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Drosophila stimulatory G protein α subunit activates mammalian adenylyl cyclase but interacts poorly with mammalian receptors: Implications for receptor–G protein interaction

(vaccinia virus expression)

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ABSTRACT **Heterotrimeric guanine nucleotide binding proteins (G proteins) transduce signals from cell-surface receptors to intracellular effector proteins. Two forms of stimulatory G protein (G_s) α-like subunit have been described in *Drosophila melanogaster*. To examine the function of these subunits we have used vaccinia virus vectors to express both proteins in cyc$^-$ cells, a murine S49 cell line deficient for $G_s\alpha$ activity. Receptor-independent activation of each *Drosophila* $G_s\alpha$ has demonstrated that both forms are capable of activating mammalian adenylyl cyclase and thus have the activity expected of stimulatory G proteins. However, the *Drosophila* $G_s\alpha$ subunits interact poorly with mammalian G_s-coupled receptors. These observations have helped to identify a region of high variability in $G_s\alpha$ proteins that may be important for receptor interactions.**

Cellular responses to a wide variety of extracellular signals are mediated by a family of guanine nucleotide binding proteins (G proteins). These proteins couple the receptors for hormones, neurotransmitters, and sensory signals to intracellular effector proteins (1, 2). The interaction of a G protein with an activated agonist–receptor complex promotes the exchange of GTP for bound GDP, causing a conformational change in the α subunit that enables it to modulate the activity of the appropriate effector proteins. The α subunit is, therefore, responsible for the specific interactions with both the receptor and effector molecules. The β–γ subunit complex is responsible for inactivation of the α subunit and is also necessary for interaction of the G protein with receptors (1, 2).

The study of the mechanism of G protein function has largely been directed toward defining the functional domains of the α subunit. These include a domain responsible for high-affinity guanine nucleotide binding and hydrolysis and domains responsible for interaction with receptors, effectors, and the β–γ subunit complex (1, 2).

The three dimensional x-ray crystal structures of the guanine nucleotide binding domains of the bacterial elongation factor Tu (3, 4), and human RAS (5) have been determined. These structures, together with the phenotypes of engineered or naturally occurring mutations of RAS proteins (6, 7), have confirmed the involvement of three highly conserved regions (8, 9) in mediating GTPase activity and conformational switches upon GTP/GDP binding. By aligning the corresponding regions of Gα subunits, models of Gα structure have been developed (10, 11). Amino-terminal and carboxyl-terminal regions are proposed to form the domains responsible for the β–γ and receptor interactions, respectively (10, 11). Specific functions have been assigned to those regions that contribute to the guanine nucleotide binding site (11). The G box is proposed to interact with the guanosine residue, while the PO_4 box makes contacts with the phosphate groups. The S box is thought to mediate the activating/inactivating conformational switch caused by GTP/GDP binding.

Experimental support for these models of Gα structure has come from biochemical, genetic, and molecular biological manipulation of Gα proteins. Partial proteolysis suggests the involvement of the amino terminus in binding of the β–γ subunit complex (12, 13). The *unc* mutation (14) and pertussis toxin (15, 16) both disrupt G protein–receptor interactions by changing or modifying amino acid residues near the carboxyl terminus. Studies of chimeric α subunits have localized the receptor domain to the carboxyl-terminal 40% of the α subunit (17). These studies also suggest that the effector domain is also located in this region (17).

The stimulatory G protein (G_s) is responsible for activation of adenylyl cyclase in response to a variety of hormonal stimuli (1, 2). The $G_s\alpha$ subunit is well suited for studies of functional domains, since the activity and mode of action of the $G_s\alpha$ subunit is the most clearly defined. In addition, the cyc$^-$ variant of the murine S49 lymphoma cell line is deficient for endogenous $G_s\alpha$ mRNA and protein (18) and thus can be used to assay the function of modified $G_s\alpha$ subunits.

We have previously described the isolation of cDNAs encoding two forms of $G_s\alpha$-like protein from *Drosophila melanogaster* (19, 20). These proteins differ primarily by the inclusion (long form, $DG_s\alpha L$) or deletion (short form, $DG_s\alpha S$) of three amino acids near the carboxyl terminus. Sequence comparisons predict that the *Drosophila* $G_s\alpha$ homologs should be capable of interacting with both the mammalian adenylyl cyclase and G_s-coupled receptors. For example, the *Drosophila* and rat $G_s\alpha$ proteins differ by only three conservative amino acid replacements over the carboxyl-terminal 59 residues (19), the region proposed to be responsible for interaction with receptors. Tests of these predictions would aid in defining α-subunit functional domains and thus provide specific tests of the various models of G_α structure and function.

