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Differential effect of antidepressants on Gqα-protein levels in rat brain

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ABSTRACT

Background: Heterotrimeric G proteins play a pivotal role in post receptor information transduction. These proteins have been implicated in the pathophysiology, diagnosis, and treatment of depression. **Aims and Objectives:** The aim of the present study was to examine the effect of chronic antidepressants treatment on the Gqα-protein levels in rat brain.

Materials & Methods: Density of $G_{q\alpha/11\alpha}$-protein levels was measured in cortex and cerebellum of rats treated with amitriptyline (AMI), desipramine (DMI) and fluoxetine (FLX) and imipramine (10 mg/Kg body wt), for 30 days, using Sodium Dodecyl Sulphate – Polyacrylamide Gel Electrophoresis (SDS-PAGE) and western blotting.

Results:In cortex, a significant decrease was observed after AMI treatment (28%, $p<0.0001$), followed by DMI (17%, $p<0.0001$) and CMI (19%, $p<0.0001$). In contrast a significant increase was observed after IMI treatment (14%, $p<0.001$) but there was no change in fluoxetine treatment. In cerebellum, a significant increase in G_q-protein levels was observed after treatment with all the above drugs. A very high increase was observed after CMI treatment (114%, $p<0.0001$) as compared to AMI (59%, $p<0.0001$), DMI (45%, $p<0.0001$) fluoxetine (28%, $p<0.0001$) and IMI (42%, $p<0.0001$).

Conclusion: The results suggest that chronic antidepressant (AD) treatment decreases the expression of cortical $G_{q\alpha/11\alpha}$-protein levels in contrast to cerebellum where these proteins are showed increased expression. However IMI treatment in cortical $G_{q\alpha/11\alpha}$protein levels predominantly over expressed by IMI treatment. The region-specific expression of $G_{q\alpha/11\alpha}$-protein levels which occurs after prolonged AD treatment, may underline the therapeutic mechanism of action.

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KEY WORDS

G-proteins, Antidepressants, SDS-PAGE, Western blotting

INTRODUCTION

In recent years research has focused upon the effects of chronic administration of ADs on various aspects of neuronal function, demonstrating alterations in the density and/or sensitivity of several neurotransmitter receptor systems[1]. Although there is considerable conflicting evidence regarding the effects on various receptor systems such as noradrenergic, serotonergic, GABAergic, dopaminergic etc., chronic administration of a variety of ADs appear to downregulate or desensitize the α-adrenergic and 5-HT receptors in rat forebrain and enhance the synaptic efficacy of serotonergic neurotransmission via 5-HT_{1A} receptors in rat hippocampus. Even these effects, however, do not completely explain the clinical efficacy of all ADs, and the dissociation between receptor number and their functional responsiveness has led to the investigation of possible post receptor sites of actions of these drugs. Neurotransmitter functions may be altered through the regulation of intracellular signaling and ADs may be effective not because they are "noradrenergic" or "serotonergic" agents per se, but because they modulate converging postsynaptic signals generated in response to multiple endogenous neurotransmitters, including NE and 5-HT. In this context the signal transducing G-proteins, which play a major role in the amplification and integration of signals in the CNS, are in a unique position to affect the functional balance between neurotransmitter systems representing attractive potential targets for AD drug effects.

Although G-proteins are present in all eukaryotic cells and control various metabolic, humoral and developmental functions, they are especially important in the CNS, where they serve critical roles of amplifying and generating neuronal signals and then transmitting these integrated signals to effectors, thereby forming the basis for a complex information processing network. Thus, given their widespread and critical roles in the regulation of neuronal function, recent research has begun to focus upon the elucidation of the role of G-proteins in the etiology and pathophysiology of various psychiatric disorders and also in the pharmacology of ADs.

