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Imbalance of the G_s and $G_{i/o}$ function in post-mortem human brain of depressed patients

Short Communication

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Summary. The amounts of various G protein subunits in postmortem brain samples from the parietal and temporal cortices were the same in controls and depressive patients as demonstrated by immunoblotting. However, photoaffinity GTP labeling (AAGTP) of $G_{i/o}\alpha$, but not $G_s\alpha$, was significantly increased in depressives in both cortex regions. Furthermore, the ratio of $G_s/G_{i/o}$ AAGTP incorporation revealed a significant reduction in depressives in these regions. The present findings suggest that an imbalance of second messengers via G protein function may be involved in the pathophysiology of depression.

Keywords: Depression, GTP binding protein, human brain, photoaffinity labeling, signal transduction.

Introduction

GTP (G) binding proteins, which are composed of a common heterotrimetric structure consisting of α-, β-, and γ-subunits, represent a class of signal transducing proteins related to the coupling of receptors to their intercellular effector subunits. Stimulatory G protein (G_s) and inhibitory G protein (G_i) are responsible for stimulation and inhibition of adenylate cyclase, respectively; G_o might regulate voltage-sensitive calcium channels; G_{i3} has an important role in the control of the K^+ channel; G_s also activates a voltage-gated calcium channel and; G_i and G_o-like proteins or G_q can regulate the phophoinositide metabolism (for reviews see Birnbaumer, 1990; Simon et al., 1991). Recently, the mechanism of action of psychoactive drugs and the pathophysiology of various neuropsy-

chiatric disorders has been related to changes in signal transduction via G proteins (Manji, 1992).

Imbalances in the second-messenger system related to G proteins have been hypothesized in the pathophysiology of affective disorders (Wachtel, 1989, 1990). Furthermore, Young et al. (1991) reported that $G_s\alpha$ subunit levels are elevated in bipolar affective disorder, indicating that Gs-mediated signal transduction may be increased in this disorder. However, few studies have directly investigated postreceptor transmembrane signaling mechanisms in the human post-mortem brain in unipolar depressive patients.

The present study examines the qualitative and quantitative alterations of G proteins utilizing western blotting with polyclonal antibodies against specific G protein subunits and photosensitive GTP labeling in membrane preparations from parietal and temporal cortical regions in post-mortem human brains obtained from depressives and controls which were matched with respect to age and post-mortem delay.

Materials and methods

The procedure used for brain dissection was described in detail by Gsell et al. (1993). Tissue samples were obtained from 12 unipolar depressives (without manic episodes) and 12 controls who were free of any neurological or psychiatric disorders. For the diagnosis of depressed patients, we used DSM-III R criteria for major depression (n = 8) and dysthymia (n = 4). Three of the 8 major depression patients attempted suicide. Four cases were on antidepressants before death. A detailed characterization of patients and controls is shown in Table 1.

Synaptic membrane-enriched fractions were prepared from human cerebral cortex as described (Ozawa and Rasenick, 1989, 1991) and stored at − 80 °C until use. Gel electrophoresis and immunoblotting were carried out according to the methods of Laemmli (1970) and Towbin et al. (1979), respectively, with minor modifications for post-mortem human brain. First, 25–50 µg of membrane protein was solubilized in 25–50 µl of Laemmli sample buffer (50 mM Tris-HCl, pH 6.8) containing 10% glycerol, 3% SDS, and 50 mM DTT. Membrane samples of equivalent protein amounts (5–10 µg) were subjected to 10% SDS-polyacrylamide gel electrophoresis. Proteins were electrophoretically transferred to nitrocellulose membranes. After transfer, the membranes were blocked for 1 h in TBS-T buffer [10 mM Tris-HCl (pH 7.5), 500 mM NaCl, 0.1% Tween-20] containing 3% BSA. They were then incubated with a dilution of 1 : 5000 polyclonal rabbit antiserum against the various G protein subunits [RM/1, AS/7, GC/2, QL, and SW/1 specific to $G_s\alpha$ {low and high molecular weight species}, $G_{i1+2}\alpha$, $G_o\alpha$, $G_q\alpha$, and Gβ-subunits, respectively; all are commercially available from NEN Dupont (Boston, MA)]. After incubation, nitrocellulose membranes were washed three times in TBS-T buffer before incubation for 1 h with a dilution of 1 : 5000 of the second antibody [anti-rabbit IgG HRP-linked $F(ab')_2$] (Amersham, Oakville, Ont.). Immunoreactivity was detected with an enhanced chemiluminescence (ECL) Western blot detection system (Amersham) followed by exposure to ECL HYPER film (Amersham). The developed autoradiographs were analyzed by laser densitometry. Under our immunoblotting conditions, the scanned signals of the various Gα- or β-subunits were proportional to the amount of protein applied to the gel within the range of 1–30 µg in human brain and rat brain. The G protein immunoreactivity was normalized against a pooled rat brain reference standard.