In this report, we have tested the ability of *Drosophila* $G_s\alpha$-like proteins to functionally complement the lack of endogenous $G_s\alpha$ function in S49 cyc$^-$ cells. The *Drosophila* $G_s\alpha$ homologs are capable of stimulating mammalian adenylyl cyclase and thus have the activity expected of stimulatory G proteins. Surprisingly, the *Drosophila* $G_s\alpha$ subunits interact poorly with mammalian receptors. These observations have helped to define a region of high variability in $G_s\alpha$ proteins that is likely to be important for efficient receptor–G protein interactions.

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Abbreviations: G protein, guanine nucleotide binding protein; G_s, stimulatory G protein; VV, vaccinia virus(es); wt, wild type; GTP[γS], guanosine 5′-[γ-thio]triphosphate; CTX, cholera toxin.

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MATERIALS AND METHODS

Viruses and Cells. Wild-type (VV:wt; WR strain) and recombinant (VV) vaccinia viruses were propagated in African green monkey BSC40 cells. Partially purified virus stocks were prepared as described (21).

Construction of Recombinant VV. cDNAs encoding the long (pDG$_s\alpha$L) and short (pDG$_s\alpha$S) forms of *Drosophila* G$_s\alpha$ have been described (19, 20). pS/Q215L was derived from pDG$_s\alpha$S by site-directed mutagenesis (see below) and encodes an α subunit in which Gln-215 is replaced by Leu. A cDNA encoding the 52-kDa form of rat G$_s\alpha$ (22) was kindly provided by R. Reed (Johns Hopkins University School of Medicine, Baltimore). Restriction fragments containing the amino acid coding regions of these cDNAs were subcloned into the *Bam*HI site of the vaccinia transfer vector pVV3 (21). The resulting plasmids were mixed with VV:wt genomic DNA and transfected by calcium phosphate precipitation into Ltk$^-$ cells that had been infected with VV:wt 3 hr previously. Recombinant viruses were purified by marker rescue (23).

Viral Infections of ck$^-$ Cells. ck$^-$ cells were spun down and washed in phosphate-buffered saline containing 1 mM $MgCl_2$ (PBS/Mg^{2+}). Cells were then resuspended in PBS/Mg^{2+}/0.1% bovine serum albumin at a density of $\approx 3 \times 10^7$ cells per ml. Infections were done in 100-mm tissue culture dishes (Nunc). VV was added at a multiplicity of infection of 40 and the infection was carried out at 30°C. After 2 hr, Dulbecco's modified Eagle's medium containing 10% heat-inactivated horse serum and gentamicin (5 μg/ml) was added directly to the infection mixture such that the final cell density was 6×10^6 cells per ml. Cells were incubated at 37°C for 14–18 hr before preparation of membranes.

Preparation of Membranes. All manipulations were carried out at 4°C. Cells were washed twice with PBS/Mg^{2+} and resuspended in 20 mM Tris·HCl, pH 7.5/2.5 mM $MgCl_2$/1 mM EDTA (TME). Cells were disrupted by Dounce homogenization and spun at $2000 \times g$ for 10 min. The pellet was resuspended in TME and reextracted. The combined supernatants were subjected to ultracentrifugation at $100,000 \times g$ for 1 hr. The pellet was resuspended in a minimal volume of TME. Protein concentrations were determined by the BCA method (24).

Western Blots. Western blots were prepared as described (25). RM is an affinity-purified rabbit antibody directed against a synthetic peptide (Arg-Met-His-Leu-Arg-Gln-Tyr-Glu-Leu-Leu) corresponding to the carboxyl terminus of vertebrate and *Drosophila* G$_s\alpha$ (26). The reaction of this antibody with both *Drosophila* G$_s\alpha$ homologs has been described (27). Cross-reacting proteins were visualized by using ^{125}I-conjugated protein A (Amersham).

