Volume 310, number 1, 66–70 FEBS 11567 September 1992

1,4-Dihydropyridines modulate GTP hydrolysis by G_o in neuronal membranes

M.I. Sweeney and A.C. Dolphin

Department of Pharmacology, Royal Free Hospital School of Medicine, Rowland Hill St., London NW3 2PF, UK

Received 5 August 1992

Several lines of evidence suggest that L-type Ca^{2+} channels (1,4-dihydropyridine receptors) are modulated by GTP-binding proteins. We have further examined this interaction by measuring the effect of 1,4-dihydropyridines on GTPase activity in brain membranes. Dihydropyridine agonists significantly increased GTPase, reflected by an increase in the maximal rate of GTP hydrolysis, without affecting the affinity for GTP or the binding of a non-hydrolysable analogue of GTP. The stimulating effect on GTPase was abolished by antisera raised against $G_o\alpha$ but not $G_i\alpha$. L-type Ca^{2+} channels may act as endogenous GTPase activating proteins (GAPs) to stimulate GTP hydrolysis by G_o.

1,4-Dihydropyridine; Calcium channel; G-protein; GTPase; G_o; Baclofen

1. INTRODUCTION

Interactions between voltage-dependent neuronal Ca^{2+} channels and various neurotransmitter receptors are mediated via regulatory GTP-binding proteins (G-proteins) which are sensitive to pertussis toxin [1–3]. Recent studies have demonstrated that a likely candidate for the subtype of G-protein involved is G_o since reconstitution of purified G_o into cells pretreated with pertussis toxin restores the inhibition of Ca^{2+} currents induced by receptor activation [4–7]. In addition, drug-induced inhibition of Ca^{2+} currents is attenuated by anti-G_o antibodies [5,8,9] and antisense oligonucleotides complementary to the mRNA of the α-subunit of G_o [10]. In the latter case, the Ca^{2+} current involved was identified as an L-type current [10] although non-L-type currents are also inhibited by neurotransmitters [2]. The interaction of 1,4-dihydropyridines with L-type Ca^{2+} channels is also modulated by a G-protein. This hypothesis was based initially on electrophysiological studies where activation of pertussis toxin-sensitive G-proteins enhanced the potentiation of Ca^{2+} currents produced by dihydropyridine agonists [11]. In binding experiments, GTP analogues increase the affinity of dihydropyridine agonist binding to membranes prepared from frontal cortex [12] and skeletal muscle [13].

G-proteins contain an intrinsic GTPase activity which hydrolyses GTP, bound to the activated state of the α-subunit, to GDP thereby inactivating and recycling the G-protein (reviewed in [14]). If an agonist-bound neurotransmitter receptor interacts with a G-protein, then an increase in GTPase usually occurs. In the present study we have demonstrated a significant stimulation of a high affinity GTPase activity by (−)-baclofen, an agonist at $GABA_B$ receptors. In addition, a profound stimulation of GTPase was produced by 1,4-dihydropyridines, particularly agonists, that act on effector L-type Ca^{2+} channels. This effect was reduced by pretreatment with antisera raised against $G_o\alpha$. Thus, this may represent a novel mechanism by which L-type Ca^{2+} channels terminate their interaction with G_o.

Correspondence address: M.I. Sweeney, Department of Pharmacology, Royal Free Hospital School of Medicine, Rowland Hill St., London NW3 2PF, UK. Fax: (44) (71) 794 6854.

Abbreviations: GTP, guanosine triphosphate; GTPγS, guanosine 5'-*O*-3-thiotriphosphate; p[NH]ppA, adenosine 5'-[β,γ-imido] triphosphate; 202–791, 2,6-dimethyl-3-nitro-5-isopropylcarboxylate-4-(2,1,3-benzoxadiazo-4-yl)-1,4-dihydropyridine; Bay K 8644, 2,6-dimethyl-3-nitro-5-methylcarboxylate-4-(2-tri-fluoromethylphenyl)-1,4-dihydropyridine; PN 200-110, 2,6-dimethyl-3-isopropyl-carboxylate-5-carbomethoxy-4-(2,1,3-benzoxadiazo-4-yl)-1,4-dihydropyridine; 4-chloro-nifedipine, 2,6-dimethyl-3,5-dicarbomethoxy-4-(4-chlorophenyl)-1,4-dihydropyridine.

