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α_1-Adrenoceptor-Induced Trafficking of Aquaporin-5 to the Apical Plasma Membrane of Rat Parotid Cells

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Incubation of rat parotid tissue with 10 μM epinephrine resulted in a transient and marked trafficking of aquaporin-5 (AQP5) from intracellular membranes to the apical plasma membrane (APM) that was maximal at 1 min. This effect of epinephrine was mimicked by phenylephrine, but not by clonidine, dobutamine, or salbutamol, and it was inhibited by phentolamine, but not by propranolol. Furthermore, the epinephrine-induced trafficking of AQP5 was inhibited by phospholipase C inhibitor U73122 as well as dantrolene and TMB-8, both of which inhibit the release of Ca^{2+} from intracellular stores. Cytochalasin D and tubulozole-C also inhibited this action of epinephrine. These results indicate that epinephrine, acting at α_1-adrenoceptors, induces the trafficking of AQP5 to the APM by triggering the release of Ca^{2+} from intracellular stores through inositol 1,4,5-trisphosphate and ryanodine receptors. In addition, the potent involvement of the cytoskeleton was shown in the epinephrine-induced trafficking of AQP5.

Stimulation of muscarinic acetylcholine, α- or β-adrenergic, or histamine receptors in salivary gland tissue with their respective agonists induces secretion of saliva (1–7). The release of electrolytes and water from salivary acini is also induced by the binding of neurotransmitters released from autonomic nerve endings to muscarinic or α-adrenergic receptors localized on the basolateral plasma membrane (BLM) of acinar cells. Stimulation of α-adrenergic or muscarinic receptors in rat parotid acinar cells results in copious fluid secretion accompanied by little protein secretion (8).

Several aquaporin (AQP) proteins that form water channels that selectively transport water across the plasma membrane have been cloned from a variety of mammalian tissues (9, 10). AQP5, one of AQP proteins, was recently shown to be present in apical plasma membranes (APMs) and intracellular membranes (ICMs) of rat parotid tissue with the use of a specific antiserum generated in response to a synthetic peptide corresponding to the COOH-terminal 21 amino acid residues of AQP5. Furthermore, acetylcholine acting at M_3 muscarinic receptors in this tissue was shown to induce the translocation of AQP5 from ICMs to the APM by increasing the cytosolic concentration of Ca^{2+} ($[Ca^{2+}]i$) (11). Muscarinic and α_1-adrenergic receptors trigger similar signal transduction pathways that involve the heterotrimetic G protein Gq-mediated activation of phospholipase C (PLC)-β, which results in the generation of inositol 1,4,5-trisphophate (IP_3) and a consequent increase in $[Ca^{2+}]i$ (4, 12). Ryanodine receptor type III (Ry3R) has also been identified in microsomal membranes of mouse parotid acini and plays an important role in Ca^{2+} homeostasis in this tissue (13). However, it has not been established whether the trafficking of AQP5 is induced by epinephrine in rat parotid tissue.

With the use of the specific antiserum to AQP5, we have now investigated the effect of epinephrine on the intracellular distribution of AQP5 in rat parotid tissue. We indicate that epinephrine acting at α_1-adrenergic receptors, induces the trafficking of AQP5 from ICMs to the APM, and that this effect appears to be mediated by the activation of IP_3 receptors (IP_3Rs) and Ry3Rs and the consequent increase in $[Ca^{2+}]i$. We further showed that epinephrine-induced trafficking of AQP5 was inhibited by cytochalasin D or tubulozole-C, suggesting the importance of the cytoskeleton in this effect.

MATERIALS AND METHODS

Materials. Epinephrine hydrochloride, clonidine hydrochloride, yohimbine hydrochloride, (1-[6[(17β)-3-methoxyestra-1,3,5(10)-trien-17-yl)amino]hexyl)-1H-pyrrole-2,5-dione] (U73122), 3,4,5-trimethoxybenzoic acid 8-(diethylamino)octyl ester (TMB-8), and cytochalasin D were obtained from Sigma (St. Louis, MO); DL-propranolol hydrochloride was from Nacalai Tesque Inc. (Kyoto, Japan); phentolamine

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hydrochloride was from Ciba-Geigy Japan (Tokyo, Japan); and (+)-ethyl cis-{4-[2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]-methylthio]phenyl} carbamate monohydrochloride (tubulozole-C) was from Janssen Biotech (Olen, Belgium); 3-[(3-cholamidopropyl)dimethyl-ammonio]propansulfonic acid (Chaps) was obtained from Dojindo (Kumamoto, Japan), and antibodies to the IP_3R and to the Ry3R were from Calbiochem-Novabiochem (San Diego, CA).