Adenylyl Cyclase Assays. Adenylyl cyclase assays were performed in 100-μl vol containing 50 mM Tris·HCl (pH 8.0), 2.5 mM $MgCl_2$, 1 mM EDTA, 2 mM 2-mercaptoethanol, 10 mM creatine phosphate, 0.4 mM ATP, phosphocreatine kinase (100 units/ml), and bovine serum albumin (100 mg/ml). Additional components were included, as indicated, at the following concentrations: 50 μM GTP, 100 μM guanosine 5'-[γ-thio]triphosphate (GTP[γS]), AlF_4^- (10 mM NaF, 10 μM $AlCl_3$), 50 μM forskolin, 100 μM (−)-isoproterenol, 10 μM prostaglandin E_1. Reactions were initiated by the addition of 50 μg of membrane protein and were allowed to proceed at 30°C for 30–40 min. Reactions were terminated by the addition of 2 vol of absolute ethanol. The ethanol extract was lyophilized and cAMP was resuspended in 10 mM Tris·HCl, pH 7.5/4 mM EDTA. cAMP levels were determined by using the Amersham [^{3}H]cAMP assay kit according to their protocol.

Cholera Toxin (CTX) Treatment. Membranes were treated with CTX (preactivated by dithiothreitol treatment) as described (28).

Site-Directed Mutagenesis. Site-directed mutagenesis was by the method of Kunkel (29) with a Bio-Rad kit according to their protocol. Codon 215 (CAG) of pDG$_s\alpha$S, encoding glutamine, was changed to CTG (leucine) using the antisense oligonucleotide 5'-CTCGTCCCGCAGGCCACCGAC-3'. Mutants were identified by dideoxynucleotide sequencing (30).

RESULTS

Expression of G$_s\alpha$ Subunits in ck$^-$ Cells. cDNAs encoding the long and short forms of *Drosophila* G$_s\alpha$ (20) and the long form of rat G$_s\alpha$ (22) were subcloned into the vaccinia transfer vector pVV3 (21) and used to generate recombinant VV by homologous recombination (23). These viruses were then used to express G$_s\alpha$ subunits in S49 ck$^-$ cells, a murine lymphoma cell line lacking endogenous G$_s\alpha$ mRNA and protein (18). Expression levels were assessed by Western blot analysis of membrane proteins probed with the RM antisera (26). The results of a representative infection are shown in Fig. 1. S49wt cells express the previously described long and short forms of vertebrate G$_s\alpha$ (31) (lane 1). As expected, no immunoreactive protein is seen in ck$^-$ cells (lane 6) or in ck$^-$ cells infected with VV:wt at high multiplicity of infection (lane 7). However, infection of ck$^-$ cells with recombinant VV resulted in the expression of RM-immunoreactive proteins of the appropriate sizes (lanes 2–4). Each of the *Drosophila* proteins is expressed at similar but reduced levels relative to the endogenous G$_s\alpha$ subunits of S49wt cells (Fig. 1, compare lane 1 to lanes 3 and 4). In contrast, the rat G$_s\alpha$ subunit is expressed at higher levels (lane 2). The relative levels of expression were quantitated by liquid scintillation counting of excised immunoreactive bands. In the membrane preparations shown in Fig. 1, the levels of *Drosophila* G$_s\alpha$ expression were $\approx$15% (DG$_s\alpha$L), and 20% (DG$_s\alpha$S) that of the VV-expressed rat α subunit (100%). The levels of VV-expressed rat α subunit are $\approx$15% higher than the levels of endogenous G$_s\alpha$ found in S49wt cells.

***Drosophila* G$_s\alpha$ Subunits Stimulate Mammalian Adenylyl Cyclase.** The ability of the virally expressed G$_s\alpha$ subunits to stimulate adenylyl cyclase in ck$^-$ membranes in response to a number of activating agents was tested. Shown in Fig. 2 are results obtained from the membranes used for the immunoblot analyses described above (Fig. 1). The activity of the *Drosophila* subunits was compared to that of the rat G$_s\alpha$ and to the activities of the endogenous G$_s\alpha$ subunits of membranes from S49wt cells. Membranes from ck$^-$ cells and ck$^-$ cells infected with VV:wt were used as negative controls.

