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# INFLUENCE OF ANTI-TUBULIN ANTIBODIES ON MUSCARINIC RECEPTOR MODULATION OF G PROTEIN GTPase ACTIVITY IN RAT STRIATUM

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**Abstract**—To understand the role of tubulin, an integral component of neural membranes, in signal transduction processes, the influence of anti-tubulin antibodies on the low $K_m$ GTPase activity associated with transducer G proteins was examined in rat striatum. Membranes were prepared from striatum by conventional procedures, and the low $K_m$ GTPase activity (EC 3.6.1.-) was determined using [$\gamma$-$^{32}$P]GTP at 37° in an ATP-regenerating buffer containing 0.2 to 2.0 $\mu$M unlabeled GTP. GTPase activity was linear for up to 30 min and was directly proportional to protein concentration. Polyclonal anti-tubulin antibodies, anti-$\alpha$-tubulin antibodies, and anti-$\beta$-tubulin antibodies (10 $\mu$g) stimulated G protein GTPase activity. Anti-$\beta$-tubulin antibody (10 $\mu$g) stimulated GTPase activity by about 60% at each time point, while 10 $\mu$g of either anti-$\alpha$-tubulin or polyclonal anti-tubulin antibodies stimulated GTPase activity by only 20–30% at each time point. The $V_{max}/K_m$ ratio, an index of the enzyme–substrate interaction, increased by only 26% with the anti-$\alpha$-tubulin antibody and by 52% with anti-$\beta$-tubulin antibody; polyclonal anti-tubulin antibodies did not affect this ratio. GTPase activity was stimulated by acetylcholine in an atropine-sensitive manner. At 100 $\mu$M, acetylcholine stimulated GTPase activity by about 50%. Polyclonal anti-tubulin, anti-$\alpha$-tubulin, or anti-$\beta$-tubulin antibodies (10 $\mu$g) potentiated acetylcholine stimulation of GTPase activity. Two possible mechanisms by which anti-tubulin antibodies could stimulate low $K_m$ GTPase activity and potentiate the stimulatory effects of acetylcholine are: (1) by inhibiting GTP binding to $\beta$-tubulin, and (2) by eliminating a chronic inhibitory effect of tubulin on G protein or receptor–G protein interaction.

Hormones and neurotransmitters stimulate adenylate cyclase through the interaction of receptors with guanine nucleotide-dependent transducer proteins (G proteins) [1–5]. G proteins are composed of three subunits, $\alpha$, $\beta$, and $\gamma$, and are associated with the plasma membrane. The binding of an agonist to the receptor facilitates, in the presence of $Mg^{2+}$, an exchange of GTP for GDP on the $\alpha$ subunit. The activated $\alpha_{GTP}$ subunit dissociates from the $\beta\gamma$ subunits, and one or both interact with effector molecules such as adenylate cyclase. An intrinsic GTPase activity of the $\alpha$ subunit hydrolyzes GTP to GDP, releasing inorganic phosphate ($P_i$); $\alpha_{GDP}$ then recombines with $\beta\gamma$, ending the activation cycle. Measurement of this increased rate of GTPase activity (EC 3.6.1.-) in response to an agonist provides a simple and direct assessment of the receptor–G protein interaction.

Tubulin, the protein subunit from which microtubules are polymerized, is a heterodimer containing an $\alpha$ and a $\beta$ subunit, with molecular weights of 50 and 60 kD respectively. Two molecules of GTP bind to tubulin, one to a site on $\beta$-tubulin where it is exchangeable with the GTP in the milieu (E-site), and one to a non-exchangeable site on $\alpha$-tubulin (N-site). The N-site GTP can be removed only by denaturation of the molecule [6]. Tubulin has the capacity to hydrolyze GTP. Bhattacharyya and Wolff [7] demonstrated that the synaptosomal fraction of rat brain contains the greatest concentration of tubulin. The tubulin found in this fraction is firmly attached to the membrane and is not an artifact of the isolation procedure. In a subsequent study, the membrane-bound tubulin was solubilized with Nonidet P-40 and shown to be capable of polymerizing into microtubules [8].

The purpose of the present study was to explore the possibility that tubulin modulates receptor–G protein–effector interactions on the cytosolic side of neural membranes. Accordingly, we examined the effect of anti-tubulin antibodies on the low $K_m$ GTPase activity (basal and muscarinic receptor-stimulated) associated with transducer G proteins in membranes from rat striatum.

