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Na⁺/K⁺-ATPase Expression Changes in the Rabbit Lacrimal Glands During Pregnancy

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Abstract

Purpose—To investigate the expressional changes of Na⁺/K⁺-ATPase subunits in the lacrimal glands (LG) of term pregnant rabbits.

Methods—LG were obtained from term pregnant rabbits and age-matched female control rabbits for laser capture microdissection (LCM), real time RT-PCR, western blot, and immunofluorescence. The mRNA and proteins of α 1, α 2, β 1, β 2, and β 3 subunits of Na⁺/K⁺-ATPase were detected and quantified.

Results—Although only the mRNA for β 3 from whole LG of pregnant rabbits was significantly different from that of normal controls, many mRNA levels for α 1, α 2, β 1, β 2, and β 3 from acini and epithelial cells from various duct segments that were collected by LCM were significantly different from those of normal control rabbits. Western blots demonstrated that the expressions of all three β subunits were significantly higher in pregnant rabbits, while both α subunits remained unchanged during pregnancy. Interestingly, immunofluorescence results showed that the distribution patterns of all Na⁺/K⁺-ATPase subunits during pregnancy were similar to those of the control rabbits.

Conclusions—Changes were found in mRNA and protein expressions of Na⁺/K⁺-ATPase subunits in LG from term pregnant rabbits and these changes suggest a role in the pregnancy-related LG secretion changes and dry eye symptoms observed in these animals.

Keywords

Dry eye; Lacrimal gland; Na⁺/K⁺-ATPase; Pregnancy

INTRODUCTION

The lacrimal gland (LG) contributes most of the water and electrolytes in tears through an osmotic process that is, like other exocrine gland secretions, mediated by aquaporins and ion transporters such as Na⁺/K⁺-ATPase.^{1–8} Deficiency of tears could lead to dry eye, the most frequently encountered problems for eye clinicians.

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LG fluid secretion is produced in two stages: (1) secretion of primary fluid in the acini, and (2) modification into the final fluid during its transit through the duct system before reaching the ocular surface. Most LG studies have focused on the acinar cells; however, recent studies have shown that ductal cells, just like acinar cells, also have substantial amounts of aquaporins and ion transporters and may play critical roles in LG secretion and dysfunction.^{1–3,7,9}

Because dry eye is closely related to changes in sex hormone milieu in the body¹⁰ and there are substantial variations of hormonal profiles during the course of pregnancy, it presents an interesting physiological environment to study dry eye. Indeed, many clinicians have an impression that pregnant women more frequently report symptoms of dry eye than non-pregnant women (personal communications), which is supported by our epidemiological study suggesting that a subpopulation of pregnant women experience increased symptoms of dry eye during the third trimester of their pregnancies (review article).¹¹ Recent reports including an ARVO (Association for Research in Vision and Ophthalmology) abstract also indicate that women with one or more pregnancies have an increased risk of having Sjögren's syndrome,^{12,13} an autoimmune disease which involves pathologies in salivary glands and LG that result in dry mouth and dry eye. Other studies have shown that the vast majority of dry eye patients are women, especially those after menopause.¹⁴ Our recent study demonstrated that basal LG fluid production decreased from 0.35 ± 0.04 in control rabbits to 0.21 ± 0.05 $\mu\text{L}/\text{min}$ in pregnant rabbits, while pilocarpine stimulated secretion increased from 5.23 ± 0.4 in control rabbits to 11.4 ± 0.56 $\mu\text{L}/\text{min}$ in pregnant rabbits.¹⁵ This was consistent with our clinical evaluations of ocular surface changes in pregnant rabbits that demonstrated the presence of dry eye symptoms (#16 is an ARVO abstract).^{16,17} Therefore, pregnancy, as a natural physiological condition, appears to be a risk factor for dry eye and can be used as a model for LG deficiency and dry eye studies.^{11,15,17}

Na^+/K^+ -ATPase is a plasma membrane enzyme that uses energy released from ATP hydrolysis to move three Na^+ out of and two K^+ into the cells.¹⁸ Na^+/K^+ -ATPase is a heterotetramer comprised of two α subunits and two β subunits. Two α subunit isoforms ($\alpha 1$ and $\alpha 2$) and three β subunit isoforms ($\beta 1$, $\beta 2$, and $\beta 3$) have been identified so far with only the β subunit being essential for its normal function.¹⁸ Na^+/K^+ -ATPase has been detected in the LG of rabbits^{1,3,8,19–26} and rats,^{7,20,27–30} and has been shown to play a critical role in LG function.^{3,8} Therefore, the aim of the present study was to investigate the expressional changes of Na^+/K^+ -ATPase subunits in the LG from pregnant rabbits with particular emphasis on the duct system.