GTP[γS] is a poorly hydrolyzed analogue of GTP that causes persistent activation of α subunits (1, 2). AlF_4^- ions also activate α subunits, perhaps by mimicking the γ-phosphate of GTP in GDP-bound forms of α subunits (32, 33). As expected, these agents are able to stimulate adenylyl cyclase activity in S49wt membranes (Fig. 2). These agents also stimulate adenylyl cyclase activity in ck$^-$ membranes con-

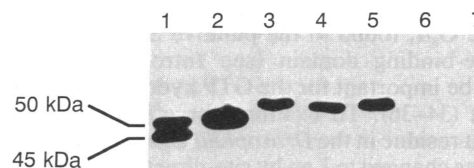

FIG. 1. Expression of Gα subunits in ck$^-$ cells using VV vectors. The infection of ck$^-$ cells with recombinant VV and preparation of membranes were as described. Membrane proteins (50 μg per lane) were separated on SDS/11% polyacrylamide gel, transferred to nitrocellulose, and probed with the RM antibody (26). ck$^-$ cells were infected with VV:wt (lane 7) or recombinant VV encoding rat G$_s\alpha$ (lane 2), DG$_s\alpha$L (lane 3), DG$_s\alpha$S (lane 4), and αS/Q215L (lane 5). Membranes were also prepared from uninfected S49wt (lane 1) and ck$^-$ cells (lane 6).

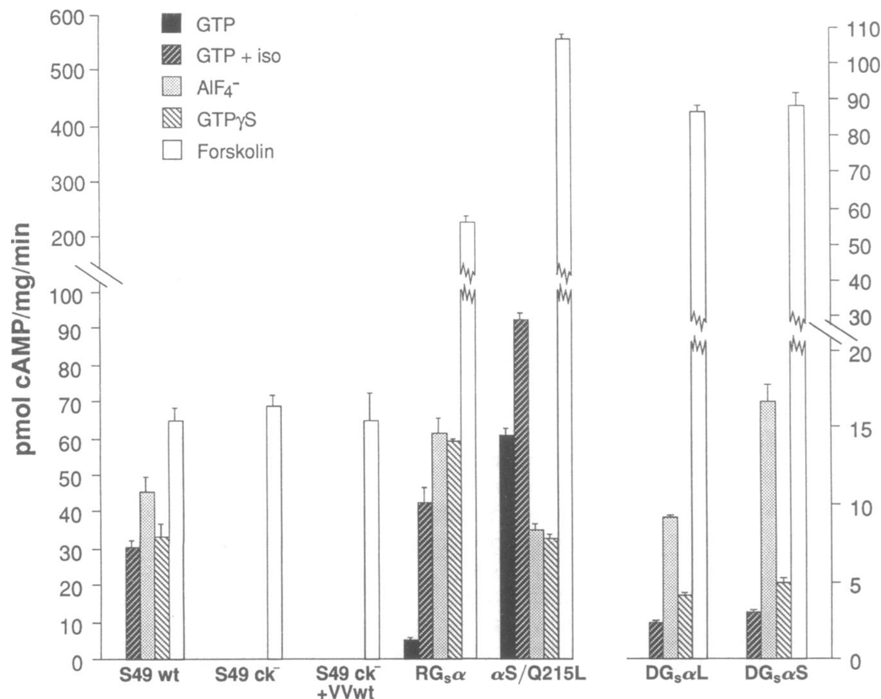

FIG. 2. Activation of adenylyl cyclase activity in S49 cell membranes. Adenylyl cyclase activity measurements were carried out as described. $G_s\alpha$ subunits expressed are as indicated. Values represent the means ± SE for duplicate or triplicate determinations using the membrane preparations characterized in Fig. 1. These results are representative of four independent experiments and, in the case of the virally expressed subunits, were done with two independent membrane preparations. Note the change in scale for membranes containing $DG_s\alpha L$ and $DG_s\alpha S$.

taining VV-expressed rat $G_s\alpha$ (Fig. 2), demonstrating that VV infection does not interfere with the coupling of $G\alpha$ subunits to effector proteins.

GTP[γS] and AlF_4^- are also each able to stimulate adenylyl cyclase activity in $DG_s\alpha S$ and $DG_s\alpha L$ membranes (Fig. 2). The effect is qualitatively similar to that seen in membranes containing mammalian $G_s\alpha$ subunits. No response is seen in membranes from ck^- cells or ck^- cells infected with VV:wt (Fig. 2). These results demonstrate clearly that *Drosophila* $G_s\alpha$-like proteins are able to stimulate mammalian adenylyl cyclase and thus have the activity expected of stimulatory G proteins.

