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Cell-specific expression of $G_{q/11}$ protein and mRNA in rat seminiferous tubules

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As part of a study to elucidate the involvement of G proteins in signal transduction in testicular cells, we have examined the cellular localization of $G_{q/11}$ within the seminiferous tubules. The somatic cells (Sertoli cells, peritubular cells) contain high amounts of both $G_{q/11}\alpha$ mRNA and immunoreactive protein. In contrast, very low levels of these G proteins and the corresponding mRNAs are present in the germ cells (pachythene spermatocytes, round spermatids). Thus, in the germ cells, receptor-regulated inositol phospholipid hydrolysis is not likely to be regulated via $G_{q/11}$, but rather through the G_o protein, which has been previously shown to be abundant in rat germ cells. Since the somatic cells are nearly devoid of G_o, the Gpp(NH)p-stimulated phospholipase C in these cells is probably regulated by G_q and/or G_{11}.

Guanine nucleotide-binding protein; Signal transduction; mRNA; Immunoreactive protein; Rat seminiferous tubule

1. INTRODUCTION

G proteins are an expanding family of GTP-binding proteins involved in signal transduction from activated receptors to effector enzymes or ion channels (reviewed in [1–3]). They consist of three distinct subunits (α, β and γ), and the diversity in these subunits contributes to a large number of possible G protein heterotrimers. Molecular cloning has revealed the existence of at least 17 genes [3].

The G_s class of proteins mediates the activation of adenylyl cyclase and voltage-gated Ca^{2+} channels [4], whereas members of the G_i class are involved in inhibition of adenylyl cyclase and stimulation of K^+ channels [5]. The G_o class may be a stimulator of phospholipase C [6,7], an inhibitor of Ca^{2+} channels [8], and a stimulator of K^+ channels [9]. Members of the recently cloned G_q family have been shown to activate phospholipase C in a pertussis toxin-insensitive manner [10–12].

As part of a study of the signal transduction in testicular cells, we have previously described the cell-specific expression of the cholera toxin-sensitive (G_s), as well as the pertussis toxin-sensitive G proteins (G_i and G_o) in rat testis [13]. We found that $G_{i3}\alpha$ is the only G_α-subunit present in all the testicular cell types examined (Sertoli cells, peritubular cells, pachythene spermatocytes and round spermatids), as demonstrated by the Western blot technique. In agreement with the fact that the germ cells lack hormone-sensitive adenylyl cyclase [14,15], $G_s\alpha$ was detected only in the somatic cell types [13,16]. Surprisingly, high levels of $G_o\alpha$ were found in the germ cells [13,17], whereas the somatic cells (Sertoli cells and peritubular cells) are almost devoid of this protein, which is highly abundant in the brain [18,19].

The α-subunits of G_q and G_{11} are present in many tissues and most abundant in brain and lung [20,21]. Far lower protein levels were found in testis, however, the cellular distribution in the testis has not been examined. In this work we have studied the distribution of $G_{q/11}\alpha$ in testicular cells by use of Northern and Western blot techniques.

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2. MATERIALS AND METHODS

2.1. *Cell preparation*

Primary cultures of Sertoli cells and peritubular cells were prepared from 19-day-old Sprague–Dawley rats as described by Dorrington et al. [22] and Hutson and Stocco [23], respectively. The cells were incubated at 34°C in a humidified atmosphere of 5% CO_2 in air. On the third day after plating in Eagle's Minimal Essential Medium (MEM; Gibco, Grand Island, NY) containing 10% foetal calf serum (Gibco), the Sertoli cells were incubated further in serum free MEM. RNA was extracted from the cells on day 5 after plating. The contamination of germ cells in the Sertoli cell preparation was less than 2%. The peritubular cells became confluent after approximately 10 days and were harvested for RNA extraction one day after confluence was reached. Sertoli cells containing lipid droplets were occasionally seen (<1%) in these preparations.

Pachytene spermatocytes and round spermatids were obtained from 32-day-old rats. The fractionation of the germ cells was performed in BSA gradients at unit gravity in a velocity sedimentation cell separator (STA-PUT) according to Grootegoed et al. [24]. RNA extraction from the cells was carried out immediately after the fractionation was completed. The germ cells were examined both by phase contrast microscopy and by regular light microscopy after fixation and staining. The purities were evaluated by counting cells; pachytene spermatocytes (PS) 80–95% and round spermatids (RST) 75–90%.