## MATERIALS AND METHODS

*Materials.* Monoclonal anti-$\beta$-tubulin antibodies (No. T-4026, clone TUB 2.1), monoclonal anti-$\alpha$-tubulin antibodies (No. T-9026, clone DM 1A), polyclonal anti-tubulin antibodies (No. T-3526), control mouse ascites fluid (No. M8273), and normal rabbit serum (No. R-4505) were purchased from the Sigma Chemical Co. (St. Louis, MO). The monoclonal antibodies utilized in this report were produced against the whole $\alpha$ or $\beta$ subunit of tubulin. The clones employed in the present study (DM 1A and TUB 2.1) were produced originally by Blose *et al.* [9] and Gozes and Barnstable [10] and are now made available through Sigma. The specificities of the antibodies were confirmed by Western blots, solid phase radioimmunoassay and immunofluorescence. Clone DM 1A (anti-$\alpha$-tubulin antibody) was shown to cross-react specifically with $\alpha$-tubulin; it exhibited a

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weak cross-reactivity with $\beta$-tubulin, approximately 10% of the activity observed with $\alpha$-tubulin. Clone TUB 2.1 (anti-$\beta$-tubulin antibody), highly specific for $\beta$-tubulin, also had a weak secondary activity against $\alpha$-tubulin.

Radiolabeled guanosine-5′-triphosphate ([$\gamma$-$^{32}$P]GTP; 1500 Ci/mmol) was purchased from ICN Radiochemicals (Irvine, CA). All other chemicals used in the present study were purchased from the Sigma Chemical Co.

*Striatal membrane preparation.* Adult, male Wistar rats (125–150 g, Harlan Sprague–Dawley, Indianapolis, IN) were housed in pairs in an environmentally-controlled room with free access to food and water. Rats were killed by decapitation and their brains removed; the striatum was dissected and homogenized in 5 mM TED buffer (Tris–HCl, 1 mM EDTA, 1 mM dithiothreitol, pH 7.4) containing 10% sucrose (w/v) using a motor-driven Teflon–glass tissue grinder. The homogenate was centrifuged at 800 *g* for 10 min. The supernatant fraction was maintained on ice, while the pellet was resuspended in TED–sucrose and centrifuged at 800 *g*. The combined supernatant fractions were then centrifuged at 9000 *g* for 20 min. The pellet was washed once with TED buffer by resuspension and centrifugation at 9000 *g* for 20 min. The pellet was resuspended in TED buffer, pH 8.0, and incubated on ice for 30 min. This suspension was centrifuged at 35,000 *g* for 10 min. The pellet was resuspended in TED buffer, pH 7.4, and used for the GTPase assay. Protein content was estimated using the method of Bradford [11].

*GTPase assay.* GTPase activity was determined in quadruplicate according to the method of Cassel and Selinger [12]. The reaction mixture (100 $\mu$L) contained 75 mM Tris–HCl (pH 7.4), [$\gamma$-$^{33}$P]GTP (70,000–100,000 cpm), unlabeled GTP at different concentrations, 2 mM $MgCl_2$, 0.5 mM ATP, 0.5 mM adenylylimidodiphosphate, 5 mM phosphocreatine, creatine phosphokinase (50 units/mL), bovine serum albumin (50 $\mu$g), 0.1 mM EDTA, 0.2 mM ethyleneglycolbis(aminoethylether)tetra-acetate (EGTA), 1 mM cAMP, 100 mM NaCl, and 10–20 $\mu$g protein. All these components were added to 12 × 75 mm glass tubes on ice, and the reaction was initiated by immersion of the tubes in a 37° water bath. The reaction was stopped by transferring the tubes to an ice bath followed by the addition of 5% activated charcoal in 20 mM phosphoric acid (pH 2.5). Samples were kept on ice for 10 min and centrifuged at 700 *g* for 10 min. An aliquot (100 $\mu$L) from the supernatant fraction was mixed with 5 mL of Scintiverse BD (Fisher Scientific, Pittsburgh, PA), and the radioactivity content was determined using a Beckman (Palo Alto, CA) liquid scintillation counter. Low $K_m$ GTPase activity (EC 3.6.1.) was routinely calculated by subtracting activity measured in the presence of 100 $\mu$M unlabeled GTP from total activity. Data were subjected to kinetic analyses employing Eadie–Hofstee plots; the $K_m$ and $V_{max}$ were determined by linear regression analysis. Statistical significance within various treatment groups was determined by analysis of variance.

