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Expression of $G_{o\alpha}$ mRNA and Protein in Bovine Tissues†

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ABSTRACT: $G_{o\alpha}$ is a 39-kDa guanine nucleotide-binding protein (G protein) similar in structure and function to $G_{s\alpha}$ and $G_{i\alpha}$ of the adenylate cyclase complex and to transducin ($G_{t\alpha}$) of the retinal photon receptor system. Although expression of $G_{o\alpha}$ protein has been reported to be tissue-specific, other workers have found $G_{o\alpha}$ mRNA in all rat tissues examined. In order to clarify this contradiction, studies to verify the distribution of $G_{o\alpha}$ mRNA and protein in bovine and rat tissues were performed. Tissues were screened for the presence of $G_{o\alpha}$ mRNA by use of a series of restriction fragments of a bovine retinal cDNA clone, λGO9, and oligonucleotide probes complementary to sequences specific among G_α subunits for the 5′ untranslated and coding regions of $G_{o\alpha}$. These probes hybridized predominantly with mRNA of 4.0 and 3.0 kb in bovine brain and retina. A 2.0-kb mRNA in retina also hybridized strongly with the cDNA but weakly with the oligonucleotide probes. In bovine lung, two mRNAs of 1.6 and 1.8 kb hybridized with the cDNA while only the 1.6-kb species hybridized with the coding-region oligonucleotide. In bovine heart, only a 4.0-kb mRNA was detected and in amounts much less than those in the other tissues. A similar distribution of $G_{o\alpha}$ mRNAs was seen in rat tissues. In bovine tissues, $G_{o\alpha}$ protein was identified with rabbit polyclonal antibodies directed against purified bovine brain $G_{o\alpha}$. An immunoreactive 39-kDa membrane protein was found principally in retina and brain, and in a lesser amount in heart. Thus, in the rat and bovine tissues examined, $G_{o\alpha}$ or $G_{o\alpha}$-like mRNAs, as well as $G_{o\alpha}$ protein, are expressed in a tissue-specific manner.

Guanine nucleotide-binding proteins (G proteins)[1] function as signal transducers from cell surface receptors to inner membrane effector enzymes in a number of regulatory systems (Stryer, 1986; Gilman, 1987). The G proteins include G_s and G_i, the stimulatory and inhibitory proteins of the adenylate cyclase system, the transducins, and G_o (Stryer, 1986; Gilman, 1987; Sternweis & Robishaw, 1984; Florio & Sternweis, 1985). Transducin activates a retinal cyclic GMP phosphodiesterase in response to photoexcitation of rhodopsin (Stryer, 1986). G_o, initially identified in brain, interacts in vitro with rhodopsin as well as muscarinic and other receptors; however, its physiological role is presently unclear (Sternweis & Robishaw, 1984; Florio & Sternweis, 1985; Kurose et al., 1986; Tsai et al., 1987; Ueda et al., 1988).

These G proteins share functional, structural, and immunological similarities (Stryer, 1986; Gilman, 1987; Moss & Vaughan, 1988). They are composed of three subunits: α, β, and γ (Stryer, 1986; Gilman, 1987; Hildebrandt et al., 1984). The β subunits appear to be very similar in all four proteins (Manning & Gilman, 1983). The γ subunits of the transducins are different from those of G_s, G_i, and G_o (Hildebrandt et al., 1985). The α subunits are unique for each G protein and presumably convey specificity to the interactions with cell surface receptors and effector enzymes (Northup et al., 1982, 1983; Brandt et al., 1983; Kanaho et al., 1984;

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[1] Abbreviations: G proteins, guanine nucleotide-binding regulatory proteins; G_s and G_i, stimulatory and inhibitory G proteins of adenylate cyclase; G_o, G protein purified from brain; $G_{s\alpha}$, α subunit of G_s; $G_{i\alpha}$, α subunit of G_i; $G_{o\alpha}$, α subunit of G_o; SDS, sodium dodecyl sulfate; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; GTPase, guanosinetriphosphatase.