METHODS

Animals and Tissue Preparation

Two groups of adult female New Zealand White rabbits (Irish Farms, Norco, CA) were used. One group consisted of six term pregnant rabbits, and the other consisted of six age- and sex-matched normal controls. Pregnant rabbits were time dated with day zero corresponding to the date of coitus. Normal gestation in the rabbit is 31 days. Term pregnant rabbits were sacrificed on the 29th day of pregnancy. The rabbits were narcotized with a mixture of ketamine (40 mg/mL) and xylazine (10 mg/mL) and given an overdose of Nembutal (80 mg/kg) for euthanasia. Inferior LG were removed and embedded in OCT, frozen in liquid nitrogen, and stored at -80°C until use. This study conformed to the standards and procedures for the proper care and use of animals by the US Public Health Service Policy on Humane Care and Use of Laboratory Animals.

Laser Capture Microdissection

Frozen sections collected with PEN membrane-coated slides (Leica Microsystems) were stained with cresyl violet with an LCM Staining Kit under RNase-free conditions (Applied Biosystems, Foster City, CA). A PixCell II LCM System (Arcturus Bioscience, Mountain View, CA) was then used to laser capture acinar cells and ductal cells from specific duct segments. Six replicate samples of approximately 100 cells each corresponding to acinar cells or a duct segment were collected for isolation of total mRNA.¹

RNA Extraction and Reverse Transcription

Total cellular RNA was isolated from RNAlater-treated samples with an RNeasy® Midi Kit plus on-column DNase digestion (Qiagen, Valencia, CA). Detailed procedures were described in our previous report.¹

Real-time RT-PCR Analysis and Pre-amplification

The sequences of primers and probes used in this study were listed in our previous publication.¹ For LCM samples, pre-amplification was performed using a TaqMan® PreAmp Master Mix Kit (Applied Biosystems). For real-time RT-PCR, TaqMan® Gene Expression Master Mix (Applied Biosystems) containing the internal dye ROX as a passive reference was used and amplification was carried out on an ABI PRISM® 7900HT Sequence Detection System (Applied Biosystems). Detailed procedures were described in our previous report.¹ Each sample was measured in triplicate and the difference between the C_T values of each target mRNA and GAPDH, an internal housekeeping gene, was used to determine the level of target mRNA relative to the level of GAPDH mRNA in the same sample.

Immunofluorescence and Microscopy

The primary antibodies used were all purchased from Santa Cruz Biotechnology, Santa Cruz, CA. The dilution for $\alpha 1$ (mouse monoclonal, sc-71638) was 1:400; for $\alpha 2$ (goat polyclonal, sc-31391), 1:400; for $\beta 1$ (mouse monoclonal, sc-21713), 1:100; for $\beta 2$ (mouse monoclonal, sc-135997), 1:100; and for $\beta 3$ (goat polyclonal, sc-66343), 1:250. Fluorescein isothiocyanate (FITC)-conjugated AffiniPure donkey anti-goat and anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) at a dilution of 1:200 were used as secondary antibodies. Rhodamine conjugated phalloidin (Invitrogen, Carlsbad, CA), at a dilution of 1:200, was also used to stain F-actin in order to discern morphological profiles of the LG. At least three control rabbits and three pregnant rabbits were stained and repeated at least three times for each subunit tested.

The immunofluorescence technique was described in detail previously.¹ Slides were observed with a Leica epi-fluorescence microscope (Leica Microsystems, Buffalo Grove, IL) and a Zeiss LSM 710 confocal laser scanning microscope (Carl Zeiss MicroImaging, Thornwood, NY). FITC-conjugated secondary antibodies were visualized by excitation with an argon laser at 488 nm. Images were analyzed with LSM image browser (Carl Zeiss MicroImaging) and PhotoShop (Adobe Systems, Mountain View, CA).