MATERIALS & METHODS

Animals and administration of drugs

Adult male Sprague-Dawley rats weighing 200-250 g, were used for all the experiments. Animals (10 for each drug), procured from Central Animal Research Facility (CARF), NIMHANS, were housed in cages (four rats per cage) and exposed to regular day/night period with food and water ad libitum. DMI (10 mg/Kg body wt), AMI (10 mg/Kg body wt), IMI (10 mg/Kg body wt and Fluoxetine (10 mg/Kg body wt), were injected intraperitoneally, once daily, for a period of 30 days. As the clinical efficacy of ADs has been observed over 3-6 weeks of administration, their effects on $G_{q\alpha/11\alpha}$-protein were studied after chronic administration for 30 days. Control rats received 0.5 ml of saline, by same route for the same period. All the animals were killed by decapitation under ether anaesthesia, 24 hrs after the last injection. Brains were removed on ice-cold Petri dish, cerebral cortex and cerebellum dissected out and used for membrane preparation. Tissues obtained from three rats were pooled for receptor binding assay.

Membrane preparation.

Crude membrane pellet was obtained from brain tissue, homogenized in 20 volumes of Tris-HCl buffer (50 mM, pH 7.4) containing 0.32 M sucrose, following the procedure described by Creese and Snyder [2] and as described earlier[3]. The pellet was resuspended in 50 mM sodium-potassium phosphate buffer (pH 7.4) for $G_{q\alpha/11\alpha}$-protein expression studies. Protein concentration was estimated by Lowry's method[4] and made to 1 mg/ml using respective buffers.

$G_{q\alpha/11\alpha}$-protein estimation.

The membrane protein samples from different regions of the rat brain were prepared and SDS-PAGE was carried out as described by Schagger H, von JG [5]. After the electrophoretic run, the gel was equilibrated in blotting buffer for 15 minutes with gentle shaking. The PVDF membrane of appropriate size was suspended in methanol, to equilibrate for about 5 minutes, taking care to avoid trapping of air bubbles. The methanol saturated PVDF membrane and Whatman No. 3 filter papers, cut according to the size of the gel, were also equilibrated in blotting buffer for 15 minutes. The Whatman No. 3 filter papers, PVDF membrane and the gel were placed in the semi-dry electroblotting apparatus, avoiding the air bubbles, according to the manufacturer's instructions and the electrodes were connected to a power supply unit. The proteins were electro blotted for 2 hours at 30V/150 mA.

The PVDF membrane was removed after electroblotting and the transfer of protein bands were visualized by staining with Ponceau S for 5 minutes. Proteins appear as light pink bands on the PVDF membrane. The molecular weight marker proteins were marked on the PVDF membrane and then the membrane was washed with distilled water to destain the protein bands. The non-specific sites on the PVDF membrane were then blocked by immersing in blocking buffer for 30 minutes with gentle shaking. The membrane was then incubated overnight at 4^0C in primary antibody solution containing both Rabbit Anti-$G_{s\alpha}$ and Rabbit Anti- $G_{q\alpha/11\alpha}$ antibodies (1: 1000 dilution). After the overnight incubation, the membrane was washed with the blocking buffer (4 X 15 minutes each). The membrane was then incubated in secondary antibody solution containing 1:2000 diluted Goat anti-rabbit IgG $F(ab')_2$-peroxidase conjugate for 1 hour and washed again with blocking buffer (4 X 15 minutes each). The PVDF membrane was then incubated with substrate chromogen solution till the intensity of the protein bands was clear (~ 15 minutes). The colour reaction was then stopped by removing the PVDF membrane from chromogen solution and washing with distilled water. The $G_{q\alpha/11\alpha}$ protein bands quantified by software which present in Geldoc machine (Bio-Rad) [5].

Data analysis

The statistical analysis was done by using SPSS software version 10.

RESULTS

The synaptosomal membrane proteins (100 µg) obtained from AMI, CMI, DMI, IMI and Fluoxetine treated rat cortex and cerebellum were resolved on 10% SDS-gels. Then $G_{q\alpha/11\alpha}$-proteins were visualized by using specific antibodies and chromogen substrate. The immunoreactivity of these proteins in drug treated rats were compared with that of the control and expressed as percentage of increase or decrease in the trace quantities of each protein (Table 1).