2. EXPERIMENTAL

2.1. *Preparation of membranes*

The frontal cortex of adult male Wistar rats (BK Universal, Hull, UK) was homogenized (8 strokes) in ice-cold 10 mM Tris-HCl/1 mM EDTA, pH 7.4 (buffer A) containing the following protease inhibitor cocktail: 5 mM benzamidine, 5 mM dithiothreitol, 100 μM chlorpromazine, 50 μM leupeptin, and aprotinin (0.25 trypsin inhibitor U/ml). Following centrifugation at 1,000 × *g* for 10 min at 4°C, the supernatant was centrifuged at 40,000 × *g* for 10 min at 4°C. The resulting membrane pellet was washed and resuspended in the homogenizing buffer (final concentration 1 mg protein/ml).

2.2. *GTPase assays*

20 μl of membrane suspension or de-ionized water were added in triplicate to 30 μl drug(s) and 50 μl of GTPase assay mix containing

 Published by Elsevier Science Publishers B.V.

approximately 100,000 cpm of [γ^{32}P]GTP (NEN). GTPase activity was quantitated by measuring [^{32}P]GTP hydrolysis at 37°C for 10 min essentially as described previously [15] except that 20 mM Tris-HCl and 1 mM creatine phosphate were used, and p[NH]ppA was omitted. [^{32}P]GTP hydrolysis was terminated by the removal to ice and addition of 900 μl of 2% charcoal in 20 mM phosphoric acid containing 5% ethanol. Aliquots (200 μl) of the supernatant resulting from centrifugation at 1,500 × g for 10 min were counted for ^{32}P content in a liquid scintillation counter. In some experiments, membranes were pre-treated for 1 h at 30°C either with non-immune serum or anti-G-protein antisera (diluted 1:50) before [^{32}P]GTP hydrolysis was assessed. Antisera used were OC1, OC2 and ON1, directed against peptide sequences in $G_o\alpha$ or the anti-$G_i\alpha$ antiserum AS7. When OC2 and AS7 were used, all sera were pre-incubated either with the peptides used for immunization (ANNLRGCGLY or KENLKDCGLF, respectively; 2 mg/ml) or vehicle (50 mM NaH_2PO_4, pH 6) for 1 h at 30°C. V_{max} and K_M for GTPase were determined using the Lineweaver–Burke analysis with GTP concentrations of 0.02, 0.2, 0.4, 0.8 and 2 μM.

2.3. *[^{35}S]GTPγS binding experiments*

These were performed for 20 min at room temperature according to the method of Avissar et al. [16] with minor modifications. Membrane suspensions (0.5 mg protein/ml in buffer A, 100 μl aliquots) were added in triplicate to 1.5 ml microfuge tubes containing 10 μl of drug, and 30 μl of buffer A containing 10 mM $MgCl_2$ and 10 nM GTPγ[^{35}S] (NEN) (final concentrations). Non-specific binding was assessed using GTP (500 μM) or unlabelled GTPγS (10 μM). Binding was terminated by diluting with 3 vols. of ice-cold 100 μM GTP in buffer A, and centrifuging immediately at 12,000 × g for 5 min at 4°C. The pellet was washed 3 times, solubilized, and then counted for bound ^{35}S in a scintillation counter. B_{max} and K_D were determined using the Scatchard analysis with free GTPγ[^{35}S] concentrations of 0.3, 1, 3 and 10 nM.

All GTPase and binding experiments with dihydropyridines were performed under infra-red lighting.

