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Hippocampal and cortical G protein ($G_s\alpha$, $G_o\alpha$ and $G_{i2}\alpha$) mRNA expression after electroconvulsive shock or lithium carbonate treatment

Stephen McGowan [a], Sharon L. Eastwood [a,b], Adam Mead [b], Philip W.J. Burnet [a,b], Cara Smith [c], Tom P. Flanigan [c], Paul J. Harrison [a,b,*]

[a] *University Department of Psychiatry, Warneford Hospital, Oxford OX3 7JX, UK*

[b] *University Department of Clinical Neurology (Neuropathology), Radcliffe Infirmary, Oxford, UK*

[c] *Oxford University – SmithKline Beecham Centre for Applied Neuropsychobiology, University Department of Clinical Pharmacology, Radcliffe Infirmary, Oxford, UK*

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Abstract

GTP-binding proteins (G proteins) are heteromers composed of α, β and γ subunits. The expression of some G protein subunits is altered both by affective disorders and by antidepressant treatments. Here we have studied three G protein α subunit mRNAs in the hippocampus and frontoparietal cortex of rats treated with lithium for 14 days or with repeated electroconvulsive shock (five shocks over 10 days). After electroconvulsive shock, the three mRNAs changed differentially in the hippocampus. Specifically, $G_s\alpha$ mRNA was decreased in CA3 and CA1, whilst $G_o\alpha$ mRNA was increased in dentate gyrus and $G_{i2}\alpha$ mRNA was reduced in dentate gyrus and CA3. Lithium carbonate treatment produced a modest, uniform increase in the three mRNAs in dentate gyrus and CA3, and a selective elevation of $G_o\alpha$ mRNA in CA1. Neither treatment altered the G protein mRNAs in the cortex nor cyclophilin mRNA in any region. These data extend the evidence that altered G protein expression is a part of the biochemical response to antidepressant treatments. Differences in the molecular and anatomical pattern of the alterations induced by electroconvulsive shock compared to lithium may contribute to their different therapeutic profiles.

Keywords: Electroshock; G-protein; GTP-binding protein; In situ hybridization; Messenger RNA

1. Introduction

Traditionally the changes in neurotransmission associated with psychiatric disorders and their treatments have been studied primarily in terms of transmitters and receptors. However, the critical molecular events could occur anywhere along the signal transduction pathway. Within this pathway, GTP-binding proteins (G proteins) are a key component. They are coupled to many neuroreceptors (Strader et al., 1994) and are a crucial point of convergence and divergence in a second messenger signalling cascade whereby activation of the receptor-G protein complex leads to a series of downstream cellular events (Milligan, 1993).

Functional G proteins are composed of an $\alpha\beta\gamma$ heterotrimer (Neer, 1994). Their nomenclature is based upon the initial discoveries of their effects on adenylate cyclase activity, and the dominant role of the α subunit. Briefly, G proteins containing a $G_s\alpha$ subunit stimulate adenylate cyclase whereas G_i proteins inhibit it. G_o has other effects, notably upon calcium channels and the phosphoinositol cycle. An important source of diversity in G proteins arises from the multiple α subunit genes, which have been shown to be differentially expressed in rat brain by in situ hybridization histochemistry (Brann et al., 1987; Largent et al., 1988; Vincent et al., 1990). Of note, G proteins are regulated at the level of gene expression as well as through post-translational and degradative processes (Hadcock and Malbon, 1993; Milligan et al., 1995).

G proteins are under investigation in several areas of psychiatry (Manji, 1992; Hudson et al., 1993; Manji et al., 1995b). In particular, changes in G protein expression and function have been reported in affective disorders, both unipolar and bipolar (manic) varieties (Avissar and

* Corresponding author. Tel.: +44-(0)1865 226463; fax: +44-(0)1865 793101.