[^{32}P]-P^3(4-azidoanilide)-P^1-5′ GTP (AAGTP) was synthesized by the method of Pfeuf-

Table 1. Quantitative and qualitative alterations of G proteins in postmortem brains of controls (C) and depressive patients (D)

	Parietal cortex		Temporal cortex	
	C	D	C	D
Subject characterization				
n	7	7	7	8
Sex (male/female)	2/5	2/5	3/4	3/5
Age (years)	72.9 ± 4.7	79.0 ± 3.7	74.1 ± 2.9	77.4 ± 2.3
PMTD (hours)	13.1 ± 5.0	6.3 ± 2.4	12.4 ± 3.6	6.6 ± 1.1
Storage time (years)	3.2 ± 0.6	4.0 ± 0.3	2.0 ± 0.5	4.2 ± 0.4
Major depression/ dysthymia	–	6/1	–	4/4
Immunoblotting (normalized immunoreactivity against a rat brain standard)				
GsH	1.06 ± 0.10	0.76 ± 0.13	0.98 ± 0.10	0.73 ± 0.32
GsL	1.02 ± 0.09	0.91 ± 0.17	0.98 ± 0.23	0.87 ± 0.43
Gi	1.12 ± 0.09	0.88 ± 0.06	1.14 ± 0.22	1.07 ± 0.11
Go	1.06 ± 0.11	1.07 ± 0.10	1.16 ± 0.07	1.10 ± 0.06
Gq	0.83 ± 0.13	0.95 ± 0.06	1.01 ± 0.16	0.95 ± 0.08
Beta	1.06 ± 0.10	1.02 ± 0.03	1.09 ± 0.37	1.05 ± 0.08
AAGTP binding (fmol/mg protein)				
G_s	375.0 ± 49.0	324.9 ± 57.0	309.6 ± 37.2	254.3 ± 20.4
$G_{i/o}$	1305.3 ± 97.7	1926.0 ± 121.4*	1158.6 ± 158.0	1826.0 ± 118.9*
Ratio of G_s to $G_{i/o}$	0.30 ± 0.05	0.17 ± 0.33*	0.28 ± 0.01	0.14 ± 0.02**

Tissue samples from the different regions were not available from all brains. Therefore, the data are listed for each region separately. There were no significant differences in age, PMDT (postmortem delay time) and storage time of m/f tissues in either parietal or temporal cortex region between controls (C) and depressive subjects (D). Mean data are given ± SEM. *, ** Significant differences compared to control subjects ($p < 0.05$, $p < 0.01$). GsH or GsL is high or low molecular weight species of stimulatory G protein (Gs), respectively

fer (1977). AAGTP binding experiments were performed as described by Ozawa and Rasenick (1989, 1991). Brain membranes (100–150 µg) were washed and resuspended in 2 mM HEPES buffer (pH 7.4) including 1 mM $MgCl_2$. Membrane suspensions were incubated with [^{32}P]-AAGTP for 5 min at 23 °C and the reaction was terminated by dilution with the above ice-cold buffer followed by centrifugation at 20,000 × **g** for 10 min to remove unbound [^{32}P]-AAGTP. Membranes were washed again and resuspended in the same buffer. Membrane suspensions were irradiated at 254 nm with a 9 w Spectroline UV lamp for 3 min on ice at a distance of 4 cm. The reaction was quenched with ice-cold 2 mM HEPES buffer including 1 mM $MgCl_2$ and 4 mM DTT. Samples were electrophoresed in 10% SDS/polyacrylamide gels by the procedure of Laemmli (1970). After electrophoresis, gels were stained with Coomassie brilliant blue, dried and autoradiographed. Based on AAGTP binding to G proteins previously identified by Hatta et al. (1986), radioactive bands corresponding to $G_s\alpha$ or $G_{i/o}\alpha$ (Giα plus Goα) were excised from the dried gel, immersed in a 4-ml scintillation cocktail and subjected to scintillation counting. Mean values are given ± SEM. Differences between controls and depressives were evaluated with the Student's t-test.