3. RESULTS

Incubation of rat frontal cortical membranes with the $GABA_B$ receptor agonist (−)-baclofen (1–30 μM) produced a dose-dependent increase in the hydrolysis of [^{32}P]GTP (Fig. 1). The effect of baclofen was maximal at 10 μM, and was due to stimulation of a high-affinity GTPase since no enhancement of GTPase was observed in the presence of 10 μM GTPγS, a non-hydrolysable analogue of GTP which binds irreversibly to the high-affinity guanine nucleotide binding site on G-proteins (Fig. 1). When Lineweaver–Burke analyses were performed (Fig. 2A), 10 μM baclofen had no effect on the apparent affinity (K_M) of GTPase for GTP (111 ± 16% of control, $P = 0.9$, $n = 7$) but produced a profound (41 ± 12%) increase in the maximal rate of GTP hydrolysis (V_{max}) (Fig. 2B). Dihydropyridines also stimulated a high-affinity GTPase activity. The agonist (+)-202–791 (0.33–10 μM) produced an 18–96% increase in V_{max} with no significant effect on K_M (Table I), and similar results were obtained using the agonist (±)-Bay K 8644, and to a lesser extent the antagonists (−)-202–791 and (+)-PN 200–110 (isradipine) (Table I). When dihydropyridine concentrations were expressed relative to the concentrations required to produce half maximal binding to cardiac membranes (K_D's taken from [17]), it was observed that agonist enantiomers produced ef-

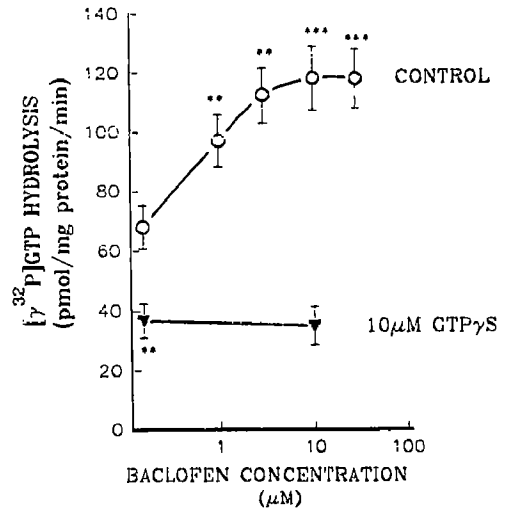

Fig. 1. Effect of (−)-baclofen and 10 μM GTPγS on GTPase activity in frontal cortical membranes. $^{**}P < 0.01$, $^{***}P < 0.001$, paired t-test, compared to basal [^{32}P]GTP hydrolysis in the absence of any drugs ($n = 5$).