Preparation and incubation of rat parotid tissue. Male Wistar rats (8 weeks old) were provided with laboratory chow (MF; Oriental Yeast, Tokyo, Japan) and water ad libitum, and were maintained in a temperature-controlled environment (22 ± 2°C) with a 12-h-light, 12-h-dark cycle (lights on at 06:00 h). All procedures were approved by the animal care committee of Tokushima University Dental School. Rats were killed by a blow to the head, and the parotid glands were rapidly removed and transferred to an ice-cold Krebs-Ringer-Tris (KRT) solution [120 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 1.0 mM $CaCl_2$, 16 mM Tris-HCl (pH 7.4), 5 mM glucose] that had been aerated with O_2 gas. Tissue slices (0.4 mm in thickness) were prepared from the parotid glands with a McIlwain Tissue Chopper (Mickle Laboratory Engineering, Surrey, UK) and equilibrated with the KRT solution for 20 min at 37°C with shaking, as described previously (2, 5–7). The slices (wet weight, 300 mg) were then incubated at 37°C in 10 ml of KRT solution in the absence or presence of epinephrine or other agents as indicated.

Preparation of APM, BLM, and ICM fractions from rat parotid tissue. APM and BLM fractions were prepared from rat parotid glands as described (14) with a slight modification as described previously (11). In brief, tissue slices were homogenized with a glass homogenizer and Teflon pestle in 20 vol of 5 mM Hepes buffer (pH 7.5) containing 50 mM mannitol and 0.25 mM $MgCl_2$, and the homogenate was filtered through a single layer of nylon bolting cloth (150-mesh). The filterate was subjected to differential centrifugation, and the pellet obtained after centrifugation at 35,000*g* for 30 min was suspended in the buffer described above.

After the addition of 1 M $MgCl_2$ to give a final concentration of 10 mM, the suspension was incubated on ice for 30 min with stirring and then centrifuged at 3000*g* for 15 min. The resultant pellet was saved as fraction 1 and the supernatant was again centrifuged at 35,000*g* for 30 min. The new pellet was saved as fraction 2, and the supernatant was centrifuged at 200,000*g* for 1 h to yield a pellet that was saved as fraction 3. The three membrane fractions were assayed for the activities of γ-glutamyltranspeptidase as a marker of the APM (15) and K^+-stimulated *p*-nitrophenyl-phosphatase as a marker of the BLM (16) as described. The specific activities of these enzymes indicated that fractions 1, 2, and 3 were enriched in BLMs, APMs and ICMs, respectively (11).

Preparation of parotid endoplasmic reticulum membranes. Endoplasmic reticulum membranes (ERMs) were prepared from rat parotid glands as described (17). In brief, tissue slices were homogenized with a glass homogenizer and Teflon pestle in 5 mM Hepes buffer (pH 7.0) containing 290 mM mannitol, 10 mM KCl, 1 mM $MgCl_2$, and 1 mM benzamide. The homogenate was filtered through a single layer of nylon bolting cloth (150-mesh), and the filterate was then centrifuged at 1000*g* for 12 min. The resultant supernatant was centrifuged at 11,000*g* for 15 min, and the new pellet was mixed with 2.0 M sucrose buffer to give a sucrose concentration of 1.25 M, layered on top of 2.0 M sucrose cushion, and overlaid with 0.3 M sucrose. The gradient was centrifuged at 140,000*g* for 90 min, and the band enriched in rough ERMs that formed at the lower interface of the density layers was collected.

Preparation of parotid microsomal membranes. Microsomal membranes were prepared from rat parotid glands as described (13). In brief, tissue slices were homogenized with a glass homogenizer and Teflon pestle in 10 mM Hepes buffer (pH 7.4) containing 250 mM sucrose, 1 mM ethylenediamine-*N,N,N′,N′*-tetraacetic acid, 1 mM dithiothreitol, and protease inhibitors of 1 mM benzamide, 1 μg/ml leupeptin, 0.7 μg/ml pepstatin A, and 0.1 mM phenylmethylsulfonyl fluoride. The homogenate was centrifuged at 250*g* for 5 min. The 250*g* supernatant was centrifuged at 10,000*g* for 20 min. The 10,000*g* supernatant was centrifuged at 100,000*g* for 1 h. The resultant pellet was collected as microsomal fraction.