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2.2. *Preparation of total RNA*

RNA from tissue and cell preparations was extracted by homogenization in guanidinium isothiocyanate. Total RNA was isolated by centrifugation through a cesium chloride cushion and purified by phenol/chloroform extractions [25].

2.3. *Probe and antiserum*

Plasmid containing a cDNA clone encoding $G_{q/11}\alpha$ was kindly supplied by Dr. Lutz Birnbaumer (Houston, Texas, USA). Gel-purified insert was labeled by using [^{32}P]dCTP (Amersham, UK) and a standard nick-translation kit (Amersham). Antiserum against the COOH terminus of G_q and G_{11} α-subunits was kindly supplied by Dr. Allan M. Spiegel. The antiserum was used at 1/1,000 final dilution for Western analysis.

2.4. *Northern analysis*

Total RNA (20 μg) was denaturated in 50% formamide and 6% formaldehyde and size-fractionated on a 1.5% agarose gel containing 6.7% formaldehyde. The RNA was visualized by staining with ethidium bromide and then transferred to BioTrans nylon filter (ICN Schwarz/Mann Biotech, Cleveland, OH) by capillary blotting technique [26]. The hybridization was performed according to the ICN procedure with the nick-translated cDNA probe. Autoradiography was carried out with Amersham HMP film.

2.5. *Western analysis*

Homogenates from whole cells were resolved SDS-polyacrylamide gels (10%) [27], transferred onto Immobilon-P filters (Millipore, USA) and immunostained as described elsewhere [28,29]. Non-fat dry milk (5%) was used as a blocking agent.

3. RESULTS AND DISCUSSION

G_q and G_{11} are highly homologous proteins and show 88% amino acid identity [20]. In this study, we used an antiserum which is specific for the C-terminal region of the α-subunits of G_q and G_{11}, in Western analysis of various testicular cell types. As shown in Fig. 1, high levels of immunoreactive $G_{q/11}\alpha$ (approx. molecular mass 42 kDa) are present in the somatic cells (Sertoli cells and peritubular cells). Far lower levels are present in pachytene spermatocytes. In round spermatids, immunoreactive $G_{q/11}\alpha$ is hardly detectable. This cellular distribution of $G_{q/11}$ explains the low levels of $G_{q/11}$ protein in whole testis tissue reported by others [21].

Northern blot analysis of total RNA from testicular cells with the $G_{q/11}\alpha$ probe reveals 3 different transcripts of size 4.0, 6.0 and 7.5 kb, respectively (Fig. 2). Previous studies by Strahtmann and Simon [20] have shown that the two largest transcripts correspond to $G_q\alpha$ mRNA, whereas the 4.0 kb band represents $G_{11}\alpha$ mRNA. In the somatic cells, the larger transcripts are abundant compared to the 4.0 kb mRNA. Only weak signals for all these transcripts are observed in the germ cells. However, pachythene spermatocytes are the only cells that contained higher levels of the $G_{11}\alpha$-type transcript (4.0 kb) compared with the G_q messages (6.0 and 7.5 kb). This indicates that the $G_{11}\alpha$ gene is expressed in meiotic germ cells, although in low amounts.

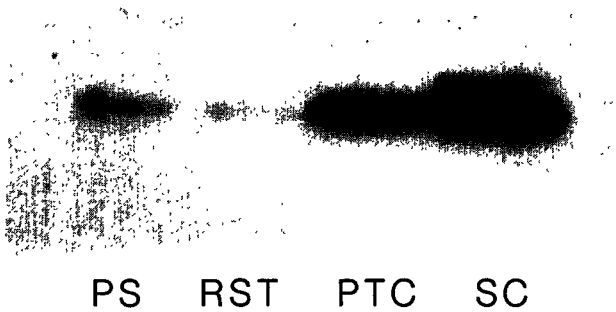

Fig. 1. Western analysis of homogenates (20 μg protein/lane) from various rat testicular cell types (PS, pachytene spermatocytes; RST, round spermatids; PTC, peritubular cells; SC, Sertoli cells). A typical immunoblot of $G_{q/11}\alpha$ is shown in the figure. The molecular mass of the proteins was detected with protein standards.