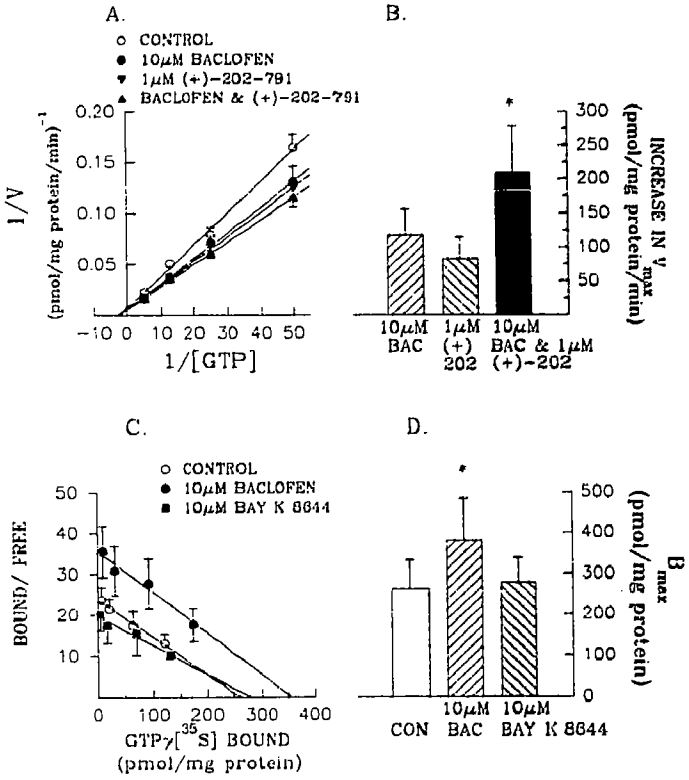

Fig. 2. Effect of (−)-baclofen and dihydropyridine agonists on GTPase activity (A,B) or [^{35}S]GTPγS binding (C,D) in frontal cortical membranes. In B, basal $V_{max} = 252.9 \pm 55.3$ pmol/mg protein/min. $^{*}P < 0.05$, analysis of variance compared to the increase in V_{max} with baclofen (BAC) or (+)-202–791 ((+)-202) alone ($n = 7$) (B) or compared to control B_{max} ($n = 6$) (D).

Table I

Effect of dihydropyridine agonists and antagonists on K_M and V_{max} of GTPase in frontal cortical membranes

	K_M (μM)	V_{max} (pmol/mg protein/min)
Agonists		
Control	0.59 ± 0.08 (100)	176.3 ± 41.5 (100)
(+)-202–791, 0.33 μM	0.56 ± 0.07 (95)	208.0 ± 62.0 (118)
(+)-202–791, 1 μM	0.62 ± 0.13 (104)	261.2 ± 50.7 (148)**
(+)-202–791, 10 μM	0.47 ± 0.06 (79)	345.6 ± 70.1 (196)**
Control	1.02 ± 0.37 (100)	231.2 ± 45.1 (100)
(±)-BayK 8644, 0.1 μM	0.97 ± 0.26 (95)	307.7 ± 49.4 (133)*
(±)-Bay K 8644, 1 μM	0.96 ± 0.21 (94)	396.1 ± 46.8 (174)***
(±)-Bay K 8644, 10 μM	0.75 ± 0.16 (74)	416.0 ± 77.5 (180)*
Antagonists		
Control	0.59 ± 0.08 (100)	176.3 ± 41.5 (100)
(−)-202–791, 0.33 μM	0.53 ± 0.07 (89)	181.6 ± 54.5 (103)
(−)-202–791, 1.0 μM	0.81 ± 0.33 (137)	248.2 ± 59.0 (141)*
(−)-202–791, 10.0 μM	0.47 ± 0.07 (80)	267.9 ± 74.0 (152)*
Control	0.55 ± 0.10 (100)	144.5 ± 28.4 (100)
(+)-PN200–110, 0.1 μM	0.52 ± 0.09 (95)	185.0 ± 43.9 (128)
(+)-PN 200–110, 1 μM	0.56 ± 0.09 (102)	204.5 ± 57.7 (141)
(+)-PN 200–110, 10 μM	0.49 ± 0.10 (89)	270.8 ± 55.1 (187)*

The K_M and V_{max} values were determined using the Lineweaver–Burke analysis described in Fig. 2. All regression lines showed regression coefficients of $r > 0.98$ ($P < 0.001$, $n = 6$–12). Values in parentheses are the K_M or V_{max} values expressed as percentages of controls (0.4% ethanol). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, paired t-test, compared to controls.

fects at concentrations which were 10–20-times their K_D for binding (96 nM for (+)-202–791, 6 nM for Bay K 8644), whereas the antagonists only produced effects at > 10^3-times their K_D (0.23 nM and 0.034 nM, respectively, for (−)-202–791 (and (+)-PN 200–110). A 4-chloro-derivative of nifedipine, which is structurally similar to 1,4-dihydropyridines but is 10,000-fold less potent at binding to dihydropyridine receptors [18], and omega-conotoxin GVIA, which inhibits N-type Ca^{2+} channels [3], had no effect on GTPase, the V_{max} being 105 ± 18% and 102 ± 11% of controls, respectively, in the presence of 10 μM of these agents ($P > 0.55$, $n = 4$–6).