Katada et al., 1984; Bokoch et al., 1983). They bind guanine nucleotides and possess GTPase activity (Gilman, 1987). The α subunits also contain sites for ADP-ribosylation by choleragen or pertussis toxin (Gilman, 1987; Moss & Vaughan, 1988). Activation of the α subunit occurs through interaction with a cell surface receptor which promotes the binding of GTP resulting in the dissociation of G_α·GTP from the $\beta\gamma$ complex (Gilman, 1987; Moss & Vaughan, 1988). The G_α·GTP subunit can then interact with the effector to modify its activity. Inactivation occurs with hydrolysis of GTP and reassociation of the $\alpha\beta\gamma$ complex (Gilman, 1987; Moss & Vaughan, 1988).

Recently, several laboratories have examined the distribution of different G proteins in various tissues using immunological techniques to identify protein (Mumby et al., 1986, 1988; Goldsmith et al., 1987) or Northern blot analysis of RNA using various probes (Jones & Reed, 1987; Beals et al., 1987; Codina et al., 1988). For most G proteins, the distributions of protein and mRNA are in good agreement; however, a discrepancy exists between these data for $G_{o\alpha}$. Mumby and co-workers (Mumby et al., 1986, 1988) report tissue-specific expression of $G_{o\alpha}$ protein in both rat and bovine species. In contrast, $G_{o\alpha}$ mRNAs were found to be present in all rat tissues examined (Jones & Reed, 1987). In order to clarify the question of $G_{o\alpha}$ expression in bovine and rat tissues, a bovine retinal cDNA clone, λGO9 encoding the entire amino acid sequence of $G_{o\alpha}$ and $G_{o\alpha}$-specific oligonucleotides were used to evaluate the distribution of $G_{o\alpha}$ mRNA. The distribution is compared with that of $G_{o\alpha}$ protein.

Materials and Methods

Materials. Pertussis toxin was purchased from List Biological Laboratories; *Bam*HI, *Eco*RI, *Hin*dIII, and *Sst*I were from New England Biolabs; [α-^{32}P]NAD and [α-^{32}P]dCTP were from New England Nuclear; horseradish peroxidase conjugated goat anti-rabbit IgG antiserum was from Bio-Rad. Random primer DNA labeling kits were from Amersham. Production and characterization of the rabbit anti-$G_{o\alpha}$ antiserum have been described (Tsai et al., 1987). The $G_{o\alpha}$ cDNA used in these experiments corresponds to the *Bss*HII–*Eco*RI restriction fragment of λGO9 described by Van Meurs et al. (1987) unless otherwise noted. Human fibroblast cytoplasmic γ-actin cDNA was kindly provided by Dr. Peter Gunning.

RNA Blot Hybridizations. Total RNA was isolated by the method of Chirgwin et al. (1979). Poly(A+) RNA was selected by oligo(dT)–cellulose chromatography, fractionated on a 1% agarose/formaldehyde gel, and transferred to nitrocellulose.

Hybridizations with ^{32}P-labeled cDNAs, prepared by the random primer method (Feinberg & Vogelstein, 1983), were performed in 40% formamide/5× SSC (1× SSC is 0.15 M NaCl/0.015 M sodium citrate)/10 mM Tris-HCl, pH 7.5/5× Denhardt's solution (1× Denhardt's is 0.02% poly(vinylpyrrolidone)/0.02% bovine serum albumin/0.02% Ficoll)/0.1% SDS/10% dextran sulfate containing denatured salmon sperm DNA, 100 μg/mL, at 42 °C for 18 h. Filters were washed once in 2× SSC/0.5% SDS at 65 °C for 20 min followed by two washes in 0.5× SSC/0.5% SDS at 65 °C for 20 min each. Filters were exposed to Kodak XAR film at −80 °C with intensifying screens.