Western Blot

LGs were homogenized in RIPA buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X 100, 1% Na deoxycholate, 0.1% SDS, 1 mM PMSF, 1 μ g/mL aprotinin, 1 μ g/mL leupeptin) and centrifuged at 2000 $\times g$ for 20 min. The supernatant was then denatured in SDS-PAGE sample buffer at 60°C for 20 min, resolved on a 7.5% or 4–20% gradient SDS-PAGE gel (Bio-Rad, Hercules, CA), and transferred onto Immobilon-P PVDF membrane (Millipore, Billerica, CA). A constant quantity of proteins from each

sample was analyzed to accurately assess transporter proteins. Membrane blots were probed with $\alpha 1$ at a dilution of 1:1,000, $\alpha 2$ at 1:500, $\beta 1$ at 1:2,000, and $\beta 2$ and $\beta 3$ at 1:500. Blots were incubated with either Alexa 680-labeled donkey anti-goat or goat anti-mouse secondary antibody (Invitrogen) and detected with an Odyssey Infrared Imaging System (Li-Cor, Lincoln, NE). Three replicates from at least 3 different animals each were used for each subunit. Densitometry analysis of the resulting gel was performed with the manufacturer's software.

Statistics

Data were presented as mean $\pm$ standard error of the mean (SEM). Student's t-test and ANOVA were used to evaluate the significance of the differences; $p < 0.05$ was considered significant.

RESULTS

Expressions of mRNA

$\alpha 1$ —mRNA level for $\alpha 1$ from whole LG of pregnant rabbits was 0.876 ± 0.022 , which is not significantly different from that of control rabbits (0.89 ± 0.064 , $p > 0.05$), as we reported earlier.²⁶ Data from LCM samples indicated that mRNA for $\alpha 1$ from pregnant rabbits was least abundant in acini (Figure 1), and its level was significantly lower in acini and intralobular duct ($p < 0.05$), while its abundance was increased in intralobar and interlobar ducts ($p < 0.05$), as compared to results from normal control rabbits.¹

$\alpha 2$ —The expression of mRNA for $\alpha 2$ was very low in LG from pregnant rabbits (0.00144 ± 0.00032); in fact, it was the least abundant of all subunits. Compared to the value (0.00136 ± 0.00013) from normal control rabbits,²⁶ no significant difference was detected ($p > 0.05$). Furthermore, we were unable to detect the presence of $\alpha 2$ mRNA in epithelial cells collected by LCM.

$\beta 1$ —mRNA level for $\beta 1$ from whole LG from pregnant rabbits was 2.721 ± 0.112 , and no significant difference ($p > 0.05$) was detected as compared to the value (2.411 ± 0.125) from normal controls that we reported.²⁶ However, data from LCM samples showed that mRNA for $\beta 1$ was more abundant in acini than ducts (Figure 1) and was significantly lower than control animals¹ in intralobular and interlobar ducts ($p < 0.05$).

$\beta 2$ —mRNA level for $\beta 2$ from whole LG of pregnant rabbits was 0.018 ± 0.002 and was not significantly different ($p > 0.05$) from the value (0.02 ± 0.003) of control rabbits.²⁶ mRNA from epithelial cells collected by LCM was most abundant in intralobular duct while least abundant in interlobar duct (Figure 1) and its levels were significantly lower than control animals¹ in interlobular and interlobar ducts ($p < 0.05$).

$\beta 3$ —mRNA for $\beta 3$ from whole LG of pregnant rabbits was 0.051 ± 0.003 , a significant decrease of 18.4% ($p < 0.05$), as compared to the value (0.062 ± 0.003) from normal control rabbits.²⁶ Data from LCM samples showed that mRNA for $\beta 3$ was least abundant in intralobular duct (Figure 1), and its abundance was significantly lower in acini and intralobular duct ($p < 0.05$), as compared to control animals.¹

Western Blot and Densitometry

By using immunoblotting of whole LG homogenates, we studied the expressions of α and β subunits. Densitometry analysis showed that expression of neither $\alpha 1$ nor $\alpha 2$ from pregnant rabbits was significantly different from those of normal control rabbits (Figure 2).

For the three β subunits, their expressions were all significantly increased in pregnant rabbits ($p < 0.05$) with the increase for β_1 being 26.8, 34.8% for β_2 , and 22.3% for β_3 (Figure 3).

Immunofluorescence

α_1 —Consistent with what we reported recently,²⁶ α_1 immunoreactivity (IR) was detected in all acinar cells, although it demonstrated a “mosaic” pattern among the acini, i.e., some acini/acinar cells showed stronger α_1 -IR, while the intensity in other acini was weaker (Figure 4). α_1 -IR was found on the basolateral membranes and absent from the apical membranes. Ducts were all stained as intensely as the acinar cells showing the most intense α_1 -IR. In LG from pregnant rabbits, the distribution pattern of α_1 -IR was similar to those in the controls, and no apparent difference was detected.