When 10 μM baclofen and 1 μM (+)-202–791 were co-incubated with the membranes, an additive increase in V_{max} of GTPase activity was observed (Fig. 2B). This suggests either that the two drugs interact with different G-proteins and the net effect is simply an additive stimulation of GTPase, or that the same G-protein whose rate of GDP–GTP exchange is stimulated by activation of the agonist-bound receptor [14] may be further modulated by interaction with a dihydropyridine receptor. In order to distinguish between effects on GTP hydrolysis and nucleotide exchange, the effect of baclofen and Bay K 8644 on the binding of [^{35}S]GTPγS to frontal cortical membranes was determined. When Scatchard analyses were performed (Fig. 2C), 10 μM Bay K 8644 had no significant effect on the apparent K_D for binding [^{35}S]GTPγS (11.1 nM in control conditions and 14.2 nM in the presence of Bay K 8644), and there was no effect on the maximal number of binding sites (B_{max}) (Fig. 2D). In contrast, 10 μM baclofen increased the B_{max} by 36 ± 5% (Fig. 2D) and increased the binding affinity to 8.9 nM, corresponding to a 31 ± 2% increase in affinity ($P < 0.03$, paired t-test, $n = 6$).

In order to identify the G-protein(s) whose GTPase activity was stimulated by baclofen and dihydropyridines, frontal cortical membranes were treated in vitro with anti-G-protein antibodies for 1 h at 30°C. The specific antisera used were OC1 or OC2 and ON1, raised against C- and N-terminal peptides, respectively, of $G_o\alpha$, and AS7, which is directed against a C-terminal peptide sequence from $G_i\alpha$ [19]. The stimulatory effect of dihydropyridine agonists on GTPase was completely eliminated by pretreatment of membranes with the anti-$G_o\alpha$ antisera. Thus, the enhancement of GTPase by 3.3 μM (+)-202–791 was attenuated by both OC1 (Fig. 3A) and ON1 (Fig. 3B), and 10 μM (±)-Bay K 8644-induced stimulation was inhibited by OC2 (Fig. 3A). Dihydropyridine-induced stimulation of GTPase was not significantly affected by the anti-$G_i\alpha$ antiserum, AS7 (Fig. 3C). In contrast, pretreatment with AS7 produced a complete inhibition of the stimulation of GTPase by 10 μM baclofen (Fig. 3C), while anti-G_o antisera, OC2 (Fig. 3A) and ON1 (Fig. 3B), produced only a partial (45 ± 1% and 77 ± 12%, respectively) reduction in the effect of baclofen. Pre-incubation (1 h, 30°C) of OC2 (Fig. 3A) and AS7 (Fig. 3C) with the respective peptides used for immunization (2 mg/ml) significantly reversed the effects of the antisera on inhibiting baclofen- or Bay K 8644-stimulated GTPase. In all cases, control membranes were treated at 30°C with non-immune serum, and anti G-protein antibodies had no effect on basal GTPase activity (see legend to Fig. 3).

4. DISCUSSION

We have demonstrated that binding of ligands to dihydropyridine receptors, located on the α_1 subunit of L-type Ca^{2+} channels [20–22], produces an increase in the activity of a high-affinity GTPase ($K_M < 1$ μM). This stimulation appears to be due to an increase in the rate of GTP hydrolysis, and not nucleotide exchange, resulting from G_o interacting directly with the L-type Ca^{2+} channel in a state which is favored by dihydropyridine agonist binding, since agonist dihydropyridines were more effective than antagonists relative to their K_D for binding to the dihydropyridine receptor. Both the C- and N-terminal regions of $G_o\alpha$ may be important in this modulation as the stimulation of GTPase by agonist dihydropyridines was completely eliminated by pretreatment of membranes with specific antisera raised against either the C- or N-terminal regions of $G_o\alpha$. Previously, both antisera have demon-