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Schreiber, 1992; Ozawa et al., 1993; Young et al., 1993; Manji et al., 1995a). To complicate matters, G proteins are also influenced by the drugs used to treat affective disorders. Thus, tricyclic antidepressants and monamine oxidase inhibitors (Lesch and Manji, 1992; Lason and Przewlocki, 1993), lithium (Colin et al., 1991; Lesch et al., 1991; Li et al., 1993) and carbamazepine (Lesch et al., 1991; Li et al., 1993) produce selective alterations in the abundance of G protein α subunits or their mRNAs. To date, however, patterns of G protein expression following treatment with these drugs remain incompletely characterised in both molecular and anatomical terms. Neither have there been equivalent studies of the effects of electroconvulsive shock, although data show that it alters various aspects of G protein functioning (Avissar et al., 1990; Nishida et al., 1990; Ozawa and Rasenick, 1991), as is the case with lithium (Jope and Williams, 1994).

In the present study we have used in situ hybridization histochemistry to investigate three α subunit mRNAs, G_s, G_o and G_{i2}, in the hippocampus and cortex of rats treated with lithium or electroconvulsive shock. Such comparative studies may be helpful in the elucidation of neurobiological mechanisms which are common to these treatments as opposed to those which distinguish them. We chose durations of administration which correspond approximately to that required for the onset of clinical therapeutic efficacy.

2. Materials and methods

2.1. Treatment of animals

Adult male Sprague Dawley rats (Harlan Olac, Bicester, UK) weighing 200–275 g were housed in groups of 4–5 with a 12 h light/dark cycle.

For administration of electroconvulsive shock, animals were anaesthetized with halothane. Bilateral electroconvulsive shock was applied through ear-clip electrodes using a Thermatronics small animal electroplexy unit delivering 150 V at 50 Hz (sinusoidal) for 1 s. A full seizure was always elicited. Control animals were handled identically but no current was passed. Treatment was given on alternate days over a 10 day period (five shocks in total). Animals were killed by decapitation 24 h after the last shock. Brains were removed, bisected coronally, frozen in embedding compound on a dry ice/alcohol slurry, and stored at −70°C.

Lithium carbonate was administered for 14 days in chow containing lithium carbonate (0.1% w/v; Special Diet Services, UK). Both treatment and control groups had free access to tap water and 0.9% (w/v) saline. Animals were sacrificed after injection with pentobarbitone (200 mg/kg i.p.) and perfused transcardially with 100 ml normal saline. Prior to perfusion, a blood sample was taken for analysis of plasma lithium carbonate levels by atomic absorption spectroscopy (Varion Spectra AA-300). Brains were snap frozen in isopentane at −40°C and stored at −70°C prior to cryosectioning

2.2. Quantitative in situ hybridization histochemistry

For each transcript, three adjacent 12 μm sections were cut and mounted onto a single slide. Sections were pretreated for in situ hybridization histochemistry as described (Najlerahim et al., 1990) by immersion in: 4% paraformaldehyde in phosphate buffered saline (5 min), 0.1 M triethanolamine hydrochloride, pH 8, containing 0.25% acetic anhydride (10 min), 70% ethanol (1 min), 80% ethanol (1 min), 95% ethanol (1 min), 100% ethanol (1 min), chloroform (10 min), 100% ethanol (2 min), and 95% ethanol (1 min). Pretreated sections were stored at −20°C.

The oligodeoxynucleotide probes were directed against regions which distinguish between the rat G protein α subunit transcripts (Itoh et al., 1986; Jones and Reed, 1987). According to the numbering of Jones and Reed (1987), the probes are complementary to nucleotides 477–521 of $G_s\alpha$, 707–751 of $G_o\alpha$, and 474–521 of $G_{i2}\alpha$. The G_s and G_o probes detect a region common to their splice variants. The probes were 3′ end-labelled with [^{35}S]dATP (~ 1500 Ci/mmol) using terminal deoxynucleotide transferase and a labelling buffer (NEN DuPont) in a 1 : 10 molar ratio.