In cortex, a significant decrease was observed with $G_{q\alpha/11\alpha}$-protein immunoreactivity in all the drug treated groups. A significant decrease was observed after AMI treatment (28%, $p<0.0001$), followed by DMI (17%, $p<0.0001$) and CMI (19%, $p<0.0001$). Interestingly a significant increase was observed after IMI treatment (14%, $p<0.001$) (Fig 1A & 2). There was no significant

change in G-protein levels in cortex after fluoxetine treatment.

In cerebellum, a significant increase in $G_{q\alpha/11\alpha}$-protein levels was observed after treatment with all the above drugs. A very high increase was observed after CMI treatment (114%, $p<0.0001$) as compared to AMI (59%, $p<0.0001$), DMI (45%, $p<0.0001$) and IMI (42%, $p<0.0001$) (Fig 1B & 3). Interestingly fluoxetine treatment also showed a significant increase (28%, $p<0.0001$) in G-protein level, but lesser than other drugs.

DISCUSSION

This study demonstrates that chronic treatment of rats with different categories of antidepressants including Desipramine (NE uptake inhibitor), Amitriptyline, Clomipramine (mixed NE/serotonin (5HT) uptake inhibitor, Imipramine and Floxetine (Selective serotonin (5HT) reuptake inhibitor alter the expression of Gqα proteins in cortex and cerebellum of the rat. This study supported by Charney et al., [6]. In contrast to this study all these drugs fails to alter the protein and mRNA levels of Gαs, Gαi, and Gβ subunits in LC of rat brain [7]. It has been reported that G-protein functions may contribute to the complex neuro adaptive mechanisms involved in the clinical actions of ADs. Previous studies suggest G-proteins as the molecular targets of many ADs [8-10]. Rasenick and colleagues have demonstrated that certain AD treatments increase the coupling of G_s to AC in rat cerebral cortex [11, 12]. Chronic treatment with AD drugs has also been shown to exert differential effects on Gα-mRNA and protein expression in rat brain, thus modifying signal transduction [8].

The mechanism of action of psychoactive drugs and the pathophysiology of various neuropsychiatric disorders related to changes in signal transduction via G-proteins has also been reported [10]. Inactivation of G_i-proteins has been shown to induce an AD-like effect in the mouse forced swimming test model. The antidepressant effect obtained was similar to that produced by AMI and CMI [13]. This resulted in the focus on the relevance of G-proteins or receptor-G-protein coupling as a potential target site for the action of many psychopharmacological agents. Majority of previous studies on effect of ADs have been restricted to the study of receptors and second messenger changes [14, 15]. In the present study the changes in G-proteins, which are important intermediates in receptor mediated signal transduction, were determined in different regions of rat brain.

The present study showed a significant decrease in the immunolabeling of cortical $G_{q\alpha}$-protein after AMI (28%), DMI (17%) and CMI (19%) treatment, whereas a significant increase was observed after IMI treatment (14%). Interestingly FLX treatment did not show any significant change in AR density in cortex. In contrast to this, several studies have shown no alteration in G_q-protein levels after AD treatment in depressed patients and animal studies [8, 16]. The potency with which ADs decreased $G_{q\alpha/11\alpha}$-protein in cortex was in the order of AMI (28%) > CMI (19%) > DMI (17%). The potency with which α_1-ARs were down regulated by ADs in cortex was also same.

In cerebellum, a significant increase in the $G_{q\alpha}$-protein was observed after CMI (114%), AMI (59%), DMI (45%), IMI (42%) and FLX (28%) treatment. In contrast, no significant effects on G alpha subunit levels following acute desipramine and moclobemide administration were observed in vitro [17].

It has been suggested that changes in $G_{q\alpha}$-protein may not be coordinately regulated in various tissues including brain [18, 19]. For this reason, we also measured the $G_{q\alpha}$-protein in cortex and cerebellum after chronic antidepressant treatment. In both cases expression of $G_{q\alpha}$-protein altered. These findings are in line with the previous reports showing

similar levels of $G_{q\alpha}$-protein expression in the cortex of rats treated with chronically with imipramine, desipramine and clomipramine compared with controls [20].

