptor-binding remained constant after electroconvulsive shock (data not shown), indicating that there is no clear correspondence between the finding in depression and those after repeated electroconvulsive shock.

The finding tachykinin NK_1 receptor densities in the cortex are increased after electroconvulsive shock has been observed for the 5-HT_{2A} receptor, and suggests there may be shared underlying processes (Burnet et al., 1999). Consistent with this possibility, preliminary investigations indicate direct and indirect associations between the serotonin and tachykinin systems. For instance, the release of substance P from interneurons in the rat hippocampus is facilitated by the activation of 5-HT_{2A} receptors on these cells (Feuerstein et al., 1996). Moreover, tachykinin NK_1 receptor antagonists influence the activity of 5-HT containing neurons by modulating the firing of noradrenergic pathways (Haddjeri and Blier, 2000).

Finally, it may seem paradoxical to suggest that antidepressant effects may be mediated by either the blockade (Kramer et al., 1998) or an increase in tachykinin NK_1 receptor densities. However, this notion is not unprecedented. For example, many antidepressants antagonise, and downregulate, the 5-HT_{2A} receptor (Roth and Ciaranello, 1991; Taylor et al., 1995) yet electroconvulsive shock markedly increases its expression (Burnet et al., 1999) and function (Goodwin et al., 1984) of this receptor. More comprehensive analyses of the effects of antidepressants versus electroconvulsive shock on tachykinin NK_1 receptor expression and function are, therefore, required before these apparent anomalies are explained.

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