Hybridization was carried out using 10^6 cpm of labelled probe in 75 μl buffer per section. Hybridization buffer comprised 4 × saline sodium citrate, 50% deionized formamide, 10% dextran sulphate, 5 × Denhardt's solution, 200 μg/ml salmon sperm DNA, 100 μg/ml polyadenylate, 25 mM sodium phosphate, 1 mM sodium pyrophosphate and 100 mM dithiothreitol. Sections were incubated overnight in a humid chamber at 35°C (for $G_s\alpha$ and $G_{i2}\alpha$ probes) or 40°C ($G_o\alpha$ probe). Post-incubation washes were performed in 1 × SSC at 57°C ($G_s\alpha$ and $G_{i2}\alpha$) or 60°C ($G_o\alpha$) for 3 × 20 min followed by 2 × 60 min at room temperature. Experimental controls comprised: hybridization with sense strand probes, pretreatment of sections with ribonuclease A (100 μg/ml at 37°C for 60 min), or incubation in the presence of 50-fold excess unlabelled probe.

Once dry, sections were apposed to autoradiographic film (Hyperfilm betamax, Amersham) for 15 days ($G_s\alpha$ mRNA), 10 days ($G_o\alpha$ mRNA), or 28 days ($G_{i2}\alpha$ mRNA) together with ^{14}C microscales (Amersham International, Little Chalfont, UK). The regions of interest selected for analysis were hippocampus (dentate gyrus, CA3 and CA1) and frontoparietal cortex. Densitometric analysis of the autoradiograms was carried out blind to treatment group by computerised image analysis (Vidas 2.1, Imaging Associates, Thame, UK), calibrated to the microscales (Eastwood et al., 1994). Each region of interest was delineated interactively on the monitor using a mouse, and was identified on both sides of the brain from each of the three sections hybridized. The mean of the resulting six readings

for each area was used in the statistical analysis. Results are expressed as ^{35}S nCi/g tissue equivalents; these units are obtained from the ^{14}C nCi/g standard values by a conversion factor of 3.0 (Miller et al., 1989).

3. Results

Distribution of each G protein α subunit mRNA showed the anticipated distinct distribution reported using oligodeoxynucleotide probes (Brann et al., 1987; Largent et al., 1988; Fig. 1A, C and E). The only significant difference was a relatively weaker signal with the $G_{i2}\alpha$ probe than previously reported (Brann et al., 1987) which may reflect variation in experimental protocols; we repeated the experiment with another $G_{i2}\alpha$ mRNA oligonucleotide and obtained a similar result (data not shown). Minimal images were seen after ribonuclease treatment (Fig. 1B), incubation with excess unlabelled probe (Fig. 1D) or hybridization with a sense strand probe (Fig. 1F). These controls, together with the differential distribution of the mRNAs, confirm the specificity of each hybridization signal.

Analysis of variance revealed a treatment-by-mRNA interaction following electroconvulsive shock in dentate gyrus ($P < 0.02$), CA3 and CA1 (both $P < 0.01$). Thus,

