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Excitotoxin lesion of nucleus basalis causes a specific decrease in G_o mRNA in cerebral cortex

Sensitivity to MK-801

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Lesions of the ascending cholinergic pathway from nucleus basalis are known to have profound effects on cortical function. In particular, a substantial potentiation of carbachol-stimulated polyphosphoinositide turnover is detected from 1 day after lesion and is maintained for several days before returning to normal by 1 month. In this study the effect of this lesion was investigated on levels of three G-protein α-subunit mRNAs. Excitotoxin lesion of the nucleus basalis caused a selective reduction in the levels of $G_o\alpha$ mRNA in cerebral cortex ipsilateral to the lesion, $G_s\alpha$ and $G_i\alpha$ mRNA being unaffected. The maximal effect was obtained at 3 days after lesion where levels of $G_o\alpha$ mRNA were decreased by 40% compared to sham-operated animals. Levels of $G_o\alpha$ mRNA returned to normal values by 28 days. Treatment with MK-801 caused a significant attenuation of the decrease in $G_o\alpha$ mRNA, indicating the involvement of NMDA receptors in this response.

G-protein; Nucleus basalis; Cerebral cortex; Messenger RNA; MK-801; *N*-methyl-D-aspartate receptor

1. INTRODUCTION

Membrane transduction systems in the nervous system demonstrate considerable adaptation in response to a number of influences such as drug administration, hormone levels and ageing. In recent studies we have characterised a response to injury which is associated with an increased postsynaptic responsiveness [1,2] and is not directly correlated with a change in receptor number or affinity [3]. In this study we have investigated whether an altered level of expression of guanine nucleotide binding proteins (G-proteins) may be involved in mediating this effect.

Increased carbachol responsiveness arises in the cerebral cortex after lesion of the afferent cholinergic pathway from nucleus basalis [1,2,4]. Excitotoxin injection into nucleus basalis which lies in the basal forebrain gives rise to a substantial cholinergic deficit in cerebral cortex with decreases of up to 70% in the level of acetylcholinesterase (AChE) and the depolarisation-induced release of acetylcholine (ACh) [3,5]. Early changes occur after the lesion in the level of expression of, initially, c-fos mRNA and later ornithine decarboxylase (ODC) mRNA and enzyme activity which are maximal within 8 h of lesion, in common with other responses to injury in the nervous system [6–8]. A later response which is first detectable at 24 h and maximal at 3–10 days is an increase in cholinergic responsiveness which is detected as an increased polyphosphoinositide (PPI) response to stimulation with carbachol. The maximal response increases 100% with no change in affinity of muscarinic receptor subtypes and a net decrease in muscarinic receptors [1–4]. The increased responsiveness represents a potential mechanism for overcoming the cholinergic deficit. Later, 'sprouting' of spared axons occurs with a partial reversal of the cholinergic deficit and the increased PPI responsiveness. In view of the importance of G-proteins in the transduction of muscarinic receptor-mediated response, we have investigated the expression of three G-protein α-subunit mRNAs at various times after lesion of the nucleus basalis. Further, in view of the observations that both changes in c-fos and ODC mRNA and PPI responsiveness are highly sensitive to treatment with the *N*-methyl-D-aspartate (NMDA) glutamate receptor antagonist, MK-801, we have examined the effect of this agent on the expression of G-protein mRNAs.

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2. MATERIALS AND METHODS

Male CFY rats (wt. 250 ± 25 g at time of surgery) were anaesthetized with Sagatal (pentobarbitone) at a dose of 90–100 mg/kg and placed in a stereotaxic frame. Unilateral lesion of the right nucleus basalis was carried out by injection of the excitotoxin, kainate (1 μg in 0.5 μl saline, pH 7.0) into the nucleus basalis (co-ordinates: AP +1.2, lateral +2.7, with respect to bregma: incisor bar at 5 mm above the intra-aural line) at a depth of 8 mm below the skull surface over a

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period of 1 min [3]. The needle was left in place for 5 min to reduce reflux of kainate. Sham-operated animals were treated as above, but using saline vehicle for injection.