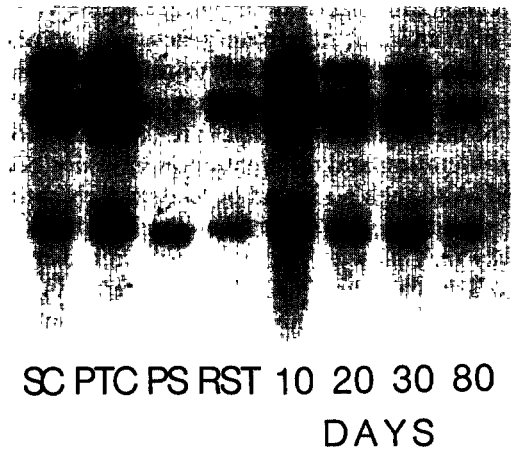

Fig. 2. Northern analysis of total RNA (20 μg RNA/lane) from various rat testicular cell types (SC, Sertoli cells; PTC, peritubular cells; PS, pachytene spermatocytes; RST, round spermatids) and whole testes of varying ages (10, 20, 30 and 80 days, respectively). The resulting filter was probed with ^{32}P-labeled cDNA for $G_{q/11}\alpha$ as described in Section 2.

Attempts to determine the relative amounts of $G_q\alpha$ and $G_{11}\alpha$ by G protein-specific oligonucleotide probes were unsuccessful due to high levels of nonspecific signals appearing at the stringency conditions used (0.5 × SSC). At present, it is unknown whether there are any functional differences between G_q and G_{11}, both of which have been shown to efficiently activate phospholipase C β1 [12]. However, Lipinsky et al. [30] have demonstrated that G_q and G_{11} differentially modulate the response to thyrotropin-releasing hormone (TRH) in *Xenopus* oocytes. The decrease in $G_{q/11}\alpha$ mRNA levels in whole testis with increasing age (Fig. 2) is compatible with our findings that $G_{q/11}\alpha$ mRNA is very low in germ cells and primarily found in somatic cells of testis. The cellular distribution of $G_{q/11}\alpha$ mRNA agrees well with the observed levels of immunoreactive protein.

In a previous study, we show that rat Sertoli and peritubular cells exhibit Gpp(NH)p-stimulated phospholipase C activity, although the actual ligand and receptor involved are unknown [13]. We also demonstrate that these cell types contain high amounts of G_s, G_{i1}, G_{i2} and G_{i3}, but very low levels of G_o, the most

likely candidate in activating phospholipase C. In view of the high amounts of immunoreactive $G_{q/11}\alpha$ observed in Sertoli and peritubular cells in this study, the phospholipase C activity in these cells may well be G_q and/or G_{11}. The mechanisms involved in the regulation of germ cell proliferation and differentiation have not been elucidated. Although no ligand or 'classical' receptor have been identified, the G protein-coupled transduction pathway may play an important role in the control of germ cell development. In fact, cDNAs encoding G protein-coupled receptors have been cloned from haploid germ cells [31,32]. Furthermore, we have previously described the presence of high amounts of G_o and G_{i3} in germ cells isolated from immature rat testis [13]. The germ cells lack the hormone-responsive adenylyl cyclase, as well as G_s. However, both meiotic and post-meiotic germ cells contain Gpp(NH)p-stimulated phospholipase C activity [13]. Since the levels of $G_{q/11}$ in the germ cells are very low, receptor-regulated inositol phospholipid hydrolysis in these cells with the formation of inositoltrisphosphate and diacylglycerol, is most probably regulated via G_o. A candidate for the corresponding ligand, as well as the receptor involved, remain to be found.