## RESULTS

Striatal membrane preparations were routinely preincubated with anti-tubulin antibodies for 10 min at 37°. At the end of this time period, the tubes were transferred to an ice bath and the remaining components of the GTPase assay were added. The tubes were then incubated at 37° for 30 min, and GTPase activity was determined. GTPase activity at 4° was negligible, ranging from 3–9 pmol $P_i$/min/mg protein. However, the 10-min preincubation at 37° decreased basal GTPase activity by 50%. Hence, it was important to determine how this treatment affected the muscarinic receptor-coupled G protein GTPase activity. Preincubated membranes responded to acetylcholine in a concentration-dependent manner (Fig. 1). Acetylcholine (100 $\mu$M) stimulated low $K_m$ GTPase activity by about 60% with an $EC_{50}$ of less than 1 $\mu$M (Fig. 1). Basal low $K_m$ GTPase activity increased linearly for up to 30 min of incubation (Fig. 2). Acetylcholine (100 $\mu$M) stimulated the GTPase activity at 10, 20 and 30 min; for example, at 30 min the level of stimulation was 58% (Fig. 2). Kinetic analysis revealed that the $V_{max}$ increased by 55% in the presence of 100 $\mu$M acetylcholine compared to the basal level (Fig. 3); the $K_m$ was not altered. $K_m$ values were 0.55 and 0.75 $\mu$M in the absence and presence of acetylcholine respectively; $V_{max}$ values were 398 and 619 pmol/min/mg in the absence and presence of acetylcholine respectively. Acetylcholine-stimulated GTPase activity was inhibited by atropine with an $IC_{50}$ of 2 $\mu$M (Fig. 4). The high concentration of atropine required to block ACh-stimulated activity is not surprising in light of the slow onset of atropine action and the lack of an atropine preincubation period.

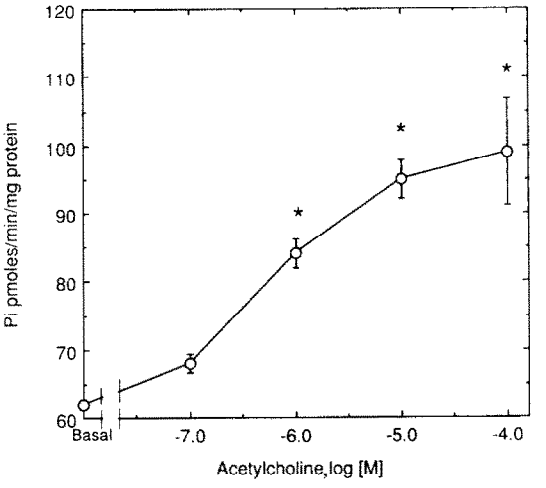


Fig. 1. Acetylcholine stimulation of G-protein GTPase activity. Rat striatal membrane protein (10 $\mu$g) was preincubated at 37° for 10 min and then transferred to an ice bath; other components of the GTPase assay, including various doses of acetylcholine, were added, and the enzyme activity was determined using 1 $\mu$M GTP. Each point is the mean ± SD of four determinations. Asterisks (*) indicate values significantly different from basal level ($P < 0.05$).

Normal mouse ascites fluid (a control for monoclonal anti-$\alpha$- and anti-$\beta$-tubulin antibodies), normal rabbit serum (a control for polyclonal anti-tubulin antibodies) and the three antibody preparations did

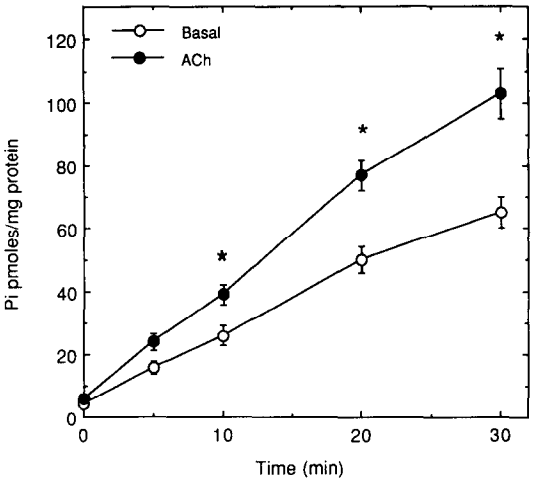


Fig. 2. Time course of high-affinity GTPase activity in the presence of acetylcholine. Rat striatal membrane protein (10 μg) was preincubated at 37° for 10 min and transferred to an ice bath; remaining components of the GTPase assay, including 100 μM acetylcholine, were added, and the enzyme activity was determined using 1 μM GTP. Each point is the mean ± SD of four determinations. Asterisks (*) indicate significantly different activities in the presence and absence of acetylcholine at that time point ($P < 0.05$).