Rats were sacrificed by stunning and cervical dislocation at 1, 3, 7 and 28 days after kainate injection. Frontoparietal cortex ipsilateral and contralateral to the site of injection and cerebral cortex, caudate and liver from unoperated animals were dissected, frozen immediately in liquid nitrogen and stored at −70°C until required.

Total RNA was isolated from tissue samples by acid guanidinium thiocyanate/phenol/chloroform extraction [9]. The resulting RNA was analysed by Northern blotting and slot blotting as previously described [7].

The following probes were used to detect G-protein α-subunit mRNAs on Northern and slot blots: a 1.7 kb *Eco*RI fragment of pGI13 derived from a cDNA sequence encoding for $G_i\alpha$; a 1.2 kb *Eco*RI fragment of pGI23 derived from a cDNA sequence coding for $G_o\alpha$ and a 1.1 kb fragment of pGI11L derived from a cDNA sequence coding for $G_s\alpha$. All cDNAs were isolated from the rat C6 glioma cell line [10] and were kindly donated by Dr Itoh. An 800 bp cDNA fragment of β-tubulin, derived from a human foetal brain library [11], was used as a reference probe.

Northern blot filters were pre-incubated in hybridisation buffer (50% formamide, 5 × SSPE (0.9 M NaCl, 0.05 M sodium phosphate, pH 7.4, 5 mM EDTA), 5 × Denhardt's solution (0.1% bovine serum albumin, 0.1% Ficoll, 0.1% polyvinylpyrrolidone), 0.5% (w/v) sodium dodecyl sulphate, 100 μg/ml salmon sperm DNA) for 2 h at 42°C. Filters were then hybridised overnight at 42°C in hybridisation buffer containing 5–7 × 10^7 dpm labelled to a specific activity of 2.5–3.5 × 10^9 dpm/μg with [α-^{32}P]dCTP using the oligolabelling method of Feinberg and Vogelstein [12,13]. Blots were washed with 1 × SSC, 0.1% SDS for 30 min at 65°C followed by 0.1 × SSC, 0.1% SDS for 30 min at 65°C and exposed to pre-flashed Hyperfilm-MP (Amersham International PLC) with intensifying screens for 72 h at −70°C.

Quantitation of G-protein α-subunit mRNA levels relative to β-tubulin mRNA levels was carried out by slot blot analysis as follows: slot blot filters were hybridised with each [^{32}P]cDNA and then washed and autoradiographed as described above. Duplicate slot blot filters were hybridised with [^{32}P]β-tubulin cDNA as described above, then washed with 1 × SSC, 0.1% SDS for 30 min at 65°C followed by 0.1 × SSC, 0.1% SDS for 30 min at 65°C and autoradiographed as described above.

The intensity of hybridisation on slot blots was measured with a scanning densitometer (Joyce Loebl Chromoscan) at 530 nm. Results are expressed as a ratio of the G-protein mRNA signal : β-tubulin mRNA signal. Statistical analysis was carried out using the Student's *t*-test.

3. RESULTS

3.1. *Northern blot analysis of G-protein α-subunit mRNAs in unoperated animals*

G-protein mRNAs were initially characterised in cerebral cortex, caudate nucleus and liver from unoperated animals. Northern blot analysis revealed major bands of 2.0 kb, 2.4 kb and 4.5 kb for $G_s\alpha$, $G_i\alpha$ and $G_o\alpha$ mRNAs, respectively (Fig. 1). These sizes are consistent with those reported previously [14]. In this study $G_o\alpha$ mRNA was found only in neuronal tissue and was not detectable in liver.