Quantitatively, the response of adenylyl cyclase to GTP[γS] and AlF_4^- in membranes expressing the *Drosophila* subunits is much less than that observed for rat $G_s\alpha$ membranes. This is likely due, at least in part, to differences in the levels of expression of the *Drosophila* and rat proteins (see Fig. 1). A correction for expression levels can be made on the basis of the quantitative immunoblot analysis described above. AlF_4^--stimulated adenylyl cyclase activity in $DG_s\alpha S$ and $DG_s\alpha L$ membranes was ≈20% ($n = 2$) and ≈15% ($n = 2$), respectively, that found for rat $G_s\alpha$ membranes (Fig. 2). After normalization for the amount of immunoreactive $G_s\alpha$ (see above), the corrected values are 135% ($DG_s\alpha S$) and 75% ($DG_s\alpha L$) of rat $G_s\alpha$ membranes. This analysis suggests that the *Drosophila* $G_s\alpha$ proteins are comparable to the vertebrate $G_s\alpha$ subunits in their ability to activate mammalian adenylyl cyclase.

Constitutive Activation of Adenylyl Cyclase. Gln-227 of vertebrate $G_s\alpha$, found in the putative S box of the guanine nucleotide-binding domain (see Introduction), has been shown to be important for the GTP hydrolytic activity of the α subunit (34–36). To examine the effect of mutating the equivalent residue in the *Drosophila* $G_s\alpha$ subunits, Gln-215 of $DG_s\alpha S$ was changed to Leu by site-directed mutagenesis (29). The mutated cDNA, designated pS/Q215L, was then inserted into a VV vector for expression in ck^- cells. Western blot analysis demonstrated that αS/Q215L was expressed in ck^- cells at levels similar to that of $DG_s\alpha S$ and $DG_s\alpha L$ (Fig. 1, compare lanes 3 and 4 with lane 5). The level of expression relative to the rat $G_s\alpha$ (100%) was ≈20%.

The expression of αS/Q215L in ck^- cells has a dramatic effect on mammalian adenylyl cyclase activity (Fig. 2). Basal adenylyl cyclase activity (GTP only) in αS/Q215L membranes is greatly elevated relative to that of *Drosophila* $G_s\alpha$ or rat $G_s\alpha$ membranes. Relative to rat $G_s\alpha$ membranes, basal activity is increased 12-fold ($n = 4$). The magnitude of this elevation is even greater (60-fold) when relative expression levels are taken into account. The Gln-215 to Leu mutation in *Drosophila* $G_s\alpha$ subunits therefore results in the constitutive activation of mammalian adenylyl cyclase.

Receptor activation (GTP plus isoproterenol) increases adenylyl cyclase activity in αS/Q215L membranes an additional 40% ($n = 4$) over basal levels (GTP only; Fig. 2). Adenylyl cyclase activity is reduced in the presence of either GTP[γS] or AlF_4^- (Fig. 2). This effect has also been observed in ck^- membranes expressing the rat Gln-227 to Leu subunit and may be due to the activation of inhibitory G proteins by these agents (35).

Potentiation of Forskolin-Stimulated Adenylyl Cyclase Activity. Activated $G_s\alpha$ subunits have been shown to potentiate the ability of forskolin to stimulate the activity of adenylyl cyclase (37, 38). This effect is also observed in ck^- cells expressing the *Drosophila* and rat $G_s\alpha$ subunits (Fig. 2). The magnitude of the effect is correlated with the activity of the expressed subunits. In ck^- membranes containing high levels of rat $G_s\alpha$ subunit (Fig. 1), forskolin-stimulated adenylyl cyclase activity was increased 3.5-fold ($n = 4$) over that of ck^- membranes. A small increase (1.3-fold) ($n = 4$) was observed in $DG_s\alpha S$ and $DG_s\alpha L$ membranes, consistent with the lower levels of expression of these subunits. Forskolin-stimulated adenylyl cyclase activity in αS/Q215L-containing membranes was increased 8.6-fold ($n = 4$) over that of ck^- membranes.