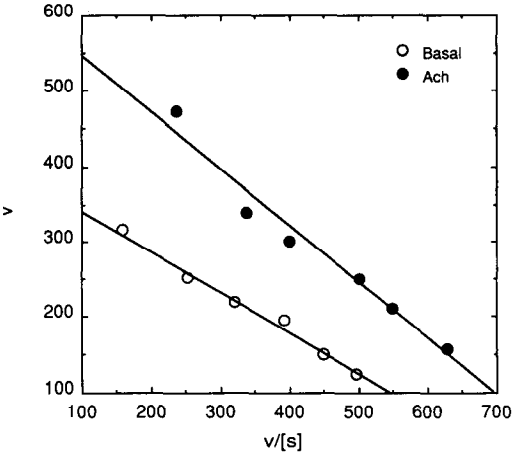


Fig. 3. Eadie–Hofstee analysis of G-protein GTPase activity in the presence and absence of acetylcholine. Rat striatal membrane protein (10 μg) was preincubated at 37° for 10 min and transferred to an ice bath; remaining components of the GTPase assay, including 100 μM acetylcholine, were added, and the enzyme activity was determined in quadruplicate at various GTP concentrations (0.2 to 2.0 μM).

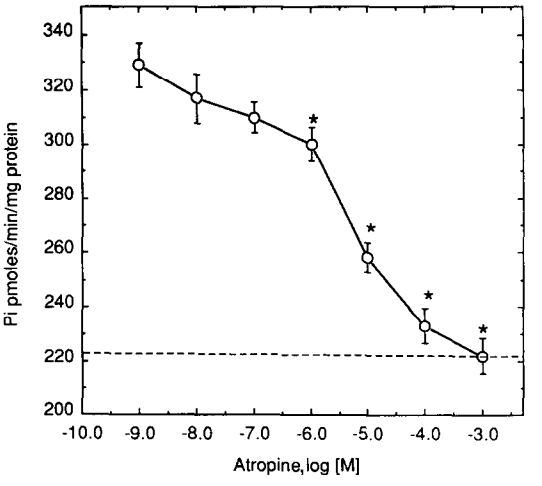


Fig. 4. Effect of atropine on acetylcholine stimulation of G protein GTPase activity. Rat striatal membrane protein (10 μg) was preincubated at 37° for 10 min and transferred to an ice bath; then various doses of atropine were added, followed by other components of the GTPase assay. Acetylcholine (100 μM) was added just before the initiation of the assay. Each point is the mean ± SD of four determinations. Asterisks (*) indicate significant inhibition of acetylcholine-stimulated activity by atropine ($P < 0.05$). Basal activity measured in the absence of acetylcholine is indicated by the dashed line (atropine did not affect this activity).

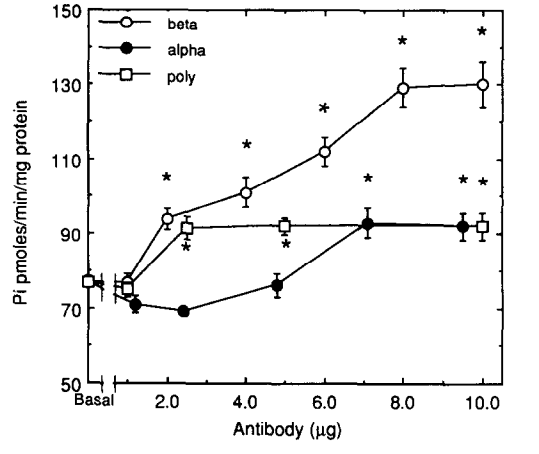


Fig. 5. Effect of anti-tubulin antibodies on G-protein GTPase activity. Rat striatal membrane protein (10 μg) was preincubated with various doses of antibodies at 37° for 10 min and then transferred to an ice bath; the remaining components of the GTPase assay were added and enzyme activity was determined using 1 μM GTP. Each point is the mean ± SD of four determinations. Asterisks (*) indicate values significantly greater than the basal level ($P < 0.05$).

not hydrolyze GTP. However, normal ascites fluid and rabbit serum stimulated low $K_m$ GTPase activity of the striatal membrane protein preparation (data not presented), albeit to a lesser extent than the antibody preparations (see below). Therefore, for each concentration of the antibody, the same concentration of either normal ascites fluid or rabbit serum was employed as a control, and the amount of stimulation that was obtained in the control tubes was subtracted from the activity in the antibody tubes. Moreover, the protein concentration of the three antibody preparations, normal ascites fluid, and serum were estimated in the same assay to eliminate any possible inter-assay variation.