3.2. *Effect of unilateral kainate injection on G-protein α-subunit mRNAs at 3 days*

Levels of the three G-protein α-subunit mRNAs were initially examined at 3 days after unilateral injection of kainate into the nucleus basalis as this is a period during which a maximal increase in PPI responsiveness is obtained [2]. RNA was isolated from cerebral cortex ipsilateral and contralateral to the kainate injection at 3 days and from unoperated animals. Levels of $G_i\alpha$, $G_o\alpha$ and $G_s\alpha$ mRNA were quantitated relative to β-tubulin mRNA levels by slot blot analysis. A significant decrease of 64% in the level of $G_o\alpha$ mRNA in ipsilateral cerebral cortex was observed at 3 days after kainate injection compared with contralateral cortex ($P < 0.001$) and of 42% when compared to the equivalent hemisphere from unoperated animals ($P < 0.001$) (Fig. 2). Levels of $G_i\alpha$ mRNA or $G_s\alpha$ mRNA in kainate injected animals were not significantly different from those in unoperated animals. These results indicate that $G_o\alpha$ mRNA is specifically reduced in response to unilateral kainate injection at 3 days.

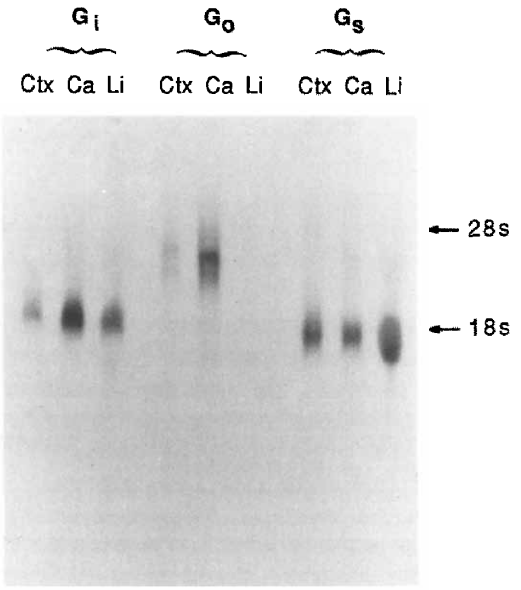

Fig. 1. Northern blot analysis of G-protein α-subunit mRNAs isolated from cerebral cortex, caudate and liver. Northern blots of RNA (20 μg/track) isolated from cerebral cortex (Ctx), caudate (Ca) and liver (Li) of control animals were hybridised with (a) [^{32}P]$G_i\alpha$-cDNA (Gi), (b) [^{32}P]$G_o\alpha$-cDNA, and (c) [^{32}P]G_s-cDNA.

3.3. *Time course of changes in $G_o\alpha$ mRNA*

The time course of changes in the level of $G_o\alpha$ mRNA was studied by slot blot analysis for a period of up to 28 days after kainate or vehicle injection into the nucleus basalis. The maximal decrease in $G_o\alpha$ mRNA was detected at 3 days after kainate injection where the level of $G_o\alpha$ mRNA in ipsilateral cortex as a ratio of that in contralateral cortex was significantly reduced by 40% ($P < 0.005$) compared to that in sham-operated animals in which vehicle only was injected into the nucleus basalis (Fig. 3). Thereafter levels of $G_o\alpha$ mRNA in ipsilateral cortex as a percentage of those in contralateral cortex returned to the baseline value by 28 days after kainate injection. No significant effect of the lesion was detected at 1 day. The levels of $G_o\alpha$ mRNA