Hybridizations with either a ^{32}P 5′ end-labeled 48-base oligonucleotide probe (5′GAAGGGCTCCGTGTCTTCCATCCGACTCACCACGTCACACACCATCTT3′) complementary to a region of the λGO9 nucleotide sequence unique to the $G_{o\alpha}$ coding sequence (nucleotides 313–360; Van Meurs et al., 1987) or a 32-base probe (5′AAAACAAAAATGTCGAGAAACCAGAACCCCCC3′) complementary to nucleotides −162 to −131 (+1 = ATG start codon) of the 5′ untranslated region were performed as described for the cDNA except formamide was deleted with the 32-mer. Filters were washed once in 2× SSC/0.5% SDS at 55 °C for 20 min followed by two washes in 0.5× SSC/0.5% SDS at 55 °C for 20 min each.

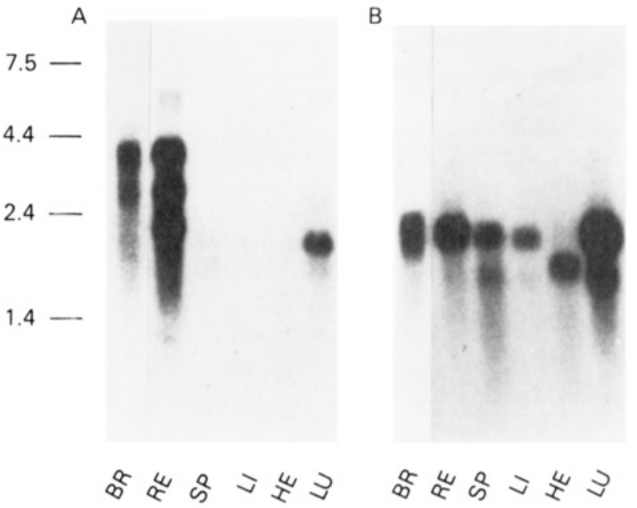

FIGURE 1: Detection of $G_{o\alpha}$ mRNA in bovine tissues with $G_{o\alpha}$ cDNA. (A) Poly(A+) RNA (5 μg) from the indicated bovine tissues was separated on a 1% agarose/formaldehyde gel, transferred to nitrocellulose, and analyzed by hybridization with ^{32}P-labeled λGO9 cDNA. (B) Shown is the blot from (A) hybridized with a ^{32}P-labeled γ-actin cDNA. Positions of RNA standards (nucleotides × 10^{-3}) are shown on the left. Abbreviations: BR, brain; RE, retina; SP, spleen; LI, liver; HE, heart; LU, lung.

Preparation of Tissue Membrane Fractions. Bovine tissues were homogenized in 4 volumes of TEND buffer (20 mM Tris-HCl, pH 8.0/1 mM EDTA/1 mM sodium azide/0.25 M sucrose/2 mM dithiothreitol/1 mM phenylmethanesulfonyl fluoride) with a Brinkmann Polytron. The homogenate was centrifuged (17000*g*, 20 min) and the resulting pellet washed twice with TEND buffer containing 0.15 M NaCl. The final pellet was used as the membrane fraction for protein analysis.

Western Blot Analysis. Proteins (100 μg) from various bovine tissues were separated by SDS–PAGE in a 12% gel (Laemmli, 1970) and transferred electrophoretically to a nitrocellulose filter in a Bio-Rad Transblot apparatus at 150 V for 10 h in 25 mM Tris/92 mM glycine buffer containing 20% methanol. Blots were incubated for 1 h at 30 °C in 20 mM Tris-HCl, pH 7.5/0.5 M NaCl/3% gelatin (TBS) followed by two rinses with TBS containing 0.05% Tween-20 (TTBS) and incubation for 4 h at 30 °C with rabbit anti-$G_{o\alpha}$ polyclonal antiserum (1:400 dilution in TTBS). After two rinses with TTBS, the blots were incubated for 2 h at 30 °C with horseradish peroxidase conjugated goat anti-rabbit IgG (diluted 1:2000 in TTBS containing 2% gelatin), rinsed twice with TTBS, and developed with peroxidase substrate (0.04% 4-chloro-1-naphthol, 0.005% hydrogen peroxide, and 15% methanol in TBS). Protein concentrations were determined according to a Coomassie blue dye-binding assay (Bio-Rad) with bovine serum albumin as standard.