Table 1

Percentage change in trace quantities of $G_{q\alpha/11\alpha}$-proteins in cortex and cerebellum of AD treated rats

Drugs	Cortex	Cerebellum
	$G_{q\alpha/11\alpha}$ (%)	$G_{q\alpha/11\alpha}$ (%)
Control	100	100
AMI	(-) 28*	(+) 59*
DMI	(-) 17*	(+) 45*
CMI	(-) 19*	(+) 114*
IMI	(+) 14**	(+) 42*
FLX	(-) 11	(+) 28*

The $G_{q\alpha/11\alpha}$-protein levels, estimated by immunoblotting in membranes obtained from cortex and cerebellum of control and AD treated rats, were calculated using 'Quantity-one' software and expressed as the percentage of increase (+) or decrease (-) in trace quantities of respective proteins in AD treated groups as compared to controls.
** p < 0.0001, ** p < 0.001*

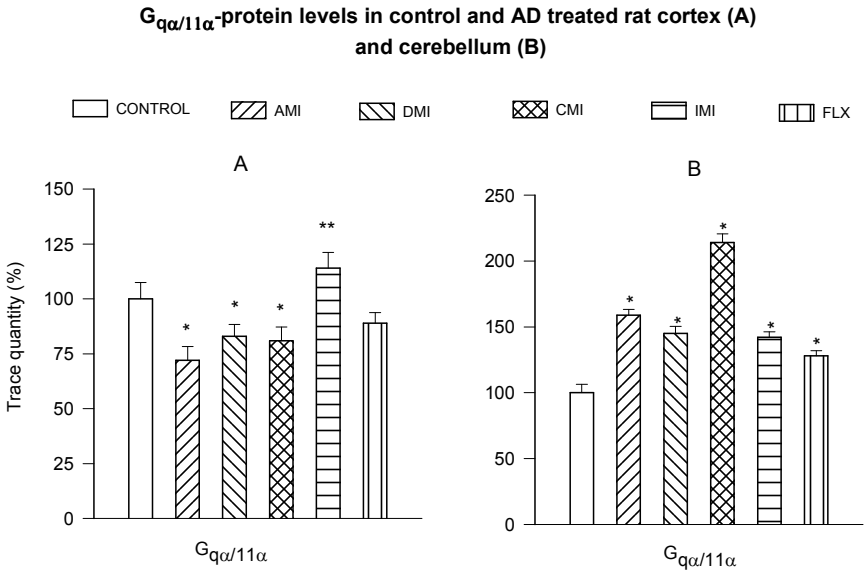

Fig 1. The $G_{q\alpha/11\alpha}$-protein levels were studied by using immunoblotting in membranes obtrained from AMI, DMI, CMI, IMI and FLX treated rat cortex (A) and cerebellum (B) and the percentage of trace quantity in each group is plotted. The values are mean and SD of 2-3 experiments.

*p<0.0001, **p<0.001

Fig 2
G_q- proteins in cortical membranes of control and AD treated rats

A representative immunoblot of $G_{q\alpha/11\alpha}$ – protein (42 kDa) using specific polyclonal rabbit antiserum, in the cortical membranes obtained from saline, AMI, FLX, CMI, DMI and IMI treated rats.

Fig 3
G_q- proteins in cerebellar membranes of control and AD treated rats

A representative immunoblot of $G_{q\alpha/11\alpha}$ – protein (42 kDa) using specific polyclonal rabbit antiserum, in the cerebellar membranes obtained from saline, FLX, AMI, CMI, IMI and DMI treated rats.

CONCLUSION

We conclude our study that differential and region-specific alteration of $G_{q\alpha}$-protein expression after treatment of various antidepressants may be considered an important factor in the achievement of therapeutic efficacy of ADs after chronic treatment. Further studies to explore the effect of ADs on receptor linked second messenger system along with alterations in CREB and BDNF expression might improve our knowledge regarding the pathophysiology of depressive disorders and mechanism of action of ADs.

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