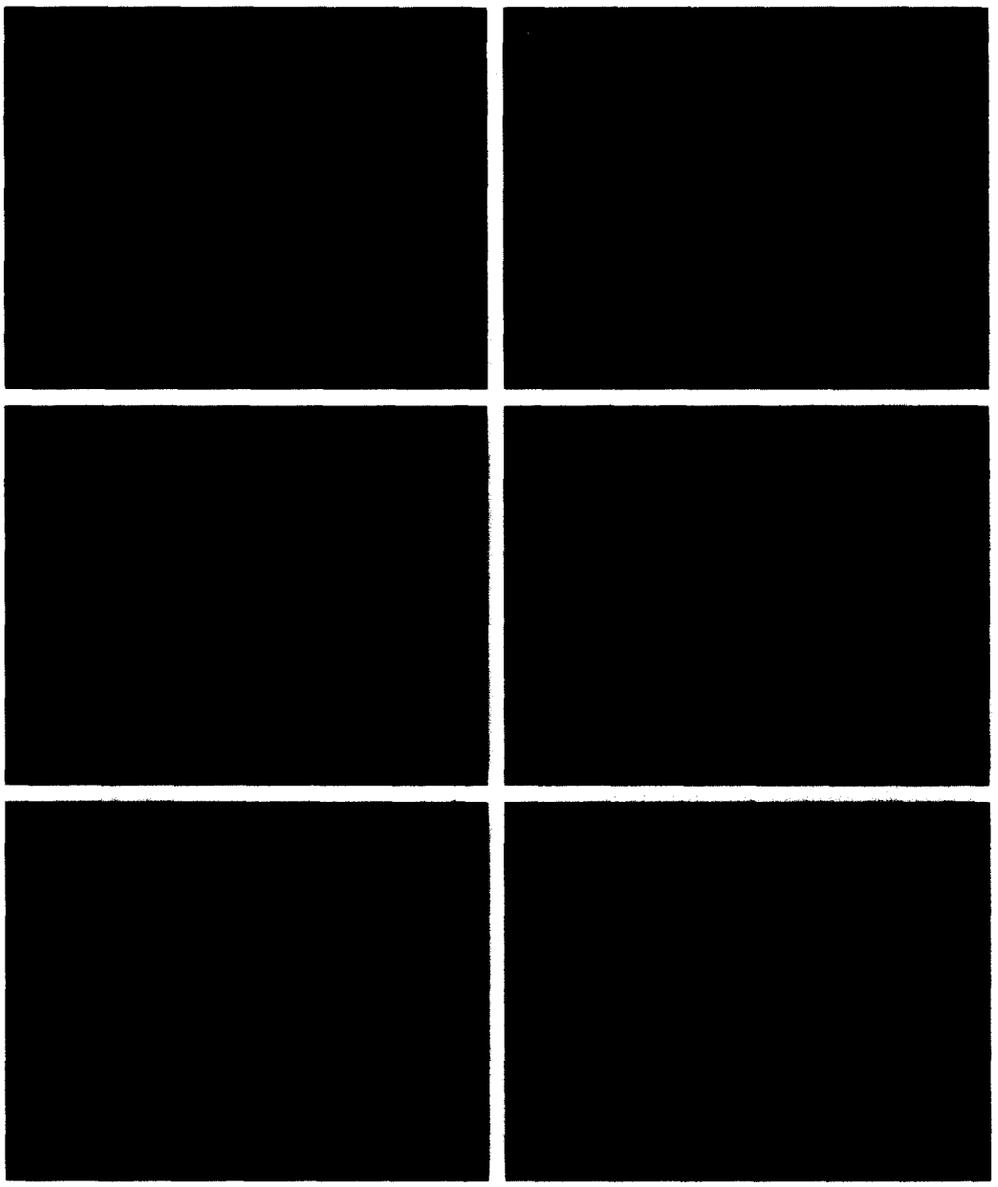

Fig. 1. Distribution of G protein α subunit mRNAs in coronal sections of rat brain at the level of the dorsal hippocampus. A: $G_s\alpha$ mRNA. B: $G_s\alpha$ mRNA, after ribonuclease pretreatment. C: $G_o\alpha$ mRNA. D: $G_o\alpha$ mRNA, after incubation in the presence of 50-fold excess unlabelled antisense probe. E: $G_{i2}\alpha$ mRNA. F: $G_{i2}\alpha$ mRNA, after sense strand hybridization.

Table 1
Effects of repeated electroconvulsive shock (five shocks over 10 days; $n = 5$–6) on G protein α subunit mRNAs compared to sham treated controls ($n = 6$)

RNA		Dentate gyrus	CA3	CA1	Frontoparietal cortex
$G_s\alpha$	Sham	601 ± 43	793 ± 72	524 ± 67	311 ± 28
	ECS	510 ± 66	580 ± 60 [a]	341 ± 43 [a]	241 ± 30
$G_o\alpha$	Sham	547 ± 27	709 ± 33	533 ± 28	321 ± 35
	ECS	728 ± 64 [a]	828 ± 58	626 ± 48	313 ± 27
$G_{i2}\alpha$	Sham	114 ± 4	111 ± 3	100 ± 3	74 ± 2
	ECS	100 ± 4 [a]	99 ± 4 [a]	90 ± 4	71 ± 3

Data are expressed as ^{35}SnCi/g, mean ± S.E.M. [a] $P < 0.05$.