Results

Detection of $G_{o\alpha}$ mRNA in Bovine Tissues. Brain (cerebrum) and retina contained two predominant mRNAs of approximately 4.0 and 3.0 kb that hybridized with the λGO9 cDNA (Figure 1A). In addition, a 2.0-kb mRNA consistently hybridized with the cDNA in retina and occasionally in brain.

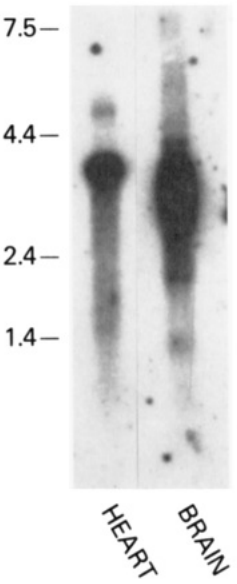

FIGURE 2: Detection of $G_{o\alpha}$ mRNA in bovine heart. Poly(A+) RNA from bovine heart was isolated by two successive passages over oligo(dT)–cellulose. Bovine brain RNA was subjected to one passage over oligo(dT)–cellulose. Heart (10 μg) and brain (5 μg) poly(A+) RNAs were hybridized as described in Figure 1. Positions of RNA standards (nucleotides $\times 10^{-3}$) are indicated on the left.

Reasons for the variable appearance of the 2.0-kb species in brain are unknown. It probably does not result from degradation of either the 3.0- or 4.0-kb species because the smaller form is always seen in retina in approximately the same quantity relative to those of the two larger RNAs. The inconsistent appearance of the 2.0-kb RNA may be due to differences in the amounts of this species present in the areas of the cerebrum used for RNA preparation. In contrast to the mRNAs observed in brain and retina, an intense band and a slightly smaller, weaker band in the 1.6–1.8-kb range were detected in lung by the cDNA probe. After prolonged autoradiographic exposure of the blot, a faint 4.0-kb band was seen in heart (data not shown). There was no detectable hybridization of the cDNA probe with mRNA from spleen or liver.

To assess the integrity of the isolated poly(A+) RNAs, the blot was stripped and reprobed with a full-length human fibroblast cytoplasmic γ-actin cDNA (Figure 1B). This probe, under the hybridization and wash conditions used in this experiment, hybridizes with all higher eukaryotic α- and β-actin mRNAs in addition to γ messages. In each tissue examined, mRNA hybridized with the actin cDNA. The pattern of mRNA bands was consistent with there being different muscle and nonmuscle forms of actin. A 2.0-kb mRNA in each nonmuscle tissue hybridized with the cDNA; in spleen and lung, an additional 1.6-kb mRNA was identified as well. In contrast, heart contained an intermediate 1.7-kb mRNA that hybridized with the actin probe, consistent with the presence of a cardiac muscle specific form of actin.

As noted, prolonged autoradiographic exposure of the blot revealed a faint 4.0-kb mRNA that hybridized with λGO9 in heart. To visualize the hybridization of the cDNA with heart RNA more clearly, poly(A+) RNA was isolated by two successive passages over oligo(dT)–cellulose. Hybridization of this RNA with the $G_{o\alpha}$ cDNA revealed a single 4.0-kb RNA species (Figure 2). On the basis of the purity and amount of RNA necessary for detection, there appears to be much less $G_{o\alpha}$ mRNA present in heart than in brain or retina.