CTX Modification. $G_s\alpha$ subunits are susceptible to modification by CTX (1, 2) at an internal arginine residue (39), resulting in a reduction in the intrinsic GTPase activity of the α subunit and constitutive activity. The conservation of the susceptible arginine in the *Drosophila* $G_s\alpha$ subunits and the high homology of the invertebrate and vertebrate proteins in the region flanking this residue suggests that the *Drosophila* $G_s\alpha$ subunits are also CTX substrates (19, 20). CTX treatment of ck^- membranes containing VV-expressed $G\alpha$ subunits (Fig. 3) results in the constitutive activation (GTP only) of adenylyl cyclase. In rat $G_s\alpha$ membranes, CTX treatment results in adenylyl cyclase activity similar to that found in the presence of AlF_4^-. In the case of the *Drosophila* $G_s\alpha$ subunits,

adenylyl cyclase activities are reduced relative to those found with AlF_4^-. This is likely due to incomplete modification of the *Drosophila* subunits. No adenylyl cyclase activity was observed in mock-treated membranes (data not shown). Since basal activity was previously observed in rat $G_s\alpha$ membranes (see Fig. 2), some G-protein function is lost as a result of the manipulations involved in CTX treatment. This effect has been observed by others (35). No effect of CTX was found in ck^- membranes or in ck^- membranes infected with VV:wt. These results demonstrate that the *Drosophila* $G_s\alpha$ subunits, like their mammalian counterparts, are substrates for functional modification by CTX.

Receptor Interactions. The ability of the *Drosophila* $G_s\alpha$ proteins to interact with mammalian G-protein-coupled receptors was tested by examining the ability of isoproterenol, a β-adrenergic agonist, to stimulate adenylyl cyclase activity. As shown in Fig. 2, basal adenylyl cyclase activity (GTP alone) was detected only in rat $G_s\alpha$ membranes. This is likely a result of the high expression levels of the rat $G_s\alpha$ achieved in ck^- cells. No receptor-stimulated (GTP plus isoproterenol) adenylyl cyclase activity is found in membranes from ck^- cells or ck^- cells infected with VV:wt (Fig. 2). As expected, receptor activation causes a large increase in adenylyl cyclase activity in both S49wt and rat $G_s\alpha$ membranes. These results again indicate that VV infection does not interfere with receptor–G-protein coupling.

Receptor activation results in a small increase in adenylyl cyclase activity in $DG_s\alpha S$ and $DG_s\alpha L$ membranes (Fig. 2). The receptor-stimulated adenylyl cyclase activity in $DG_s\alpha S$ and $DG_s\alpha L$ membranes was ≈7% (n = 2) and 5% (n = 2), respectively, that observed for rat $G_s\alpha$ membranes. After correcting for the amount of immunoreactive $G_s\alpha$ (see above), the receptor-stimulated activity is 35% ($DG_s\alpha S$) and 25% ($DG_s\alpha L$) that observed for rat $G_s\alpha$ membranes. Alternatively, the relative efficiency of coupling to the receptor can be compared by using the ratio of receptor-dependent activation (isoproterenol plus GTP) to receptor-independent activation (AlF_4^-). In the case of both S49wt and rat $G_s\alpha$ membranes, this ratio is 0.68 (n = 4). For $DG_s\alpha S$ and $DG_s\alpha L$ membranes, this ratio is 0.18 (n = 2) and 0.25 (n = 2), respectively. This analysis suggests that the *Drosophila* $G_s\alpha$ subunits interact inefficiently with the mammalian β-adrenergic receptor.

The prostaglandin E_1 receptor stimulates adenylyl cyclase through $G_s\alpha$ in S49 cells (17). Adenylyl cyclase is activated by prostaglandin E_1 in cells expressing rat $G_s\alpha$ to levels similar to that observed with isoproterenol (data not shown). However, in cells expressing either of the *Drosophila* $G_s\alpha$ proteins, adenylyl cyclase is poorly activated though this receptor (data not shown), indicating that the interaction of the *Drosophila* $G_s\alpha$ subunits with mammalian G-protein-coupled receptors is generally inefficient.