Table 2
Effects of 14 days' lithium treatment ($n = 8$) on G protein α subunit mRNAs compared to controls ($n = 7$)

mRNA		Dentate gyrus [a]	CA3 [a]	CA1	Frontoparietal cortex
$G_s\alpha$	Control	252 ± 31	332 ± 40	199 ± 29	141 ± 9
	Lithium	282 ± 25	366 ± 26	205 ± 13	137 ± 20
$G_o\alpha$	Control	404 ± 12	532 ± 29	368 ± 25	298 ± 17
	Lithium	459 ± 17	616 ± 29	456 ± 15 [b]	322 ± 30
$G_{i2}\alpha$	Control	66 ± 6	83 ± 4	68 ± 6	43 ± 4
	Lithium	82 ± 3	99 ± 4	77 ± 2	55 ± 4

Data are expressed as ^{35}SnCi/g, mean ± S.E.M. [a] Overall effect of treatment, $P < 0.05$. [b] $P < 0.01$.

the treatment has differential effects upon the three mRNAs in the hippocampus as summarised in Table 1. $G_s\alpha$ mRNA was reduced by electroconvulsive shock, significantly so in CA3 and CA1 (both $P < 0.05$). Smaller decreases in $G_{i2}\alpha$ mRNA were observed in dentate gyrus and CA3 (both $P < 0.05$). In contrast to $G_s\alpha$ and $G_{i2}\alpha$ mRNAs, $G_o\alpha$ mRNA was increased by repeated electroconvulsive shock, particularly in the dentate gyrus ($P < 0.05$). Fig. 2 illustrates the changes in hippocampal $G_s\alpha$ and $G_o\alpha$ mRNAs seen following electroconvulsive shock. None of the mRNAs were affected significantly by the treatment in the frontoparietal cortex (Table 1).

Plasma lithium carbonate levels at death ranged from 0.48–0.59 mmol/l in the treated animals and were < 0.05 mmol/l in the controls. In CA1, lithium carbonate treatment affected the G protein mRNAs ($P < 0.01$) and there was also a treatment-by-mRNA interaction ($P < 0.05$), explained by a selective increase in $G_o\alpha$ mRNA ($P < 0.01$). There is an effect of lithium carbonate on the G protein mRNAs in dentate gyrus and CA3 (both $P < 0.05$) but no treatment-by-mRNA interaction, reflecting the uniform increases in all three mRNAs in these subfields. Although the lack of an interaction in dentate gyrus and CA3 means that further analysis should be carried out with