The three types of tubulin antibody stimulated low $K_m$ GTPase activity (Fig. 5). Anti-β-tubulin antibody

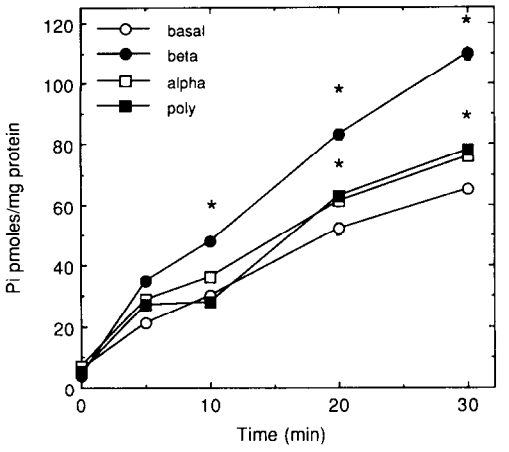


Fig. 6. Time course of G protein GTPase activity in the presence of anti-tubulin antibodies. Rat striatal membrane protein (10 μg) was preincubated with 10 μg each of polyclonal anti-tubulin, anti-β-tubulin or anti-α-tubulin antibodies at 37° for 10 min; the assay tubes were then transferred to an ice bath and the remaining components of the GTPase assay were added. Enzyme activity was determined using 1 μM GTP. Each point is the mean ± SD of four determinations. Asterisks (*) indicate values significantly greater than the basal value at that time point ($P < 0.05$).

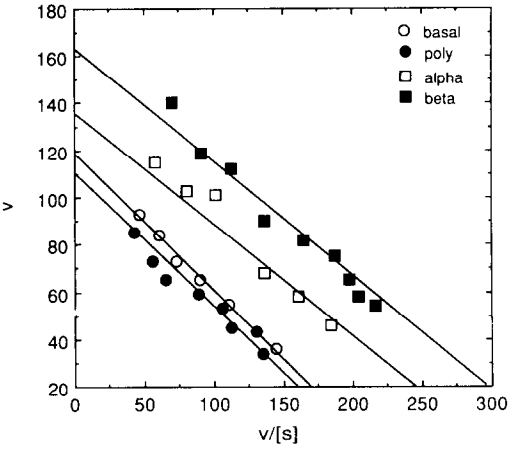


Fig. 7. Eadie–Hofstee analysis of the effect of anti-tubulin antibodies on G-protein GTPase activity. Rat striatal membrane protein (10 μg) was preincubated with 10 μg each of polyclonal anti-tubulin, anti-β-tubulin or anti-α-tubulin antibodies at 37° for 10 min; the assay tubes were then transferred to an ice bath, the remaining components of the GTPase assay were added, and the enzyme activity was determined in quadruplicate at various GTP concentrations (0.2 to 2.0 μM).

was more effective than anti-α-tubulin and polyclonal anti-tubulin antibodies in stimulating the GTPase activity. Two micrograms of anti-β-tubulin increased GTPase activity by 20%; 8.0 μg of anti-β-tubulin stimulated the GTPase activity by 67%, and no further stimulation was observed at higher concentrations. In contrast, 7.0 μg of anti-α-tubulin stimulated the GTPase activity by only 20%, and no further stimulation was observed at higher concentrations. The low $K_m$ GTPase activity was stimulated by 20% by 2.5 μg of polyclonal anti-tubulin antibody with no further stimulation at higher concentrations (Fig. 5). A time course study revealed that basal low $K_m$ GTPase activity was linear for up to 30 min and that the three types of antibody stimulated GTPase activity at each time point (Fig. 6). Anti-β-tubulin antibody (10 μg) stimulated GTPase activity by about 60% at each time point, whereas 10 μg of either anti-α-tubulin or polyclonal antibodies stimulated the GTPase activity by only 20–30% at each time point (Fig. 6). The kinetics of the reaction were studied by varying the substrate concentration in the absence or presence of the antibodies, and the data were analyzed by Eadie–Hofstee plots and linear regression analysis (Fig. 7). Regression analysis revealed the following kinetic parameters: $K_m$ (μM) basal, 0.57; anti-α-tubulin antibody, 0.58; anti-β-tubulin antibody, 0.55; and polyclonal anti-tubulin antibody, 0.48. $V_{max}$ (pmol/min/mg) basal, 118; anti-α-tubulin antibody, 151; anti-β-antibody, 173; and polyclonal anti-tubulin antibody, 101. None of the three types of antibody, employed at a concentration of 10 μg, produced a significant change in $K_m$ compared to the basal value. $V_{max}$ increased by 28 and 47% in the presence of 10 μg anti-α- and 10 μg anti-β-tubulin antibodies respectively; there was no change in the $V_{max}$ with 10 μg polyclonal anti-tubulin antibodies (Fig. 7).

Concentration–response curves for acetylcholine stimulation of GTPase activity were obtained in the presence of saturating concentrations of each of the three antibodies. In the absence of antibody, acetylcholine stimulated the G protein GTPase in a concentration-dependent manner; at 100 μM, acetylcholine stimulated the GTPase activity by 50% (Fig. 8A). Polyclonal anti-tubulin, anti-α-tubulin, or anti-β-tubulin antibodies (10 μg) stimulated the GTPase activity by 30, 15 and 45% respectively. Acetylcholine stimulation was potential in the presence of each antibody (Fig. 8B). For example, at 10 and 100 μM acetylcholine stimulated GTPase activity by 32 and 50% respectively. In the presence of anti-β-tubulin antibody, acetylcholine stimulation was increased to 61 and 64% respectively (above the level of activity measured in the presence of anti-β-tubulin antibody alone).