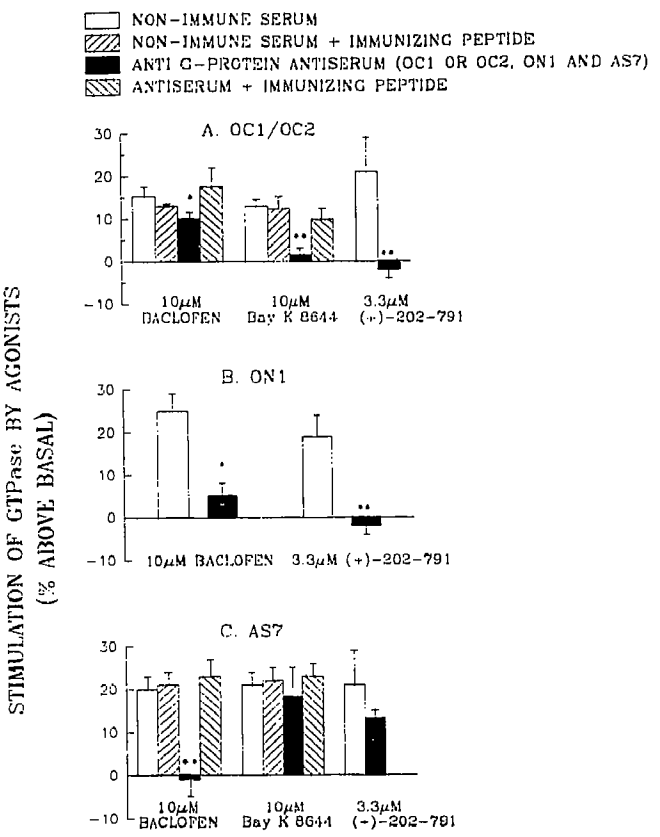

Fig. 3. Effect of incubation of frontal cortical membranes with anti-$G_o\alpha$ antisera, OC1 or OC2 (A) and ON1 (B), anti-$G_i\alpha$ antiserum AS7 (C), or non-immune serum on GTPase activity stimulated by (−)-baclofen and dihydropyridine agonists. Values are means ± S.E.M. of the % increase above basal GTPase. Values for basal GTPase activity were (pmol/mg protein/min): 51.5 ± 4.2, 59.5 ± 3.0, 58.6 ± 5.1, and 55.9 ± 6.8 for non-immune serum, non-immune serum plus $G_o\alpha$ peptide, OC1/OC2 and OC2 plus $G_o\alpha$ peptide, respectively (A); 46.1 ± 5.4 and 46.3 ± 5.7 for non-immune serum and ON1, respectively (B); and 43.0 ± 1.3, 49.5 ± 2.3, 43.1 ± 2.1, and 46.2 ± 1.1 for non-immune serum, non-immune serum plus $G_i\alpha$ peptide, AS7 and AS7 plus $G_i\alpha$ peptide, respectively (C). $^*P < 0.05$, $^{**}P < 0.01$, paired t-test, compared to drug-stimulated GTPase in membranes treated with non-immune serum (n =5–8).

strated a high specificity and selectivity to recognize a 39 kDa protein corresponding to $G_o\alpha$ while not cross-reacting with G_i [19]. Our results have been extended by showing that Bay K 8644 stimulates GTPase in membranes prepared from cultured cerebellar granule neurons, and that this effect is prevented by pretreatment of the cells with pertussis toxin (M. Silver, M.I.S. and A.C.D., manuscript in preparation). We are currently examining the effect of dihydropyridines on GTPase in a purified reconstituted membrane system.

Neurotransmitters inhibit voltage-dependent Ca^{2+} channels in neurons and secretory cells via G_o ([4,8–10]; see Introduction). The Ca^{2+} channel type which is modulated appears to be N-type in some neurons because of its sensitivity to omega-conotoxin, and because dihydropyridine-agonist-enhanced tail currents do not display this modulation [2,23]. However, there is also good evidence that L-type currents which are dihydropyridine-sensitive can also be modulated by G_o [3,10,24]. In the experiments described here, we have not addressed the interaction of G_o with N-type Ca^{2+} channels because of the lack of state-dependent ligands for these channels, and omega-conotoxin had no effect on GTPase activity.