The presence in several tissues of multiple mRNAs that hybridize with λGO9 raises the question of whether the cDNA

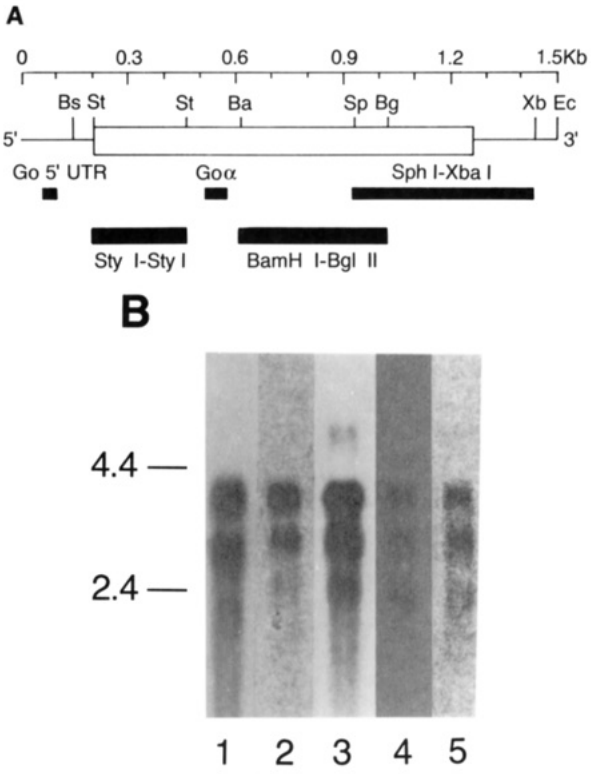

FIGURE 3: Hybridization of retinal mRNA with various λGO9-specific probes. (A) The schematic depicts a map of the bovine retinal cDNA clone, λGO9 (open bar represents the coding region), and the positions of the various restriction fragment and oligonucleotide probes (indicated by the shaded bars). (B) Bovine retinal poly(A+) RNA (10 μg) was analyzed as described in Figure 1 except that hybridizations were with the indicated probes: (1) oligonucleotide Go5′UTR; (2) *Sty*I–*Sty*I; (3) oligonucleotide Goα; (4) *Bam*HI–*Bgl*II; (5) *Sph*I–*Xba*I. Positions of RNA standards (nucleotides $\times 10^{-3}$) are shown on the left.

specifically hybridizes to $G_{o\alpha}$ mRNA under these experimental conditions. The cDNA coding sequences of different G_α subunits are very similar relative to those of unrelated proteins. To address this question of specificity, a series of probes were used to hybridize with retinal poly(A+) RNA (Figure 3A). Three of the probes were restriction fragments of λGO9 that span much of the coding and 3′ untranslated regions. The other two probes were oligonucleotides unique among G proteins to $G_{o\alpha}$. Probe Go5′UTR, consisting of 32 bases, is complementary to nucleotides −162 to −131 (+1 = ATG start codon) of the 5′ untranslated region of λGO9 and exhibits <30% identity with other α subunits. Probe Goα consists of 48 bases complementary to nucleotides 313–360 of the λGO9 coding region and is ≤50% identical with the corresponding regions of other G proteins. Each of these probes hybridized with three mRNAs of approximately 2.0, 3.0, and 4.0 kb (Figure 3B), consistent with the results observed with the *Bss*HII–*Eco*RI cDNA (Figure 1A). The intensities of the autoradiographic signals of the mRNA bands were observed to vary with the different probes, indicative of nonuniform hybridization; the basis for this is unknown.

To ensure that all mRNAs detected by the *Bss*HII–*Eco*RI cDNA in tissues other than retina were $G_{o\alpha}$ or $G_{o\alpha}$-related, the blot in Figure 1 was rehybridized with probe Goα (Figure 4). This probe hybridized with mRNAs of 3.0 and 4.0 kb in both retina and brain and the 2.0-kb mRNA in retina; in lung, the probe hybridized with the 1.6-kb mRNA but not the 1.8-kb species. No mRNAs were detected with probe Goα in equivalent amounts of poly(A+) RNA from liver, heart, or spleen.