## DISCUSSION

The present results demonstrate that anti-tubulin antibodies, and especially anti-β-tubulin antibodies, stimulated the low $K_m$ GTPase activity of striatal membranes. Moreover, these antibodies enhanced the ability of acetylcholine to stimulate this activity. Striatal neurons predominantly express muscarinic receptors of the m4 and m1 subtypes [13]. These receptors are coupled most efficiently, via G proteins, to adenylate cyclase (inhibition) and phospholipase C (stimulation) respectively [14]. The GTPase activity measured using 1 μM GTP as substrate appears to be associated with muscarinic receptor-linked G protein insofar as it (1) possesses a low $K_m$, and (2) is stimulated by acetylcholine.

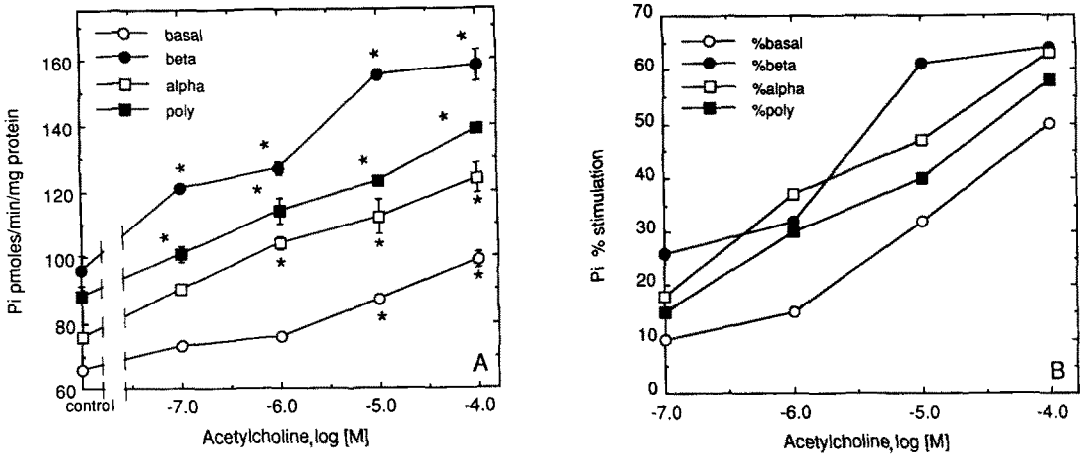


Fig. 8. Effect of acetylcholine on G-protein GTPase activity in the presence of anti-tubulin antibodies. Rat striatal membrane protein (10 μg) was preincubated with 10 μg each of polyclonal anti-tubulin, anti-β-tubulin or anti-α-tubulin antibodies at 37° for 10 min as indicated. The assay tubes were transferred to an ice bath, the remaining components of the GTPase assay (including various concentrations of acetylcholine) were added, and the enzyme activity was determined using 1 μM GTP. Each point is the mean ± SD of four determinations. The actual values obtained are shown in panel A. The percent stimulation obtained at each concentration of acetylcholine over the corresponding control level (i.e. the activity measured in the presence of 10 μg antibody but no acetylcholine) is depicted in panel B. Asterisks (*) in panel A indicate values significantly different from corresponding control values ($P < 0.05$).

In contrast, tubulin-associated GTPase activity is inhibited by anti-tubulin antibodies [15].

Two possible mechanisms by which anti-tubulin antibodies could stimulate low $K_m$ GTPase activity and potentiate the stimulatory effects of acetylcholine are (1) by inhibiting GTP binding to β-tubulin, and (2) by eliminating a chronic inhibitory effect of tubulin on G protein function or receptor–G protein interaction.

The anti-β-tubulin antibody stimulated low $K_m$ GTPase activity to the greatest extent. The GTP binding site (E-site) on the β subunit is exchangeable with the GTP present in the reaction mixture. The regions associated with GTP binding are located within the N-terminal domain of β-tubulin. Residues in regions β63-67 bind to the purine moiety, side chains in the region β155-174 bind the ribose, and a flexible loop in β137-146 appears to regulate access to the binding site [16]. Antibodies to β-tubulin residues β154-165 inhibit GTP binding to the E-site [16] and anti-tubulin antibodies (polyclonal) inhibit GTPase activity of phosphocellulose purified tubulin by about 80% [15]. In synaptosomal membranes, anti-β-tubulin antibodies may prevent the binding of GTP to tubulin. Since GTP has a 14-fold higher affinity for tubulin than for $G_i$ [6, 17], anti-tubulin antibodies may remove a GTP sink, increasing the concentration of available GTP in the milieu. The excess of GTP thus made available is hydrolyzed by the G protein. Based on the results of Bhattacharyya and Wolff [7], we estimate that 1.0 to 2.0 μM tubulin is present in the GTPase assay reaction mixture. The GTPase assay was routinely conducted with 1.0 μM GTP. Thus, there is sufficient tubulin present in the reaction mixture to act as a significant GTP sink. The observation of an exchange of a nonhydrolyzable GTP analogue between tubulin and $G_i$ also supports this possibility [18].