α_2 —The distribution of α_2 -IR in LG from pregnant rabbits was very similar to that of control animals, i.e., minimal α_2 -IR was detected in acinar cells, while strong α_2 -IR was detected in the ducts that appeared as intense clustered punctate staining (Figure 5), results consistent with our previous report from normal control rabbits and rabbits with induced autoimmune dacryoadenitis (IAD), a model of Sjögren’s syndrome.²⁶

β_1 —For β_1 -IR, we found LG from pregnant rabbits showed similar results to that of normal control animals (Figure 6). Consistent with our previous reports,^{1,26} β_1 -IR was detected in the basolateral membranes of all acinar and ductal cells, but not on the apical membranes, and the level of β_1 -IR demonstrated a “mosaic” pattern, i.e., higher in some acinar cells and/or acini than in other acini, a pattern similar to that of α_1 . β_1 -IR in the ductal cells was much stronger, while numerous punctate staining was also present within the cytoplasm.

β_2 —In LG from pregnant rabbits, β_2 -IR was detected in all acinar cells as numerous punctate staining that aggregated toward the apical cytoplasm, with much higher intensity observed in the ducts (Figure 7), a distribution pattern that was similar to that of control rabbits and consistent with our previous report.²⁶

β_3 —For β_3 -IR, we found its distribution pattern was not different from that of the control rabbits, i.e., β_3 -IR was detected in all acinar cells in a diffuse pattern as numerous punctate staining within the cytoplasm, while minimal β_3 -IR was detected in the ducts (Figure 8).

DISCUSSION

Na^+/K^+ -ATPase is an enzyme located in the plasma membrane of all animals. It plays an important role in maintaining resting potential, regulating cell volume, and functioning as a signal transducer to regulate MAPK pathway as well as intracellular calcium.³¹ The resulting Na^+ gradient provides the driving force for secondary active transporters and carrier processes, e.g., intestine, kidney tubules, and exocrine glands, which power either absorption or secretion. Therefore changes of Na^+/K^+ -ATPase have been implicated in the pathogenesis of many diseases.³¹

Our recent report indicated that in rabbits with IAD, a model of Sjögren’s syndrome, Na^+/K^+ -ATPase showed changes at both gene and protein levels in the LG.²⁶ A recent study from patients with primary Sjögren’s syndrome also showed that Na^+/K^+ -ATPase was one of the main targets of anti-M₃ IgG in salivary glands, resulting in compromised Na^+/K^+ -ATPase activity that lead to Sjögren’s syndrome-related dry mouth.³²

In accordance with our previous reports in normal control rabbits¹ and rabbits with IAD,²⁶ we demonstrated the presence of all five subunits of Na⁺/K⁺-ATPase in the LG of pregnant rabbits, with $\alpha 1$ being the dominant α subunit and $\beta 1$ being the dominant β subunit. Ductal cells also demonstrated intense staining with both $\alpha 1$ and $\beta 1$. These results were generally in agreement with earlier studies that documented the presence and importance of Na⁺/K⁺-ATPase in LG of rats^{7,20,27–30} and rabbits.^{1,3,8,19–25,33}

Recent reports indicate that women with one or more pregnancies have an increased risk of having Sjögren's syndrome.^{12,13} Our own epidemiological study also indicated that some pregnant women experience increased symptoms of dry eye during the third trimester of their pregnancies.¹¹ We have also found that many pregnant rabbits demonstrated typical clinical symptoms of dry eye,^{16,17} e.g., decreased Schirmer's test score and tear break-up time as well as increased Rose Bengal staining. Basal LG fluid production was also found reduced in pregnant rabbits, while pilocarpine stimulated secretion rate increased and protein concentration decreased.¹⁵ Therefore, pregnancy, as a normal physiological state, appears to be a natural model for LG deficiency and dry eye studies.