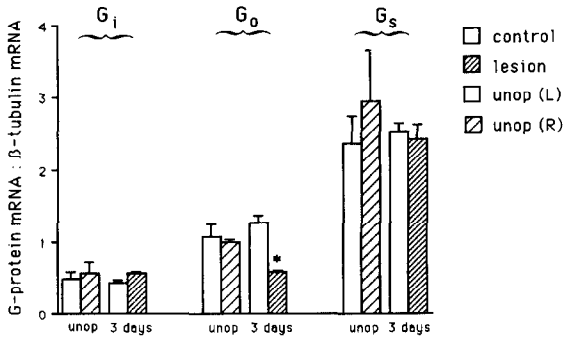

Fig. 2. Effect of unilateral kainate injection on G-protein α-subunit mRNAs. Slot blots of RNA isolated from ipsilateral (lesion) and contralateral (control) cerebral cortex at 3 days after unilateral kainate injection and from the equivalent hemispheres (left (L) and right (R)) of unoperated animals were hybridised with [^{32}P]$G_i\alpha$ cDNA (G_i), [^{32}P]-$G_o\alpha$ cDNA (G_o) and [^{32}P]$G_s\alpha$ cDNA (G_s). Levels of G-protein mRNA are expressed relative to β-tubulin mRNA levels. Results are means ± SE for 5 animals in the unoperated groups and 7 animals in groups injected with kainate. *Indicates that, at 3 days, unilateral kainate injection into nucleus basalis caused a decrease in the level of G_o mRNA in ipsilateral cerebral cortex compared with contralateral cortex ($P < 0.001$) and compared with the equivalent hemisphere in unoperated animals ($P < 0.001$).

in cerebral cortex of sham-operated animals in which vehicle only was injected into the nucleus basalis showed no significant changes relative to unoperated control animals.

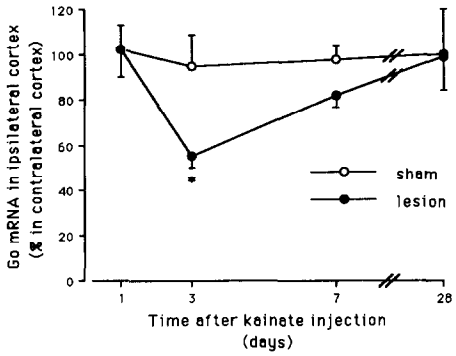

Fig. 3. Time course of the effect of unilateral kainate injection on levels of $G_o\alpha$ mRNA in cerebral cortex. $G_o\alpha$ mRNA was measured in cerebral cortex at 1, 3, 7 and 28 days after injection of vehicle (sham) or kainate (lesion) into the nucleus basalis. Levels of G_o mRNA were quantitated relative to levels of β-tubulin mRNA by slot blot analysis. Results are expressed as levels of G_o mRNA in cortex ipsilateral to the site of kainate injection as a percentage of levels of G_o mRNA in contralateral cortex. Values are means ± SE for 5 animals in all groups except for the groups of lesioned animals at 1, 3 and 7 days where $n = 11$. *Indicates that unilateral injection of kainate caused a significant decrease in G_o mRNA in lesioned cortex compared with sham-operated animals in which vehicle only was injected into the nucleus basalis ($P < 0.005$). This result reflects a net decrease in the level of G_o mRNA in ipsilateral cortex since the level in contralateral cortex at 3 days was not significantly different from that in either hemisphere of unoperated animals. However, at 7 days the level of G_o mRNA in contralateral cortex was significantly increased by 24% ($P < 0.040$) compared with that in ipsilateral cortex. Hence the apparent decrease in the proportion of $G_o\alpha$ mRNA in ipsilateral cortex actually reflects a slight increase in the level of G_o mRNA in contralateral cortex at 7 days.

In order to confirm that the reduction in the level of $G_o\alpha$ mRNA in ipsilateral cerebral cortex was dependent on the activation of the ascending pathway from the nucleus basalis, and not due to local tissue damage around the site of needle entry, $G_o\alpha$ mRNA was measured in a strip of ipsilateral frontoparietal cortex in the immediate vicinity of the site of needle entry and in two strips of ipsilateral frontoparietal cortex immediately adjacent to this region. The level of $G_o\alpha$ mRNA was significantly reduced in all three of these regions compared to contralateral cortex at 3 days (Fig. 4). Since these regions coincide with the area of innervation from the nucleus basalis, the reduction in $G_o\alpha$ mRNA is likely to be initiated by kainate-induced excitation of this ascending pathway.