Stimulation of GTPase by the dihydropyridines was not affected by AS7 which recognizes G_i [19], strongly suggesting that L-type Ca^{2+} channels do not interact directly with G_i. Baclofen-induced stimulation of GTPase was reduced substantially by anti-$G_i\alpha$ antisera, but also was reduced partially by anti-$G_o\alpha$ antisera, suggesting that $GABA_B$ receptors interact with G_i, and to a lesser extent with G_o, to stimulate a high affinity GTPase. It has been shown previously that a baclofen-mediated increase in GTPase was blocked by pretreatment with pertussis toxin [25]. Activation of G_i by baclofen would lead to an inhibition of adenylate cyclase (reviewed in [25]), while recent studies in our laboratory [9] have indicated that the baclofen-mediated inhibition of Ca^{2+} currents in dorsal root ganglion neurons is mediated via G_o.

The data described here suggest that the L-type Ca^{2+} channel may act as a GTPase activating protein (GAP) when in the dihydropyridine–agonist-bound state. This may represent a mechanism whereby the channel, having interacted with the G-protein which modulates its gating kinetics [26], limits the temporal effectiveness of the modulating signal. Thus, the life-time of the channel/$G_o\alpha$ complex will be reduced because the intrinsic GTPase activity of α_o has been increased. Dihydropyridine agonists bind preferentially to the resting state of the channel [27], and their binding and actions are modified by subunit composition [28]. Their ability to enhance GTPase may indicate that $G_o\alpha$ also interacts with the Ca^{2+} channel in the same state. This hypothesis could explain why dihydropyridine agonists increased the binding of [^{3}H]baclofen to $GABA_B$ receptors in rat cerebral cortical membranes [29], i.e. by increasing the proportion of G-proteins in the ground state. From studies on G_k activation of the cardiac K^+ currents ($I_{k(M)}$) [30] it has been observed that the k_{cat} determined from the rate of decay of $I_{k(M)}$ is over 100 min^{-1}, whereas this value is about 2 min^{-1} in purified preparations of G_i or G_o [14,31]. A similar mechanism, where the effector (phosphodiesterase) accelerates the normally slow rate of GTP hydrolysis by a heterotrimeric G-protein (transducin) has recently been proposed [32,33]. In addition, there exists a family of GAPs which stimulate the very slow endogenous GTP hydrolysis by small G-proteins, including $p21^{ras}$ (reviewed in [14]), and may also act as effectors for this G-protein [34]. Our results indicate that interaction with effector (in this case L-type Ca^{2+} channels) may represent a widespread mechanism

by which intrinsic G-protein GTPase activities are enhanced.

Acknowledgements: This work was supported by the MRC (UK) and the Wellcome Trust in grants to A.C.D. M.I.S. is a Fellow of the MRC of Canada. We are very grateful to G. Milligan, D.J. Triggle, U. Rüegg, and N. Bowery for their gifts of antisera, dihydropyridines and baclofen, and to M.S. (Sam) Fazeli for useful discussions.