Results

Figure 1 a shows representative immunoblots from depressives and controls of G protein α- and β-subunits which migrated at expected molecular masses ($G_s\alpha$: 52 and 45 kDa, $G_{i1\text{ and }2}\alpha$: 40–41 kDa, $G_o\alpha$: 39 kDa, $G_{q\text{ and }11}\alpha$: 42 kDa, and Gβ-subunits: 35–36 kDa, respectively).

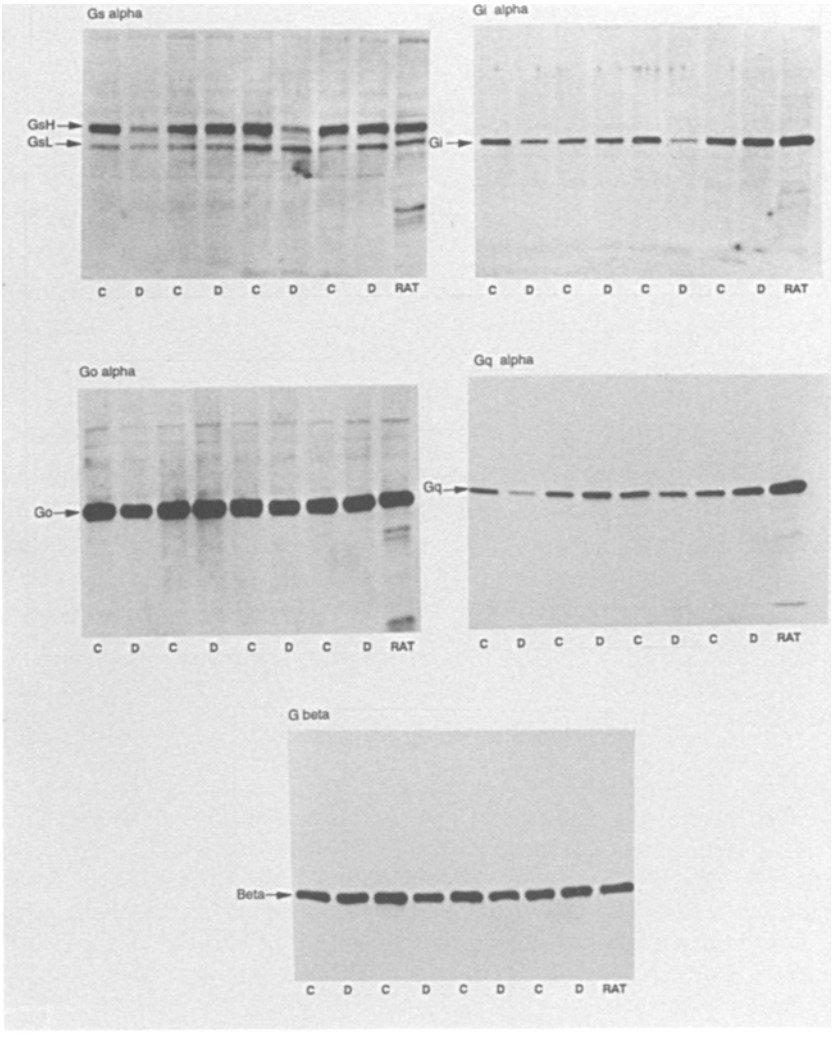
Table 1 summarizes quantitative and qualitative changes of G proteins in parietal and temporal cortex areas from brains of controls and depressives. There were no significant differences in immunoreactivities of any G protein subunits in either cortex region between depressives and controls.

As reported previously for rat brain (Hatta et al., 1986; Ozawa and Rasenick, 1989, 1991), we were able to identify both 42 kDa and 39–40 kDa proteins as $G_s\alpha$ and $G_{i/o}\alpha$ (mixed band of $G_i\alpha$ and $G_o\alpha$), respectively, in human brain membranes by AAGTP labeling (Fig. 1 b). AAGTP binding to $G_{i/o}\alpha$ but not $G_s\alpha$ showed significant increases in depressives compared with that in controls in both cortex areas. In addition, the ratio of $G_s/G_{i/o}$ AAGTP incorporation revealed a significant reduction in depressives in these regions.