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REFERENCES

[1] Taylor, C.W. (1990) Biochem. J. 272, 1–13.
[2] Simon, M.I., Strahtmann, M.P. and Gautam, N. (1991) Science 252, 802–808.
[3] Hepler, J.R. and Gilman, A.G. (1992) Trends Biochem. Sci. 17, 383–387.
[4] Mattera, R., Graziano, M.P., Yatani, A., Zhou, Z., Graf, R., Codina, J., Birnbaumer, L., Gilman, A.G. and Brown, A.M. (1989) Science 243, 804–807.
[5] Yatani, A., Mattera, R., Codina, J., Graf, R., Okabe, K., Padrell, E., Iyengar, R., Brown, A.M. and Birnbaumer, L. (1988) Nature 336, 680–682.
[6] Kikuchi, A., Kozawa, O., Kaibuchi, K., Katada, T., Ui, M. and Takai, Y. (1986) J. Biol. Chem. 261, 11558–11562.
[7] Moriarty, T.M., Padrell, E., Carty, D.J., Omri, G., Landau, E.M. and Iyengar, R. (1990) Nature 343, 79–82.
[8] Hescheler, J., Rosenthal, W., Trautwein, W. and Schultz, G. (1987) Nature 325, 445–447.
[9] VanDongen, A.M.J., Codina, J., Olate, J., Mattera, R., Joho, R., Birnbaumer, L. and Brown, A.M. (1988) Science 242, 1433–1437.
[10] Smrcka, A.V., Hepler, J.R., Brown, K.O. and Sternweis, P.C., Science 251, 804–807.
[11] Taylor, S.J., Chae, H.Z., Rhee, S.G. and Exton, J.H. (1991) Nature 350, 516–518.
[12] Lee, C.H., Parks, D., Wu, D., Rhee, S.G. and Simon, M.I. (1992) J. Biol. Chem. 267, 16044–16047.
[13] Paulssen, R.H., Paulssen, E.J., Gordeladze, J.O., Hansson, V. and Haugen, T.B. (1991) Biol. Reprod. 45, 566–571.
[14] Gordeladze, J.O. and Hansson, V. (1980) Int. J. Androl. 3, 539–552.
[15] Gordeladze, J.O., Purvis, K., Clausen, O.P.F., Rommerts, F.F.G. and Hansson, V. (1981) Int. J. Androl. 4, 172–182.
[16] Haugen, T.B., Paulssen, E.J., Gordeladze, J.O. and Hansson, V. (1990) Biochem. Biophys. Res. Commun. 168, 91–98.
[17] Haugen, T.B., Eskild, W. and Hansson, V. (1992) Biochem. Biophys. Res. Commun. 183, 41–47.
[18] Sternweis, P.C. and Robishaw, J.D. (1984) J. Biol. Chem. 259, 13806–13813.
[19] Neer, E.J., Lok, J.M. and Wolf, L.G. (1984) J. Biol. Chem. 259, 14222–14229.
[20] Strathmann, M. and Simon, M. (1990) Proc. Natl. Acad. Sci. USA 87, 9113–9117.
[21] Gutowski, S., Smrcka, A., Nowak, L., Wu, D., Simon, M. and Sternweis, P.C. (1991) J. Biol. Chem. 266, 20519–20524.
[22] Dorrington, J.H., Roller, N.F. and Fritz, I.B. (1975) Mol. Cell. Endocrinol. 3, 57–70.
[23] Hutson, J.C. and Stocco, D.M. (1981) Endocrinology 108, 1362–1368.
[24] Grootegoed, J.A., Grollé-Hey, A.H., Rommerts, F.F.G. and van der Molen, H.J. (1977) Biochem. J. 168, 23–31.
[25] Chirgwin, J.M., Przybyk, A.E., MacDonald, R.J. and Rutter, W.J. (1979) Biochemistry 18, 5294–5299.
[26] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning (A Laboratory Manual), Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[27] Laemmli, U.K. (1970) Nature 227, 680–685.
[28] Towbin, H., Staehelin, T. and Gordon, J. (1979) Proc. Natl. Acad. Sci. USA 76, 4350–4354.
[29] Gierschik, P., Milligan, G., Pines, M., Goldsmith, P., Codina, J., Klee, W. and Spiegel, A.M. (1986) Proc. Natl. Acad. Sci. USA 83, 2258–2262.
[30] Lipinsky, D., Gershengorn, M.C. and Oron, Y. (1992) FEBS Lett. 307, 237–240.
[31] Meyerhof, W., Müller-Brechlin, R. and Richter, D. (1991) FEBS Lett. 284, 155–160.
[32] Parmentier, M., Libert, F., Schurmans, S., Schiffmann, S., Lefort, A., Eggerickx, D., Ledent, C., Mollereau, C., Gérard, C., Perret, J., Grootegoed, A. and Vassart, G. (1992) Nature 355, 453–455.