In the pregnant rabbit model we presented here, real time RT-PCR results demonstrated changes of mRNA for Na⁺/K⁺-ATPase subunits in both acini and ducts as compared to control rabbits.¹ Western blot also indicated that the expressions of all three β subunits were increased during pregnancy. The substantial presence of Na⁺/K⁺-ATPase in ductal cells and their changes during pregnancy suggest active Na⁺ and K⁺ transport in the ductal cells and their potential contribution to pregnancy-related LG secretion changes and dry eye symptoms. It should be noted that although up to 15% of all LG epithelial cells are ductal cells,^{34–36} most LG studies have focused on acinar cells which represent ~80% of all epithelial cells and only a few have paid sufficient attention to the duct system.^{1,3,7,9,37,38}

Our whole LG mRNA results showed only $\beta 3$ subunit decrease in pregnant rabbits while LCM data displayed decreases among all the beta subunits in various duct segments. This differential expression of the β subunits demonstrates the variability of each duct segment's contribution towards the final tear fluid. The apparent discrepancy between our whole LG western blot which demonstrated increases in all beta subunits as opposed to the decreases we saw in our whole LG mRNA and LCM results, a phenomenon common in many organ systems, could be the result of a number of mechanisms such as disruption in protein translation and post-translational modification.^{39,40} Protein and mRNA expression often only have a low to moderate correlation, and higher protein expression also does not necessarily correlate with increased function. Therefore more studies of the functional aspect of Na⁺/K⁺-ATPase in the LG are needed to identify the exact effect of its varying expression during dry eye.

The profound changes of hormone levels experienced during the course of pregnancy likely play a role in the substantial changes of Na⁺/K⁺-ATPase and subsequent LG functional status that contribute to the alterations of LG fluid secretion and dry eye symptoms.^{11,15,17} Sex hormones changes, e.g., androgens, have been well documented to play a role in dry eye.^{10,30,41,42} In pregnant rabbits, LG undergoes an immunophysiological transformation similar to that of the mammary gland as it prepares to deliver milk-prolactin immunoreactivity in the ductal cell increases and redistributes from the apical to basolateral cytoplasm.^{43,44} Other reports also showed that lymphocytes in the rabbit LG redistribute from their normal periductal foci to the interacinar space during pregnancy.^{15,43}

In addition to LG, pregnancy-related Na⁺/K⁺-ATPase changes have also been documented in other tissues and organs.⁴⁵ In women with pregnancy-induced hypertension, Na⁺/K⁺-ATPase activity in red blood cells was increased and may be linked to the increased

numbers of Na^+/K^+ -ATPase or its active subunits.⁴⁶ Defects in Na^+/K^+ -ATPase expression and activity were also reported in women with pregnancy and preeclampsia.⁴⁷

In addition to pregnancy, the endocrine and neural systems also have great influence on Na^+/K^+ -ATPase expression in LG. In female rat with hypophysectomy, LG showed rapid atrophy with total Na^+/K^+ -ATPase activity reduced by half while dihydrotestosterone and prolactin treatment partially restored its activity.³⁰ In rabbits with ovariectomy, however, dihydrotestosterone treatment increased Na^+/K^+ -ATPase activity by 29%, while the synthetic estrogen diethylstilbestrol decreased total Na^+/K^+ -ATPase by 12%.²⁴ Cholinergic stimulation in rat caused translocation of Na^+/K^+ -ATPase from intracellular pools to basolateral membranes²⁰ while in rabbit acinar cells it caused a decrease of total content of Na^+/K^+ -ATPase²⁵ and its redistribution from cytoplasmic reserves to basolateral membranes.^{19–21}

In summary, our findings revealed changes within the LGs of pregnant rabbits in mRNA and protein expressions of Na^+/K^+ -ATPase in both acinar and ductal cells that suggest functional changes of this protein may contribute to changes of LG secretion and dry eye symptoms. More functional studies are certainly required to identify how the changes in each duct segment as well as in the acini during pregnancy contribute towards dry eye.

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FIGURE 1.

Real-time RT-PCR of Na^+/K^+ -ATPase subunits from LG epithelial cells of pregnant rabbits collected by LCM. Data from control rabbits is from our previous publication (used with permission).¹ $\alpha 1$, mRNA level for $\alpha 1$ was predominantly localized in the ducts, and during pregnancy, its level was significantly decreased in acini and intralobular duct ($p < 0.05$), while its level was significantly increased in intralobar and interlobar ducts ($p < 0.05$). $\alpha 2$, mRNA level for $\alpha 2$ was extremely low and we were unable to detect any $\alpha 2$ in epithelial cells collected by LCM. $\beta 1$, mRNA level for $\beta 1$ from pregnant rabbits was most abundant in acini and its level was significantly decreased in intralobular and interlobar ducts ($p < 0.05$). $\beta 2$, During pregnancy, mRNA level for $\beta 2$ was most abundant in intralobular duct while least abundant in interlobar duct and its level was significantly decreased in interlobular and interlobar ducts as compared to normal controls ($p < 0.05$). $\beta 3$, mRNA level for $\beta 3$ was most abundant in intralobar and interlobar ducts, while its level was significantly reduced in acini and intralobular duct during pregnancy ($p < 0.05$). D4, intralobular duct; D3, interlobular duct; D2, intralobar duct; D1, interlobar duct. Data were presented as mean $\pm$ SEM.