Preparation of antibodies to AQP5. Rabbit polyclonal antibodies to AQP5 were generated in response to a synthetic peptide (KGTYEPEEDWEDHREERKKTI) corresponding to the deduced COOH-terminal amino acid sequence of AQP5 (18).

Immunoblot analysis. APM, ICM, and BLM fractions were treated with the solubilizing buffer (19) and subjected to SDS–polyacrylamide gel electrophoresis (PAGE) on a 12.5% gel. The ERM fraction was subjected to SDS–PAGE on a 2 to 12% gradient gel. After PAGE, the separated proteins were electrophoretically transferred from the unstained gel to a nitrocellulose membrane (Hybond ECL; Amersham Pharmacia Biotech UK Limited, Little Chalfont, Buckinghamshire, UK) with the use of a Trans-Blot apparatus (Bio-Rad, Hercules, CA). The blots were probed with antibodies to AQP5 (1:1,500 dilution), or with antibodies to either the IP_3 receptor (1:250 dilution) or the Ry3R (1:100 dilution). Immune complexes were detected with horseradish peroxidase-conjugated secondary antibodies and ECL reagents (Amersham Pharmacia Biotech UK Limited, Little Chalfont, Buckinghamshire, UK).

The antibodies to AQP5 recognized a single protein with a mobility corresponding to a molecular mass of 27 kDa in the APM and ICM fractions, but not in the BLM fraction, consistent with previous results (11). Detection of this protein was prevented by preadsorption of the antibodies to AQP5 with excess immunizing peptide.

Statistical analysis. Data are presented as means ± SE and were analyzed for statistical significance with Student's *t* test or analysis of variance at all time point. *P* value of <0.05 was considered statistically significant.

RESULTS

Effect of epinephrine on the intracellular distribution of AQP5 in rat parotid tissue. Rat parotid tissue slices were incubated for 1 min at 37°C with 10 μM epinephrine to investigate the effect of this agent on the intracellular distribution of AQP5. The tissues was then immediately frozen to stop the reaction before homogenization and preparation of membrane fractions. Immunoblot analysis with antibodies to AQP5 revealed that epinephrine induced a marked increase in the amount of AQP5 in the APM (233.8 ± 21.0% of control) and an associated decrease in the abundance of this protein in the ICM fraction (80.2 ± 3.0% of control) (Fig. 1). Incubation of the tissue did not affect either the yield of APM, BLM or ICM fraction, or the immunoblot analysis per se. These observations indicated that epinephrine induces a trafficking of AQP5 from ICMs to the APM in rat parotid cells.

Effects of α- and β-adrenergic antagonists on the epinephrine-induced trafficking of AQP5. The subtype of adrenergic receptors responsible for the epinephrine-induced increase in the amount of AQP5 in the APM of rat parotid cells was investigated with the use of specific α- and β-adrenergic receptor antagonists. The α-adrenergic antagonist phentolamine (100 μM) markedly inhibited the increase in the abundance of AQP5 induced by incubation of tissue with 10 μM epinephrine

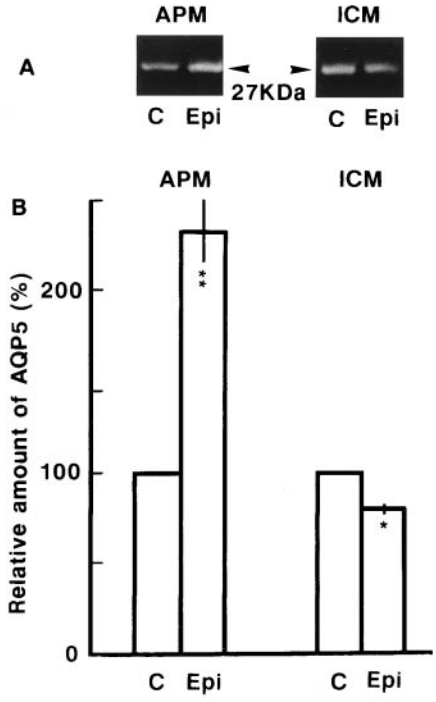

FIG. 1. Effect of epinephrine on the amounts of AQP5 in the APM and ICM fractions of rat parotid tissue. (A) Rat parotid tissue slices were incubated in the absence (C, control) or presence of 10 μM epinephrine (Epi) for 1 min at 37°C, after which APM (5 μg of protein) and ICM (40 μg of protein) fractions were prepared and subjected to immunoblot analysis with antibodies to AQP5. The position of the 27-kDa immunoreactive protein is indicated. (B) Immunoblots similar to those shown in (A) were subjected to densitometric analysis, and the amount of AQP5 in each fraction, based on the intensity of the chemiluminescence signal, was expressed as a percentage of the value for control tissue. Data are means ± SE of five independent experiments. $*p < 0.05$, $**p < 0.01$ versus the value for control cells.

for 1 min (Fig. 2). In contrast, β-adrenergic antagonist propranolol (100 μM) had a much smaller inhibitory effect on this action of epinephrine. Thus, the epinephrine-induced trafficking of AQP5 in rat parotid cells appears to be mediated by α-adrenergic receptors.