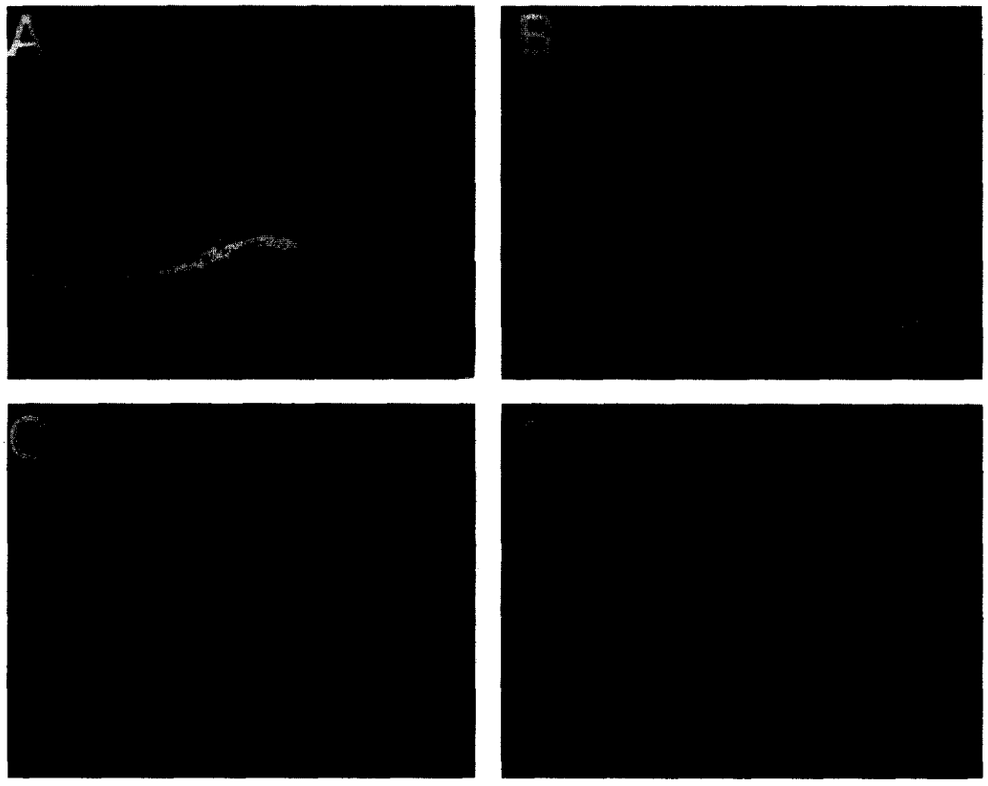

Fig. 2. $G_s\alpha$ (A, B) and $G_o\alpha$ (C, D) mRNAs in the hippocampus of sham controls (A, C) and animals treated with repeated electroconvulsive shock (B, D), showing the reduction of $G_s\alpha$ mRNA and increase of $G_o\alpha$ mRNA in the treated animals.

caution in these subfields, one-way analysis of variance indicates significant elevations of $G_o\alpha$ and $G_{i2}\alpha$ mRNAs in dentate gyrus (both $P < 0.05$). $G_{i2}\alpha$ mRNA, $G_s\alpha$ mRNA, and $G_o\alpha$ mRNA were not altered in the frontoparietal cortex by lithium carbonate (Table 2).

The values for each mRNA in Table 2 differ from those in Table 1, reflecting differences in the way the brains in the lithium carbonate and electroconvulsive shock groups were processed (see Section 2) as well as variation in probe labelling efficiency between experiments. It does not affect the quantitative analyses since all animals in a given experiment (i.e. electroconvulsive shock vs. sham, and lithium carbonate vs. control) underwent identical protocols.

4. Discussion

These data show that administration of electroconvulsive shock or lithium to rats in a regimen approximating that necessary for onset of therapeutic efficacy produces selective changes in the level of G protein α subunit mRNAs in the hippocampus but not frontoparietal cortex.

4.1. Hippocampal G protein mRNAs after electroconvulsive shock or lithium

Only the increase in hippocampal $G_o\alpha$ mRNA was common to both treatments (Tables 1 and 2). The other two G protein mRNAs changed differentially, with $G_s\alpha$ mRNA being decreased by electroconvulsive shock and unchanged by lithium, whilst $G_{i2}\alpha$ mRNA was reduced by electroconvulsive shock but increased by lithium. The similar change in $G_o\alpha$ mRNA expression induced by electroconvulsive shock and lithium may be a biochemical correlate of the therapeutic overlap of these treatments (which comprises resistant depression and second-line treatment of mania). Equally, the distinct changes in $G_s\alpha$ and $G_{i2}\alpha$ mRNAs may be indicative of mechanisms by which the two treatments differ, accompanying their separate main areas of efficacy, viz. electroconvulsive shock for severe depression and lithium for prophylaxis of bipolar disorder. Determining whether any given hippocampal G protein expression change is common to all physical treatments for affective disorders will require parallel studies of other agents, such as tricyclic antidepressants (Lesch and Manji, 1992; Lason and Przewlocki, 1993), monoamine oxidase inhibitors (Lesch and Manji, 1992) and selective serotonin uptake inhibitors.

Further interpretation of our hippocampal data is hindered by the absence of other reports on hippocampal G protein mRNAs after electroconvulsive shock or lithium. Neither are there many data concerning the abundance of the encoded subunits following these treatments. Lesch et al. (1991), using an enzyme-linked immunosorbent assay, found an increase in hippocampal $G_i\alpha$ abundance after 21 days' lithium, which suggests that the increase of $G_{i2}\alpha$ mRNA translates to the protein level. On the other hand, they found no change in $G_o\alpha$ to accompany our elevation of $G_o\alpha$ mRNA (Table 2). Comparison of our data with immunochemical measurements is in any event made difficult by the differential changes in mRNA compared to protein which have been reported. Although an altered mRNA level is usually accompanied by differences in synthesis of the encoded protein, and regulation at the level of gene expression clearly occurs for G proteins (Hadcock and Malbon, 1993), this is not always the case. For example, Li et al. (1993) found lowered $G_i\alpha$ mRNA but not $G_i\alpha$ after lithium therapy. Conversely, protein degradation and recycling are mechanisms of G protein regulation not requiring altered gene expression (Milligan et al., 1995). Thus, unchanged G protein mRNA levels after lithium or electroconvulsive shock (Tables 1 and 2) do not mean that these subunits are not altered in other ways by these treatments. Ultimately, therefore, it is necessary to combine measurements of gene expression with multiple other approaches to G proteins (Manji et al., 1995b).