FIGURE 2.

Western blots of α subunits from whole LG homogenates. No significant difference was detected in either $\alpha 1$ or $\alpha 2$ subunits between normal control and pregnant rabbits ($p > 0.05$). β -actin was used as loading control. Data are representative images of at least three replicates from at least 3 different animals each. Preg, pregnant.

FIGURE 3.

Western blots of β subunits from whole LG homogenates. All β subunits were significantly increased in LG from pregnant rabbits ($p < 0.05$). β -actin was used as loading control. Data are representative images of at least three replicates from at least three different animals each. Preg, pregnant.

FIGURE 4.

Immunofluorescence of α_1 -IR. Control: α_1 -IR (green) was present in every acinar cell, most prominently in the basolateral membranes but not on the apical membranes. The α_1 -IR was much stronger in some acinar cells (arrowheads) while much weaker in other acini (arrows), giving the gland a “mosaic” pattern. Ducts (D) were all stained as strong as those acinar cells showed the most intense α_1 -IR. Pregnancy: the distribution pattern of α_1 -IR in LG from pregnant rabbits was similar to that of control rabbits and no significant difference was detected. At least three control rabbits and three pregnant rabbits were stained and repeated at least three times. Scale bar = 50 μ m.

FIGURE 5.

Immunofluorescence of α_2 -IR. Control: Intense staining of α_2 -IR was detected in duct cells as clustered punctate staining (arrowheads) while minimal staining was found in acinar cells (arrows). Pregnancy: In pregnant rabbits, the staining pattern of α_2 -IR was similar to that of control animals, i.e., α_2 -IR was mostly found in duct cells as clusters of punctate staining (arrowheads), while minimal staining was found in acini (arrows). No significant difference of α_2 -IR was observed between control and pregnant rabbits. At least three control rabbits and three pregnant rabbits were stained and repeated at least three times. Scale bar = 25 μ m.

FIGURE 6.

Immunofluorescence of β_1 -IR. Control: β_1 -IR was present in basolateral membranes of all acinar and ductal cells as well as numerous punctate staining within the cytoplasm. However, levels of β_1 -IR differed in a “mosaic” pattern, higher in some acinar cells and/or acini (arrowheads) than others. β_1 -IR in ductal cells was uniformly higher (arrow). Pregnant: like in control animals, β_1 -IR was also present in all acinar cells and demonstrated a similar “mosaic” pattern (arrowheads). Ducts also showed a much higher β_1 -IR (arrow). However, no significant difference of β_1 -IR was observed between control and pregnant animals. At least three control rabbits and three pregnant rabbits were stained and repeated at least three times. Scale bar = 50 μ m.

FIGURE 7.

Immunofluorescence of β_2 -IR. Control: β_2 -IR (green) was present in every acinar cell as numerous punctate staining that aggregate towards the apical cytoplasm (arrowheads). The intensity of β_2 -IR was much higher in ducts (arrow). Pregnant: the distribution pattern of β_2 -IR in LG from pregnant rabbits was similar to that of control rabbits, i.e., numerous punctate β_2 -IR was detected in the cytoplasm of all acinar cells (arrowheads) and β_2 -IR was much stronger in duct cells (arrow). Red: F-actin that has been stained with rhodamine. At least three control rabbits and three pregnant rabbits were stained and repeated at least three times. Scale bar = 50 μm .

FIGURE 8.

Immunofluorescence of β_3 -IR. Control: β_3 -IR (green) was present in every acinar cell as numerous punctate staining within the cytoplasm (arrowheads) in a diffuse pattern. However, minimal β_3 -IR was detected in the duct cells (arrow). Pregnant: the distribution pattern of β_3 -IR in LG from pregnant rabbits was similar to that of control rabbits, i.e., numerous punctate β_3 -IR was detected in the cytoplasm of every acinar cell (arrowheads) while minimal β_3 -IR was present in the duct cells (arrow). Red: F-actin that has been stained with rhodamine. At least three control rabbits and three pregnant rabbits were stained and repeated at least three times. Scale bar = 50 μ m.