3.4. *Effect of MK-801 on the level of $G_o\alpha$ mRNA in cerebral cortex in response to kainate injection into the nucleus basalis*

The NMDA receptor antagonist MK-801 was tested for its effect on the $G_o\alpha$ mRNA response to unilateral kainate injection at 3 days. At a dose of 1 mg/kg, MK-801 administered 2 h after unilateral kainate injection, significantly attenuated the reduction in $G_o\alpha$

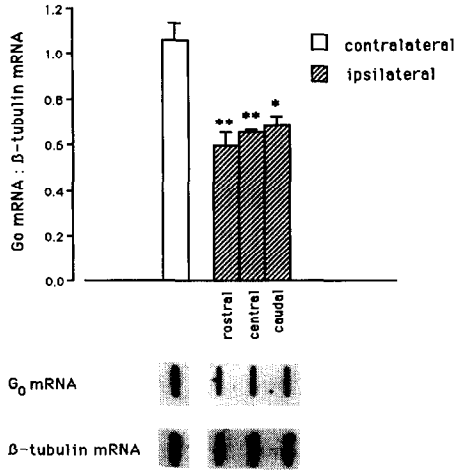

Fig.4. Localisation of G_o mRNA in cerebral cortex following unilateral injection of kainate into nucleus basalis. In order to determine whether the decrease in the level of G_o mRNA in ipsilateral cortex at 3 days was due to the excitation of the pathway by kainate injection into the nucleus basalis, rather than due to non-specific tissue damage around the site of needle entry, G_o mRNA was assayed in regions of cerebral cortex coinciding with areas receiving an innervation from the nucleus basalis. Levels of G_o mRNA were quantitated relative to levels of β-tubulin mRNA in a strip of ipsilateral cerebral cortex in the immediate vicinity of the site of needle entry (central), in two strips of ipsilateral frontoparietal cortex immediately adjacent to this region (rostral and caudal) and in contralateral cerebral cortex at 3 days. Values are means ± SE for 3 animals. * and ** indicate that unilateral injection of kainate caused a significant reduction in G_o mRNA ($P < 0.014$ and $P < 0.001$, respectively) in all three regions of ipsilateral cerebral cortex compared with contralateral cortex. Autoradiographs of representative slot blots loaded with x μg RNA probed with [^{32}P]$G_o\mu$ cDNA and [^{32}P]β-tubulin cDNA are shown below the histograms.

Table I

Effect of MK-801 on the reduction of G_o mRNA levels in cerebral cortex in response to unilateral injection of kainate into nucleus basalis

	G_o mRNA in cerebral cortex ipsilateral to kainate injection (% contralateral cortex)
Sham	95.4 ± 13.5
Vehicle	51.0 ± 5.8
MK-801 (1 mg/kg)	79.4 ± 8.9*

MK-801 at a dose of 1 mg/kg or vehicle (5% ethanol) was administered i.p. at 2 h after kainate injection into nucleus basalis. Results are expressed as the ratio of G_o mRNA/β-tubulin mRNA in the ipsilateral cortex as a percentage of the ratio of G_o mRNA/β-tubulin mRNA in contralateral cortex. Values are means ± SE for 5 animals in each group. *Indicates that MK-801 significantly attenuated the reduction in $G_o\alpha$ mRNA in cerebral cortex compared with vehicle treated animals ($P < 0.029$).

mRNA seen at 3 days by 64% ($P < 0.029$) (Table I). This result indicates that a major component of the reduction in $G_o\alpha$ mRNA in ipsilateral cerebral cortex is mediated by NMDA receptors.

4. DISCUSSION

G-Proteins are abundant in the nervous system, in keeping with the importance of transduction mechanisms in neuronal communication. Eight G-protein genes have been identified by molecular cloning of cDNAs and the three G-proteins which are most abundant in the mammalian brain, G_s, G_i and G_o, have been extensively characterised [10]. They are composed of three subunits, α, β and γ. The α subunit contains the guanine nucleotide binding site and the same β and γ subunits are common to many G-proteins. The molecular sizes of the α subunits are 45, 41 and 39 kDa for the G_s, G_i and G_o subunit, respectively. The $G_s\alpha$ subunit directly activates adenylate cyclase and is ADP ribosylated by cholera toxin. The $G_i\alpha$ subunit inhibits adenylate cyclase and both and $G_i\alpha$ and $G_o\alpha$ can be ADP-ribosylated by pertussus toxin in certain systems. $G_i\alpha$ and $G_o\alpha$ are also thought to have a direct effect on phospholipase C, K^+-channel gating and voltage-dependent calcium channels. The distribution of α subunit mRNAs in the mammalian brain has been characterised by in situ hybridisation, G_s, G_{i2} and G_o being more abundant than G_{i1} and localised especially in regions such as cerebral cortex [14], hippocampus and cerebellum. G_o differs from G_s and G_{i2} in that it is mainly localised to brain whereas G_s and G_{i2} are also abundant in other tissues. G-Proteins are involved in the three main responses of muscarinic receptors, inhibition of adenylate cyclase, opening of K^+ channels and the activation of phospholipase C which is affected in the experimental model investigated in the present study. Muscarinic agonist stimulation of PPI hydrolysis in rat cerebral cortex shows similar characteristics to that occurring in other tissues and G-protein involvement is indicated since guanosine 5′-(β-imido)-triphosphate (p[NH]ppG) and guanosine 5′-(γ-thio)-triphosphate (GTP[S]) potentiate the hydrolysis [15].