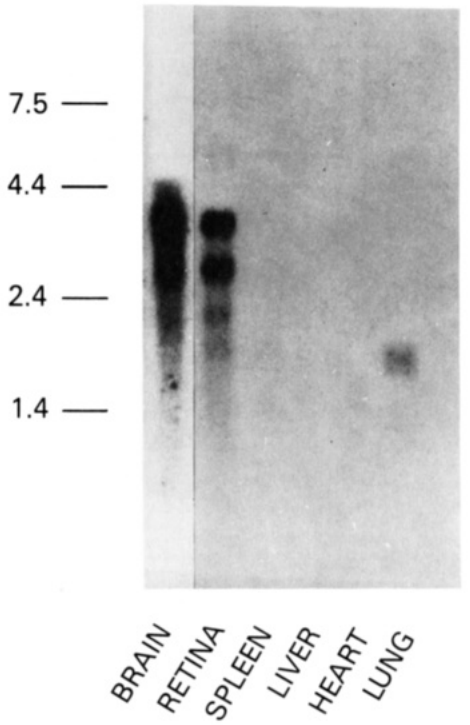

FIGURE 4: Detection of $G_{o\alpha}$ mRNA in bovine tissues with a $G_{o\alpha}$-specific oligonucleotide. Poly(A+) RNA (5 μg) from the indicated tissues was analyzed as described in Figure 1 except that a ^{32}P 5′ end-labeled 48-base oligonucleotide was used as the hybridization probe as described under Materials and Methods. Positions of RNA standards (nucleotides $\times 10^{-3}$) are shown on the left.

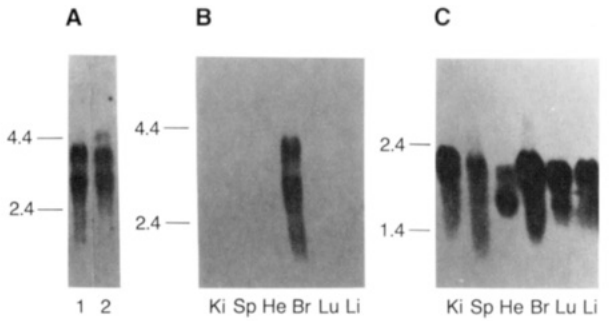

FIGURE 5: Detection of $G_{o\alpha}$ mRNA in rat tissues. (A) Rat brain poly(A+) RNA (10 μg) was analyzed as described in Figure 1 with (1) ^{32}P-labeled λGO9 cDNA or (2) ^{32}P 5′ end-labeled probe Goα. (B) Poly(A+) RNA (10 μg) from the indicated rat tissues was analyzed as in (A) with the λGO9 cDNA. (C) Shown is the blot from (B) hybridized with a ^{32}P-labeled γ-actin cDNA. Positions of RNA standards (nucleotides $\times 10^{-3}$) are shown to the left. Abbreviations: Ki, kidney; Sp, spleen; He, heart; Br, brain; Lu, lung; Li, liver.

To determine whether $G_{o\alpha}$ is expressed differently among rat tissues compared to bovine tissues, poly(A+) RNA from various rat tissues was evaluated for the presence of $G_{o\alpha}$ mRNA. mRNAs in the 3.0- and 4.0-kb range were detected in brain with both the *Bss*HII–*Eco*RI cDNA and probe Goα (Figure 5A); probe Go5′UTR failed to hybridize with either mRNA under experimental stringency conditions (data not shown). Comparison of the nucleotide sequence of probe Go5′UTR with the corresponding region of the rat $G_{o\alpha}$ cDNA (Jones & Reed, 1987) indicates an 81% identity. Among the rat tissues examined for the presence of $G_{o\alpha}$ mRNA by hybridization with the *Bss*HII–*Eco*RI cDNA, only brain displayed a detectable amount (Figure 5B). The integrity of the RNAs from each tissue was confirmed by hybridization with the γ-actin cDNA (Figure 5C).