The alterations in a particular G protein mRNA after repeated electroconvulsive shock were similar in each hippocampal subfield (Table 1). This is in contrast to the effects of electroconvulsive shock on 5-HT_{1A} (Burnet et al., 1995) and KA1 and KA2 (Porter et al., 1996) receptor mRNAs, for which the changes varied markedly between different subfields. The latter pattern of response is indicative of mechanisms specific to particular hippocampal cell populations, whereas the present data suggest that the changes in G protein expression after electroconvulsive shock may be attributable to a process operating more uniformly upon the hippocampus.

Table 2 shows that values for all three G protein mRNAs were somewhat higher in the hippocampus following lithium treatment compared to controls. To investigate if this observation were due to a generalised, small increase in overall hippocampal mRNA, we measured cyclophilin mRNA, a housekeeping gene transcript. Cyclophilin mRNA was unaffected by lithium treatment in all subfields (data not shown), as it was by repeated electroconvulsive shock (Porter et al., 1996). The cyclophilin data confirm that the G protein mRNA changes reported here do not merely reflect an overall alteration in hippocampal gene expression induced by either treatment.

4.2. Cortical G protein mRNAs after electroconvulsive shock or lithium

Repeated electroconvulsive shock affects some receptor mRNAs in the cortex, including 5-HT_{2A} (Butler et al., 1993; Burnet et al., 1995), β_1 adrenergic (Hosoda and Duman, 1993) and KA2 (Porter et al., 1996). However, unlike the changes in the hippocampus, electroconvulsive shock produced no alterations in G protein mRNAs in the

frontoparietal cortex (Table 1). This provides a further indication of the regional specificity of changes in G protein expression occurring after affective disorder treatments (Lesch et al., 1991; Lesch and Manji, 1992).

Lithium also had a lack of effect on expression of the three G protein mRNAs in the cortex (Table 2). In agreement with earlier studies, we found no alteration in cortical $G_o\alpha$ mRNA (Colin et al., 1991; Li et al., 1993). On the other hand we did not replicate the reduction of cortical $G_{i2}\alpha$ mRNA induced by lithium (Colin et al., 1991; Li et al., 1993). We also found cortical $G_s\alpha$ mRNA to be unaffected by lithium (Table 2), in agreement with Colin et al. (1991) but not with Li et al. (1993), who found a modest decrease. We cannot account for the discrepancies other than to point out certain differences between the studies, for example, the species of rat, duration of treatment, area of cortex analysed, or method used for mRNA quantification. Regardless, electroconvulsive shock and lithium treatments seem unlikely to explain the *increased* G_s level reported in the cerebral cortex of patients with bipolar affective disorder, many of whom will have received these treatments during their illness (Young et al., 1993).

4.3. Future studies

The potential pathophysiological and therapeutic significance of G protein changes in this field indicates the need for further investigation. For example, alterations in other α subunit mRNAs, as well as differential expression of their isoforms, or of β and γ subunits (Clapham and Neer, 1993), might be important determinants of abnormal signal transduction. Anatomically, more localised changes in G protein expression may exist, for example, within subpopulations of neurons within a cytoarchitectonic region. Such issues could be addressed with cellular resolution in situ hybridization histochemistry and immunocytochemistry.

4.4. Summary

Levels of G protein α subunit mRNAs are altered by repeated electroconvulsive shock and by lithium in the hippocampus but not in the frontoparietal cortex of the rat. The data confirm that treatments for affective disorders have localised and differential effects on G protein expression. The effects should be taken into account when studying G protein involvement in the pathophysiology of these disorders.

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