Effects of α_1-, α_2-, β_1-, and β_2-adrenergic agonists on the alteration of the amount of AQP5 in the APM. To identify the specific α-adrenergic receptor subtype responsible for the epinephrine-induced translocation of AQP5, we incubated rat parotid tissue slices with specific agonists of α_1- and α_2-adrenergic receptors (Fig. 3). Exposure of tissue for 1 min to 10 μM phenylephrine, an α_1-adrenergic agonist, induced a 2.2-fold increase in the amount of AQP5 in the APM. This effect of phenylephrine was inhibited by the α_1-adrenergic antagonist prazosin (100 μM). In contrast, the α_2-adrenergic agonist clonidine (10 μM) had no significant effect on the amount of AQP5 in the APM, either in the absence or presence of the α_2-adrenergic antagonist yohimbin (100 μM). As additional controls, we showed that neither the β_1-adrenergic agonist dobutamine (10 μM), in the absence or presence of the β_1-adrenergic antagonist atenolol (100 μM) nor the β_2-adrenergic agonist salbutamol (10 μM), in the absence or presence of the β_2-adrenergic antagonist butoxamine (100 μM), had any significant effect on the amount of AQP5 in the APM. These observations indicate that epinephrine acts at α_1-adrenergic receptors to induce the trafficking of AQP5 to the APM in rat parotid cells.

Effects of U73122, dantrolene, and TMB-8 on the epinephrine-induced trafficking of AQP5. To investigate the signaling pathway by which the interaction of epinephrine with α_1-adrenoceptors triggers the trafficking of AQP5 from ICMs to the APM of rat parotid cells, we examined the effect of the PLC inhibitor U73122 (10 μM) (20). This agent prevented the epinephrine-induced increase in the amount of AQP5 in the APM (Fig. 4). Dantrolene (30 μM), a hydantoin derivative that inhibits Ca^{2+}-induced Ca^{2+} release from the sarcoplasmic reticulum of skeletal muscle and from unidentified Ca^{2+} stores in other cell types (21), inhibited by ~70% the trafficking of AQP5 from ICMs to the

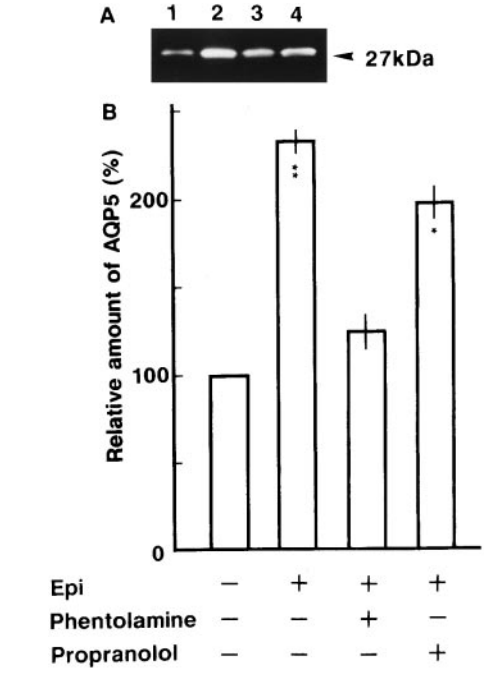

FIG. 2. Effects of phentolamine and propranolol on the epinephrine-induced increase in the amount of AQP5 in the APM. (A) Rat parotid tissue slices were incubated for 1 min at 37°C in the absence (lane 1) or presence of 10 μM epinephrine, either alone (lane 2) or in the additional presence of 100 μM phentolamine (lane 3) or 100 μM propranolol (lane 4). The APM fraction (5 μg of protein) was then prepared and subjected to immunoblot analysis with antibodies to AQP5. (B) Immunoblots similar to those shown in (A) were subjected to densitometric analysis, and the amount of AQP5 was expressed as a percentage of the value for control cells. Data are means ± SE of three independent experiments. $*p < 0.05$, $**p < 0.01$ versus the value for control cells.