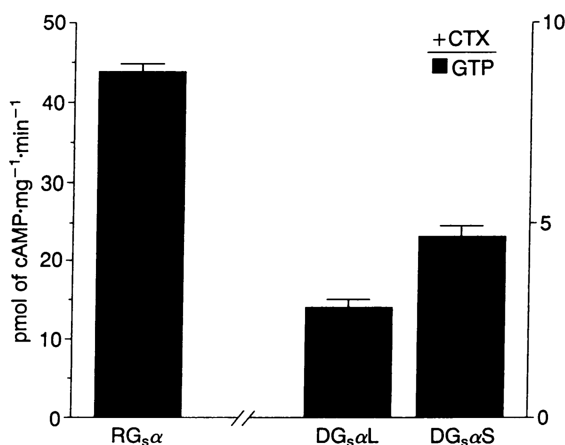

FIG. 3. CTX activation of $G_s\alpha$ subunits. Membranes were treated with CTX and adenylyl cyclase activities were determined, in the presence of GTP, as described. Values represent means ± SE of duplicate determinations and represent two independent experiments.

DISCUSSION

In this study, we have shown that the *Drosophila* $G_s\alpha$-like subunits function in a manner similar to their mammalian homologs. Both forms of *Drosophila* $G_s\alpha$ are able to activate mammalian adenylyl cyclase and can be constitutively activated either by mutating a residue shown to be important for regulating GTPase activity in the mammalian subunits (34–36) or by treatment with CTX. Surprisingly, the *Drosophila* subunits interact with mammalian receptors in an inefficient manner.

To study *Drosophila* $G_s\alpha$ subunit function, recombinant VV were used to express these subunits in S49 cyc^- cells, a murine lymphoma cell line deficient for endogenous $G_s\alpha$ activity. As shown in Fig. 1, the VV system used here efficiently expresses $G_s\alpha$ subunits in ck^- cells. In particular, the rat $G_s\alpha$ subunit is expressed at levels that exceed those of the endogenous $G_s\alpha$ subunits in S49wt cells. In addition, VV infection does not interfere with G-protein-coupled signal transduction events since VV-expressed rat $G_s\alpha$ is able to mediate efficient receptor-dependent and receptor-independent activation of adenylyl cyclase in cyc^- cells. VV vectors therefore can be used to efficiently express cDNAs in ck^- cells. This system should be particularly useful for rapidly assessing the activity of modified or chimeric $G_s\alpha$ proteins.

The expression levels of the *Drosophila* subunits, while substantial, are consistently lower than those of the rat $G_s\alpha$, and this may be a reflection of different patterns of codon usage in mammalian and invertebrate genes or of differences in the stabilities of the mRNAs due to differences in untranslated regions. Alternatively, as a distinct consensus sequence for *Drosophila* translation initiation codons has been reported (40), the translational initiation of *Drosophila* mRNAs in mammalian cells may be inefficient.

Both long and short species of *Drosophila* $G_s\alpha$ are able to stimulate adenylyl cyclase activity in ck^- cells in response to agents that activate $G\alpha$ subunits directly (GTP[γS], AlF_4^-). When relative expression levels are taken into account, each subunit is able to activate adenylyl cyclase with an efficiency similar to that observed for the rat $G_s\alpha$ subunit. The *Drosophila* $G_s\alpha$ subunits therefore have the activity expected of stimulatory G proteins as predicted from the high level of homology (70% identity) between the *Drosophila* and mammalian $G_s\alpha$ subunits.

Mutational analysis has identified $G\alpha$ residues involved in guanine nucleotide binding and hydrolysis (34–36). For example, replacement of Gln-227 by Leu reduces the k_{cat} for GTP hydrolysis by >100-fold and results in the constitutive activation of adenylyl cyclase (35, 36). The Gln-227 to Leu mutation does not appear to completely abolish GTPase activity as receptor activation increases adenylyl cyclase activity (35).