Since the N-site GTP on the α-tubulin is non-exchangeable with that in medium, we would not expect to observe any effect with the anti-α-tubulin antibody. However, 7 μg of anti-α-tubulin antibody stimulated the GTPase activity by 20%. A similar amount of stimulation was achieved by the anti-β-subunit antibody at a concentration of only 2 μg. It is possible that the anti-α-tubulin antibody cross-reacted with the β-subunit. In fact, it was reported previously that the monoclonal anti-α-tubulin antibody (clone DM 1A) employed in the present study exhibits a weak cross-reactivity with the β-tubulin, approximately 10% of the activity observed with the α-tubulin [9]. This would not be surprising considering the 40–50% homology in the primary structures of α and β tubulin [19]. Furthermore, it was reported previously that anti-α-subunit antibodies cross-react with the β-tubulin in certain regions of the N-terminal domain, although the anti-α-subunit antibody binds to the β-tubulin two orders of magnitude less tightly than the anti-β-subunit antibody [20]. A 20% stimulation in the GTPase activity was observed with 2.5 μg polyclonal anti-tubulin antibodies; no further stimulation of the GTPase activity was obtained even with 10 μg antibody. This weak activity of the polyclonal antibody may reflect its low titer. Eadie–Hofstee analysis of the kinetics appears to be in agreement with results obtained under steady-state conditions: the $K_m$ was not altered appreciably with any of the antibodies; the $V_{max}/K_m$ ratio, an index of enzyme–substrate interaction, increased by 26% with the anti-α-tubulin antibody and by 52% with anti-β-tubulin antibody; there was no change in this ratio with polyclonal anti-tubulin

antibody. Thus, it appears that among the three antibodies, anti-$\beta$-tubulin antibody promoted the best conditions for enzyme–substrate interaction.

A second explanation which could account for the observed results is that tubulin exerts a chronic inhibitory effect on G protein GTPase activity. Binding of neurotransmitter or hormone to the receptor facilitates the exchange of GTP for GDP on the $\alpha$ subunit of protein, ultimately leading to increased hydrolytic activity [3]. GDP release is the rate-limiting step in the G protein cycle. It is possible that tubulin interacts with the G protein to depress this nucleotide release. Agonist stimulation of the receptor might eliminate this inhibitory effect through an interaction with either the G protein or tubulin itself. Such a scheme could account for the synergistic actions of tubulin antibodies and acetylcholine on G protein GTPase activity.

Our observations that normal mouse ascites fluid and rabbit serum stimulate the low $K_m$ GTPase activity of the striatal membrane protein preparation are similar to those reported by McKenzie *et al.* [21]. The protein present in the fluid may protect the heat-labile G-protein from inactivation during the 10-min preincubation period. We have observed previously that exposure of striatal membranes to 50° inactivates the G-protein, resulting in a rapid and complete loss of low $K_m$ activity [22]. Hence, it was essential to examine the effect of preincubation of the membrane preparation at 37° on the GTPase activity. The kinetics of the basal GTPase activity in the preincubated samples were similar to those reported previously [23]. Although the 10-min preincubation resulted in a 50% loss in the basal GTPase activity, the ability of the membranes to respond to acetylcholine was not impaired. The fact that acetylcholine stimulation of the GTPase activity was concentration-dependent and atropine-sensitive is consistent with previous results obtained with striatal membranes not preincubated at 37° [23, 24].

In summary, we have demonstrated that anti-tubulin antibodies, especially anti-$\beta$-tubulin antibody, stimulate the low $K_m$ GTPase activity associated with G-proteins in rat striatal membranes and potentiate the ability of acetylcholine to stimulate this activity. We suggest that tubulin may be acting as a GTP sink or may exert a chronic inhibitory influence on G protein function or receptor–G protein interactions. Given the ubiquitous and highly conserved nature of both tubulin and G proteins, similar regulatory mechanisms may be present in a variety of other receptor systems as well.