REFERENCES

[1] Dolphin, A.C. (1990) Annu. Rev. Physiol. 52, 243–255.
[2] Schultz, G., Rosenthal, W., Trautwein, W. and Hescheler, J. (1990) Annu. Rev. Physiol. 52, 257–292.
[3] Tsien, R.W., Lipscombe, D., Madison, D.V., Bley, K.R. and Fox, A.P. (1988) Trends Neurosci. 11, 431–438.
[4] Hescheler, J., Rosenthal, W., Trautwein, W. and Schultz, G. (1987) Nature 325, 445–447.
[5] Harris-Warwick, R.M., Hammond, C., Paupardin-Tritsch, D., Homburger, V., Rouot, B., Bockaert, J. and Gerschenfeld, H.M. (1988) Neuron 1, 27–32.
[6] Ewald, D.A., Sternweis, P.C. and Miller, R.J. (1988) Proc. Natl. Acad. Sci. USA 85, 3633–3637.
[7] Toselli, M., Lang, J., Costa, T. and Lux, H.D. (1989) Pflüger's Arch. Physiol. 415, 255–261.
[8] McFadzean, I., Mullaney, I., Brown, D.A. and Milligan, G. (1989) Neuron 3, 177–182.
[9] Menon-Johansson, A.S. and Dolphin, A.C. (1992) J. Physiol. 452, 177P.
[10] Kleuss, C., Hescheler, J., Ewel, C., Rosenthal, W., Schultz, G. and Wittig, B. (1991) Nature 353, 43–48.
[11] Dolphin, A.C. and Scott, R.H. (1989) J. Physiol. 413, 271–288.
[12] Bergamaschi, S., Govoni, S., Cominetti, P., Parenti, M. and Trabucchi, M. (1988) Biochem. Biophys. Res. Commun. 156, 1279–1286.
[13] Dunn, S.M.J. and Bladen, C. (1991) Biochemistry 30, 5716–5721.
[14] Bourne, H.R., Sanders, D.A. and McCormick, F. (1990) Nature 348, 125–132.
[15] Koski, G. and Klee, W.A. (1981) Proc. Nat. Acad. Sci. USA 78, 4185–4189.
[16] Avissar, S., Schreiber, G., Danon, A. and Belmaker, R.H. (1988) Nature 331, 440–442.
[17] Triggle, D.J. and Rampe, D. (1989) Trends Pharmacol. Sci. 10, 507–511.
[18] Yousif, F.B. and Triggle, D.J. (1986) Can. J. Physiol. Pharmacol. 64, 273–283.
[19] Milligan, G., Tanfin, Z., Goureau, O., Unson, C. and Harbon, S. (1989) FEBS Lett. 244, 411–416.
[20] Tanabe, T., Takeshima, H., Mikami, A., Flockerzi, V., Takahashi, H., Kangawa, K., Kojima, M., Matsuo, H., Hirose, T. and Numa, S. (1987) Nature 328, 313–318.
[21] Perez-Reyes, E., Kim, H.S., Lacerda, A.E., Horne, W., Wei, X., Rampe, D., Campbell, K.P., Brown, A.M. and Birnbaumer, L. (1989) Nature 340, 233–236.
[22] Kim, H.S., Wei, X., Ruth, P., Perez-Reyes, E., Flockerzi, V., Hofmann, F. and Birnbaumer, L. (1990) J. Biol. Chem. 265, 11858–11863.
[23] Plummer, M.R., Logothetis, D.E. and Hess, P. (1989) Neuron 2, 1453–1463.
[24] Offermanns, S., Gollasch, M., Hescheler, J., Spicher, K., Schmidt, A., Schultz, G. and Rosenthal, W. (1991) Mol. Endocrinol. 5, 995–1002.
[25] Bowery, N.G., Hill, D.R. and Moratalla, R. (1989) in: Allosteric Modulation of Amino Acid Receptors: Therapeutic Implications (Barnard, E.A. and Costa, E. eds.) pp. 159–172, Raven Press, New York.
[26] Lipscombe, D., Kongsamut, S. and Tsien, R.W. (1989) Nature 340, 639–642.
[27] Sanguinetti, M.C., Krafte, D.S. and Kass, R.S. (1986) J. Gen. Physiol. 88, 369–392.
[28] Varadi, G., Lory, P., Schultz, D., Varadi, M. and Schwartz, A. (1991) Nature 352, 159–162.
[29] Al-Dahan, M.I. and Thalmann, R.H. (1989) J. Neurochem. 53, 982–985.
[30] Breitwieser, G.E. and Szabo, G. (1988) J. Gen. Physiol. 91, 469–493.
[31] Higashijima, T., Ferguson, K.M., Smigel, M.D. and Gilman, A.G. (1987) J. Biol. Chem. 262, 757–761.
[32] Vuong, T.M. and Chabre, M. (1991) Proc. Natl. Acad. Sci. USA 88, 9813–9817.
[33] Arshavsky, V.Y. and Bownds, M.D. (1992) Nature 357, 416–417.
[34] Yatani, A., Okabe, K., Polakis, P., McCormick, F. and Brown, A.M. (1990) Cell 61, 769–776.