Discussion

In the present report we have shown that there were no quantitative alterations in any G protein subunit in temporal and parietal cortices between controls and depressives. Recently some studies reported alterations in the concentrations or biosynthesis of certain G protein subunits at the gene-expression level in psychiatric diseases or after treatment of experimental animals with psychotropic drugs (Lesch et al., 1991; Young et al., 1991; Li et al., 1991) suggesting that the responsiveness of the G protein second-messenger system might also change with alterations of the protein or mRNA concentrations. A change in the protein or mRNA amount, however, does not always imply defective protein function, but rather often appears to reflect compensatory consequences. Therefore, we introduced the hydrolysis-resistant photoaffinity GTP analog, AAGTP to elucidate the real functional qualities of G proteins. This compound has been used to identify adenylate cyclase activation associated with G proteins in synaptic membranes. We have used this compound as a functional probe as well as a photoaffinity compound (Hatta et al., 1986; Ozawa and Rasenick, 1989, 1991). In contrast to the immunoblotting study, a significant elevation in AAGTP binding to $G_{i/o}\alpha$ was seen in depressives compared with controls in temporal and parietal cortices. In addition, the ratio of $G_s/G_{i/o}$ AAGTP incorporation was significantly decreased in depressives.

One might explain the present observations as resulting from the effects of antidepressants or lithium since these drugs may influence G protein-related signal transduction (Ozawa and Rasenick, 1989, 1991; Lesch et al., 1991, 1992; Avissar et al., 1992). However, no cases in the present study took lithium, and elevations of AAGTP binding to $G_{i/o}$ and reductions of the ratio of $G_s/G_{i/o}$ AAGTP incorporation were seen in depressives independent of previous antidepressant medication.



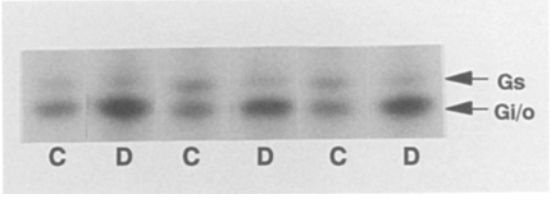


Fig. 1. a A representative immunoblotting of the several G protein subunits using polyclonal rabbit antiserum against G protein subunits (RM/1, AS/7, GC/2, QL, and SW/1 specific to $G_s\alpha$, $G_{i1\text{ and }2}\alpha$, $G_o\alpha$, $G_{q\text{ and }11}\alpha$: and G$\beta$-subunits, respectively) in temporal cortex. Control (C) and Depression (D) (four different subjects in each group). Detailed methods are explained in the text. The exposure time to ECL HYPER films was 30 sec–1 min except for $G_s\alpha$ which was exposed for 3–10 min. **b** A representative autoradiogram SDS-PAGE analysis of [^{32}P]-AAGTP-labeled human synaptic membranes in parietal cortex. Control (C) and Depression (D) (three different subjects in each group). Detailed methods are described in the text

A lack of balance in the second messenger signaling of affective disorders has suggested that affective disorders may be caused by a functional disproportion of the two major second signaling systems, with depression resulting from hypofunction of adenylate cyclase pathways with absolute or relative dominance of the phospholipase C (PLC) pathways, and mania resulting from contrasting conditions for depression, hypothesized from the mechanisms of action of therapeutic agents and peripheral tissues studies in affective disorders (Wachtel, 1989, 1990). Avissar and Schreiber (1992) also proposed that hyperfunction of G proteins, either as a trait or as a state function, leads to characteristics of a manic or depressive state caused by instability in the activities of protein kinases A and C.

Adenylate cyclase is regulated by the balance between G_s and G_i functions. In addition, G_i and G_o-like proteins, which are ADP-ribosylated substrates of pertussis toxin, are presumed to activate the PLC system since this toxin affects the phosphoinositide metabolism (Birnbaumer, 1990). Thus, the present study showing a decrease in the proportions of G_s and $G_{i/o}$ in depressives suggests that depression may be a state occurring during hypofunction of the adenylate cyclase system and hyperfunction of the PLC system through altered equilibrium of G protein functions.

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