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## REFERENCES

1. Stryer L and Bourne HR, G Proteins: A family of signal transducers. *Annu Rev Cell Biol* **2**: 391–419, 1986.
2. Birnbaumer L, Codina J, Mattera R, Yatani A, Scherer N, Toro MJ and Brown AM, Signal transduction by G-proteins. *Kidney Int* **32**: S-14–S-37, 1987.
3. Gilman AG, G Proteins: Transducers of receptor-generated signals. *Annu Rev Biochem* **56**: 615–649, 1987.
4. Neer EJ and Clapham DE, Roles of G-protein subunits in transmembrane signalling. *Nature* **333**: 129–134, 1988.
5. Spiegel A, Carter A, Brann M, Collins R, Goldsmith P, Simonds W, Vinitsky R, Eide K, Rossiter K, Weinstein L and Woodward C, Signal transduction by guanine nucleotide-binding proteins. *Recent Prog Horm Res* **44**: 337–375, 1988.
6. Carlier MF, Guanosine-5′-triphosphate hydrolysis and tubulin polymerization. Review article. *Mol Cell Biochem* **47**: 97–113, 1982.
7. Bhattacharyya B and Wolff J, Membrane-bound tubulin in brain and thyroid tissue. *J Biol Chem* **250**: 7639–7646, 1975.
8. Bhattacharyya B and Wolff J, Polymerisation of membrane tubulin. *Nature* **264**: 576–577, 1976.
9. Blose SH, Meltzer DI and Feramisco JR, 10-nm Filaments are induced to collapse in living cells microinjected with monoclonal and polyclonal antibodies against tubulin. *J Cell Biol* **98**: 847–858, 1984.
10. Gozes I and Barnstable CJ, Monoclonal antibodies that recognize discrete forms of tubulin. *Proc Natl Acad Sci USA* **79**: 2579–2583, 1982.
11. Bradford MM, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**: 248–254, 1976.
12. Cassel D and Selinger Z, Catecholamine-stimulated GTPase activity in turkey erythrocyte membranes. *Biochim Biophys Acta* **452**: 538–551, 1976.
13. Brann MR, Buckley NJ and Bonner TI, The striatum and cerebral cortex express different muscarinic receptor mRNAs. *FEBS Lett* **230**: 90–94, 1988.
14. Peralta EG, Ashkenazi A, Winslow JW, Ramachandran J and Capon DJ, Differential regulation of PI hydrolysis and adenylyl cyclase by muscarinic receptor subtypes. *Nature* **334**: 434–437, 1988.
15. Ravindra R, Effect of estradiol on corpus luteum function in pregnant hamster. *Ph.D. Thesis*. Indian Institute of Science, Bangalore, India, 1982.
16. Hesse J, Theirauf M and Ponstingl H, Tubulin sequence region $\beta$ 155–174 is involved in binding exchangeable guanosine triphosphate. *J Biol Chem* **262**: 15472–15475, 1987.
17. Bokoch GM, Katada T, Northrup JK, Vi M and Gilman AG, Purification and properties of the inhibitory guanine nucleotide binding regulatory component of adenylate cyclase. *J Biol Chem* **259**: 3560–3567, 1984.
18. Rasenick M and Wang N, Exchange of guanine nucleotides between tubulin and GTP-binding proteins that regulate adenylate cyclase: Cytoskeletal modification of neuronal signal transduction. *J Neurochem* **51**: 300–311, 1988.
19. Dustin P, *Microtubules*. Springer, Berlin, 1984.
20. Mandelkow EM, Herrmann M and Riehl V, Tubulin domains probed by limited proteolysis and subunit-specific antibodies. *J Mol Biol* **185**: 311–327, 1985.
21. McKenzie FR, Kelly ECH, Unson CG, Spiegel AM and Milligan G, Antibodies which recognize the C-terminus of the inhibitory guanine-nucleotide binding protein ($G_i$) demonstrate that opioid peptides and foetal-calf serum stimulate the high-affinity GTPase activity of two separate pertussis-toxin substrates. *Biochem J* **249**: 653–659, 1988.
22. Dennison RL, Boswell E, Harmer N, Wright E, Narayanan TK and Aronstam RS, Selective inactivation of G-proteins associated with muscarinic receptors by heat. *Soc Neurosci Abstr* **14**: 228, 1988.
23. Onali P, Olianas MC, Schwartz JP and Costa E, Involvement of a high affinity GTPase in the inhibitory

coupling of striatal muscarinic receptors to adenylate cyclase. *Mol Pharmacol* **24**: 380–386, 1983.
24. Anthony BL, Dennison RL, Narayanan TK and Aronstam RS, Halothane inhibition of acetylcholine-stimulated, pertussis toxin-sensitive GTPase activity in rat brain. *Soc Neurosci Abstr* **14**: 811, 1988.