A mutation corresponding to the vertebrate Gln-227 to Leu mutation was introduced into the short form of *Drosophila* $G_s\alpha$ by site-directed mutagenesis (αS/Q215L). The functional consequences of this mutation are similar to those of the vertebrate Gln-227 to Leu mutation. A strong constitutive activation of adenylyl cyclase activity is observed when the mutant subunit is expressed in ck^- cells. Receptor activation results in a slight increase in adenylyl cyclase activity over basal levels. By analogy to the vertebrate Gln-227 to Leu mutation, the Gln-215 to Leu mutation is likely to dramatically reduce but not abolish the intrinsic GTPase activity of the *Drosophila* subunit. It seems reasonable to conclude that the putative guanine nucleotide binding domain of the *Dro-*

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DGsαL (296-326) LSEYFSEFNKYQTPIDTGDAIMESNDDPEVI
                o•o  •  •o •  • ••••••      •  •
  Gsα (308-335) IEDYFPEFARYTTPEDATPEPGE---DPRVT
                       o • •      o•       o
Golfα (295-322) IEDYFPEYANYTVPEDATPDAGE---DPKVT
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FIG. 4. Comparison of amino acid sequences of *Drosophila* and vertebrate Gα subunits in a variable region of the putative receptor binding domain. Amino acids are indicated by the single-letter code. Solid dots indicate nonconservative differences, and open dots indicate conservative substitutions found in the DG$_s\alpha$L/rat G$_s\alpha$ and rat G$_s\alpha$/rat G$_{olf}\alpha$ comparisons.

sophila subunits is similar to that of the vertebrate Gα subunits. Additional evidence for the similarity of the guanine nucleotide binding domains of the vertebrate and *Drosophila* subunits is provided by CTX studies. CTX catalyzes the ADP ribosylation of vertebrate G$_s\alpha$ and G$_t\alpha$ at an arginine residue that forms part of the guanine nucleotide binding site (39). The *Drosophila* G$_s\alpha$ subunits are also subject to modification by CTX, as shown by the constitutive activation of adenylyl cyclase in DG$_s\alpha$L or DG$_s\alpha$S membranes treated with CTX.

A large body of evidence suggests that receptor interaction occurs at the carboxyl-terminal region of Gα subunits (14–17). Since the *Drosophila* and mammalian G$_s\alpha$ subunits are essentially identical over their carboxyl-terminal 59 residues and *Drosophila* G$_s\alpha$ subunits are capable of receptor-independent activation of adenylyl cyclase, it was expected that mammalian receptors could be coupled to an activation of adenylyl cyclase through the *Drosophila* G$_s\alpha$ proteins. Surprisingly, the *Drosophila* subunits couple poorly to both β-adrenergic and prostaglandin E$_1$ receptors. A comparison of the *Drosophila* G$_s\alpha$ and rat G$_s\alpha$ subunits over the receptor interaction domain identifies a 28-amino acid region that may be important for interactions with receptors (Fig. 4). This region follows the G box of the guanine nucleotide binding site (11) and includes an insertion of 13 amino acids specific to G$_s\alpha$ subunits (1, 2). This region also differentiates the long and short forms of *Drosophila* G$_s\alpha$ (20). As shown in Fig. 4, DG$_s\alpha$L and rat G$_s\alpha$ differ in 16 of 28 residues (3 conservative replacements). Differences in this region then may be responsible for the inefficient coupling of mammalian receptors to *Drosophila* G$_s\alpha$. Sequence comparisons of rat G$_s\alpha$ with G$_{olf}\alpha$, an olfactory-specific Gα (41), are consistent with this idea. G$_{olf}\alpha$ is 88% identical to rat G$_s\alpha$. When expressed in cyc$^-$ cells, G$_{olf}\alpha$ also interacts inefficiently with β-adrenergic receptors but to a greater extent than *Drosophila* G$_s\alpha$ subunits (42). Over the region of variability, rat G$_s\alpha$ and G$_{olf}\alpha$ differ in 6 residues (3 conservative) (Fig. 4).

In summary, the *Drosophila* G$_s\alpha$ subunits are similar in function to their mammalian counterparts. The efficiency of their interaction with mammalian adenylyl cyclase is similar to that of vertebrate G$_s\alpha$ subunits. The guanine nucleotide binding domain, as assessed by mutational analysis and CTX treatment, appears to be similar to that of mammalian G$_s\alpha$ proteins. Surprisingly, the *Drosophila* G$_s\alpha$ subunits are unable to interact with mammalian G$_s$-coupled receptors in an efficient manner. Amino acid sequence comparisons between the *Drosophila* G$_s\alpha$ subunits, vertebrate G$_s\alpha$, and G$_{olf}\alpha$ suggest a region in the putative receptor binding domain that may be responsible for these differences.

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