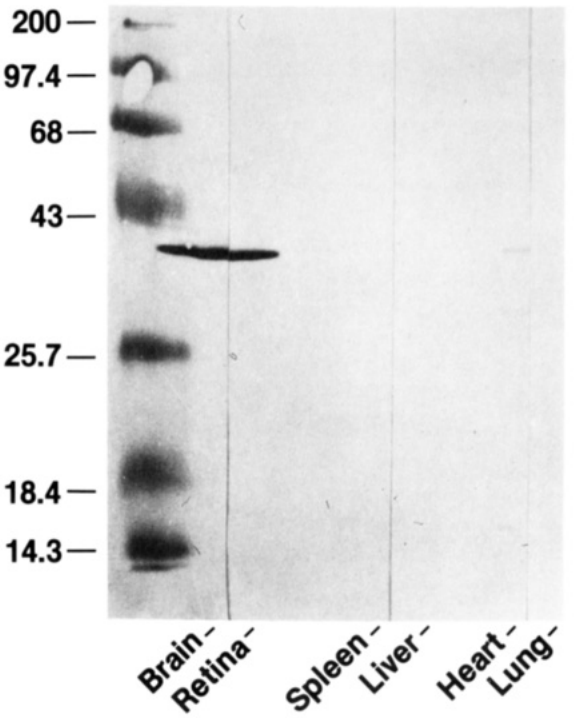

FIGURE 6: Detection of $G_{o\alpha}$ protein in bovine tissues. Membrane proteins (100 μg) from the indicated bovine tissues were separated by electrophoresis, transferred to a nitrocellulose filter, and reacted with rabbit anti-$G_{o\alpha}$ polyclonal antiserum. The immunoreactive protein bands were detected by the horseradish peroxidase method. Positions of protein standards ($M_r \times 10^{-3}$) are indicated on the left.

Expression of $G_{o\alpha}$ protein in the bovine tissues was evaluated with a rabbit anti-bovine brain $G_{o\alpha}$ polyclonal antiserum that has been shown not to react with G_s, G_i, or rod outer segment transducin (Tsai et al., 1987). This antiserum reacted with a 39-kDa protein in membranes from brain, retina, and heart (Figure 6); however, the quantity of $G_{o\alpha}$ in heart, on the basis of the intensity of the immunoreactive band, was much less than that in either retina or brain. Liver, lung, and spleen membranes did not contain detectable amounts of $G_{o\alpha}$ protein.

Discussion

Using a series of hybridization probes, including a bovine retinal $G_{o\alpha}$ cDNA encoding the entire amino acid sequence, we have examined the distribution of $G_{o\alpha}$ mRNA in various bovine tissues. In brain and retina, a predominant 4.0-kb mRNA and a 3.0-kb mRNA hybridized with the λGO9 cDNA. The cDNA hybridized to a third 2.0-kb species found consistently in retina and infrequently in brain. Lung poly(A+) contained two mRNAs between 1.6 and 1.8 kb in size that hybridized with the cDNA. Bovine heart contained only the 4.0-kb RNA species and in an amount much smaller than was found in brain and retina; $G_{o\alpha}$ mRNA was not detectable in liver or spleen.