In this study, we have shown that lesion of the nucleus basalis causes a significant decrease in the level of $G_o\alpha$ mRNA in cerebral cortex. The effect is specific for this α-subunit and no similar change occurs in the expression of $G_i\alpha$ and $G_s\alpha$ mRNAs. This effect also has a similar time course to that shown for the increased PPI responsiveness and is transient in nature, decreasing to control levels by 1 month. Although a direct link between the decrease in $G_o\alpha$ and the increased PPI responsiveness has not yet been demonstrated, it might be speculated that $G_o\alpha$ mediates an inhibitory regulation of PPI responsiveness. Although G_o is extremely abundant in the cerebral cortex its precise function has not yet been elucidated. However, studies on *Xenopus* oocytes indicate that $G_o\alpha$ couples muscarinic receptors to PPI turnover [16] and an effect on voltage-dependent calcium channels has also been recently demonstrated. Noradrenaline, dopamine and γ-aminobutyric acid (GABA) inhibit the voltage-dependent calcium channel in dorsal root and sympathetic ganglia, which effect can be mimicked by intracellular guanine nucleotides and blocked by pertussis toxin. A similar effect can be demonstrated with opioid peptides in neuroblastoma cells where it has been specifically shown that the pertussis toxin block can be prevented by intracellular application of G_i and G_o, the latter effect of G_o being 10 times more potent than G_i [17]. This is further supported from the evidence that rat brain G_o is a substrate of pertussis toxin ADP-ribosylation [18]. The loss of an inhibitory regulation mediated through G_o which enhances calcium entry would facilitate PPI responses since depolarisation which induces Ca^{2+} entry is known to activate PPI turnover [19]. Hence the temporary suppression of G_o function would promote cholinergic action over the period when cholinergic function is depressed.

The nature of possible intermediary processes initiating a change in G_o expression is indicated from the effect of treatment with MK-801. Although treatment with MK-801 does not modify the neurotoxic effect of kainate on nucleus basalis cells and hence the subsequent development of a cholinergic deficit in cerebral cortex, this treatment does prevent the induction of c-fos and ODC mRNA and the increased postsynaptic responsiveness [2,6,7]. Thus a clear association is seen between the latter process and the decrease in the level of G_o mRNA seen here, indicating the involvement of an NMDA receptor in both processes. The NMDA receptor is directly linked to a cation channel and the mechanisms through which NMDA receptor activation could affect G_o expression are likely to involve intracellular events induced by NMDA receptor activation, e.g. Ca^{2+} flux. Injection of rat brain mRNA into *Xenopus* oocytes has shown evidence of coupling of the

metabotropic glutamate receptor, which is activated by glutamate or quisqualate, to a G-protein [20] and this system may well be activated during the excitatory phase induced by kainate injection.

These results indicate that a selective and transient change can occur in response to a lesion of the nervous system and suggest that regulation of G-protein expression offers a sensitive mechanism for a potentially useful adaptation. A differential regulation of $G_s\alpha$ and $G_i\alpha$ mRNA and protein also occurs in brain after corticosterone treatment with no effect on $G_o\alpha$ mRNA [21], indicating a further example of an adaptive response depending on a specific regulation of G-protein expression.

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