Since G proteins exhibit considerable nucleotide identity, it was of concern that the cDNA, even under stringent conditions, might in fact hybridize to non-$G_{o\alpha}$ mRNA. To verify the identification of the $G_{o\alpha}$ mRNA, RNA from the various tissues was hybridized with a series of small restriction fragments of λGO9 as well as $G_{o\alpha}$-specific oligonucleotide probes complementary to a 48-base region of the cDNA sequence where G protein α subunits display minimal nucleotide identity (probe Goα) and a 32-base sequence of the 5′ untranslated region of λGO9 (probe Go5′UTR). On the basis of alignments of cDNA sequences, it is apparent that there is a high degree of interspecies conservation of the 5′ untranslated regions of

the individual G_α subunits. In contrast, the untranslated regions of the different G proteins are quite dissimilar. Hybridization patterns of these probes with retinal poly(A+) RNA were in each case consistent with that observed with the *Bss*HII–*Eco*RI cDNA. Probe Goα, when hybridized to poly(A+) RNA from various tissues, displayed a pattern very similar to, but not identical with, that of the *Bss*HII–*Eco*RI probe. Two of the mRNAs detected by the cDNA, the 2.0-kb retinal species and the 1.8-kb mRNA, hybridized weakly or not at all, respectively. The basis for the different hybridization patterns is unclear and is being examined further.

These studies also utilized a rabbit polyclonal antiserum against bovine brain $G_{o\alpha}$ to examine the distribution of $G_{o\alpha}$ protein. This antiserum, which has been shown not to react with $G_{s\alpha}$, $G_{i\alpha}$, or the transducins, reacted with 39-kDa proteins in membranes from bovine brain, retina, and heart. Paralleling the amounts of $G_{o\alpha}$ mRNA, the quantity of immunoreactive $G_{o\alpha}$ protein in heart was much less than that in either retina or brain. Although two lung mRNAs were identified as $G_{o\alpha}$-like, immunoreactive protein was not detected by the $G_{o\alpha}$ antibodies. The discrepancy between the apparent mRNA and protein distributions may result from hybridization of the cDNA and oligonucleotide probes to mRNAs that encode proteins closely related to $G_{o\alpha}$. Other investigators, using antibodies prepared against a $G_{o\alpha}$-specific 15 amino acid peptide, have seen a similar distribution for $G_{o\alpha}$ protein in rat and bovine tissues (Mumby et al., 1986, 1988).

These data reported here for the distribution of $G_{o\alpha}$ mRNA in bovine and rat tissues are contradictory to those of Jones and Reed (1987) for rat tissues. Our studies identify multiple $G_{o\alpha}$ or $G_{o\alpha}$-like mRNAs present in rat brain which hybridized with the *Bss*HII–*Eco*RI cDNA as well as probe Goα. In contrast to the results of Jones and Reed, who observed $G_{o\alpha}$ mRNA in all tissues examined, mRNA were not detected with the *Bss*HII–*Eco*RI probe in the other rat tissues. The reason for this discrepancy is not known. It could be argued that under low stringency conditions a cDNA containing as many bases as the *Bss*HII–*Eco*RI cDNA and as similar in nucleotide sequence as $G_{o\alpha}$ is with other G_α subunits might hybridize with non-$G_{o\alpha}$ mRNA. We have shown using a series of $G_{o\alpha}$-specific probes that under the hybridization conditions used in these studies only $G_{o\alpha}$ or $G_{o\alpha}$-like mRNAs were detected. In fact, probe Go5′UTR which is 81% identical with the corresponding region of the rat cDNA reported by Jones and Reed failed to hybridize with any mRNA present in rat brain. Therefore, probe Go5′UTR is not only specific for $G_{o\alpha}$ but specific for bovine $G_{o\alpha}$ under these experimental conditions. Moreover, a comparison of the 600-base rat $G_{o\alpha}$ 5′ untranslated region probe used by Jones and Reed against the Genbank databank indicated that their probe is no more specific against non-G proteins than probe Goα or Go5′UTR used in these studies. In conclusion, we have observed that both $G_{o\alpha}$ or $G_{o\alpha}$-related mRNAs and protein appear to be expressed in a tissue-specific fashion; expression, however, is not confined to neural tissue alone.

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