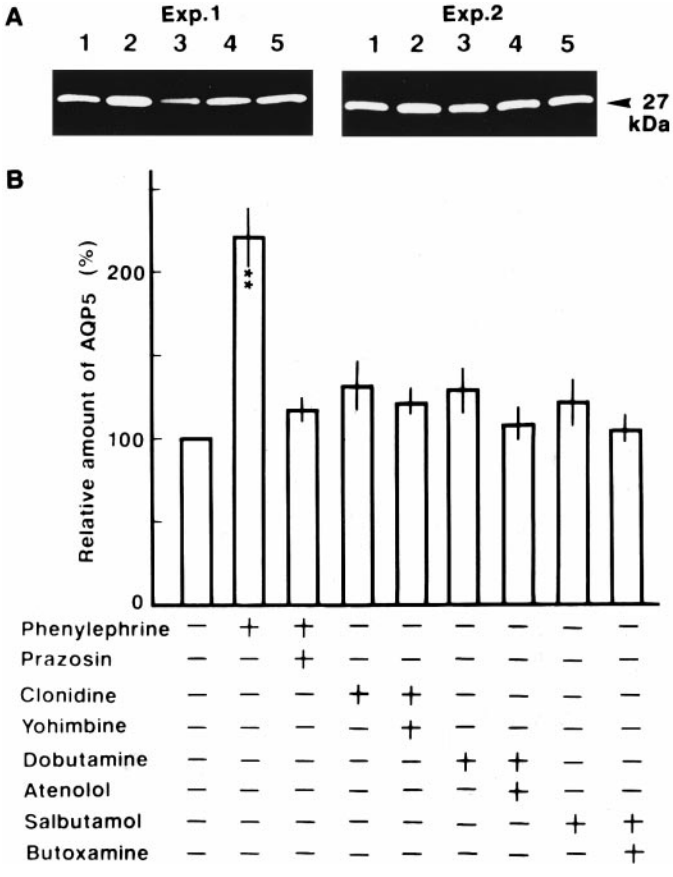

FIG. 3. Effects of α- and β-adrenergic agonists and antagonists on the amount of AQP5 in the APM. (A) Rat parotid tissue slices were incubated for 1 min at 37°C in the absence (Exp. 1 and 2, lane 1) or presence of 10 μM phenylephrine (Exp. 1, lane 2), 10 μM phenylephrine and 100 μM prazosin (Exp. 1, lane 3), 10 μM clonidine (Exp. 1, lane 4), 10 μM clonidine and 100 μM yohimbine (Exp. 1, lane 5), 10 μM dobutamine (Exp. 2, lane 2), 10 μM dobutamine and 100 μM atenolol (Exp. 2, lane 3), 10 μM salbutamol (Exp. 2, lane 4) and 10 μM salbutamol and 100 μM butoxamine (Exp. 2, lane 5). The APM fraction (5 μg of protein) was then prepared and subjected to immunoblot analysis with antibodies to AQP5. (B) Immunoblots similar to those shown in (A) were subjected to densitometric analysis, and the amount of AQP5 was expressed as a percentage of the value for control cells. Data are means ± SE of three independent experiments. $^{**}p < 0.01$ versus the value for control cells.

APM induced by 10 μM epinephrine. Finally, TMB-8 (30 μM), an inhibitor of Ca^{2+} release from intracellular compartments, completely blocked epinephrine-induced increase in the amount of AQP5 in the APM. These observations suggest that activation of PLC, the consequent production of IP_3 and Ca^{2+} release mediated by the IP_3Rs and Ry3Rs, contribute to the trafficking of AQP5 induced by epinephrine.

Effect of epinephrine on the time-dependent changes in the amounts of AQP5 in APM, IP_3Rs in ERMs, and Ry3Rs in microsomal membranes. To investigate further the contributions of IP_3Rs and Ry3Rs to the epinephrine-induced trafficking of AQP5 to the APM, we examined the effect of epinephrine on the abundance of these receptors in ERMs and microsomal membranes. Immunoblot analysis with respective and specific antibodies revealed that incubation of rat parotid tissue slices with 10 μM epinephrine induced marked increases in the amounts of IP_3Rs and Ry3Rs in the ERMs and microsomal membranes, respectively (Fig. 5). The increases were transient, with the abundance of IP_3Rs peaking at 1 min after exposure to

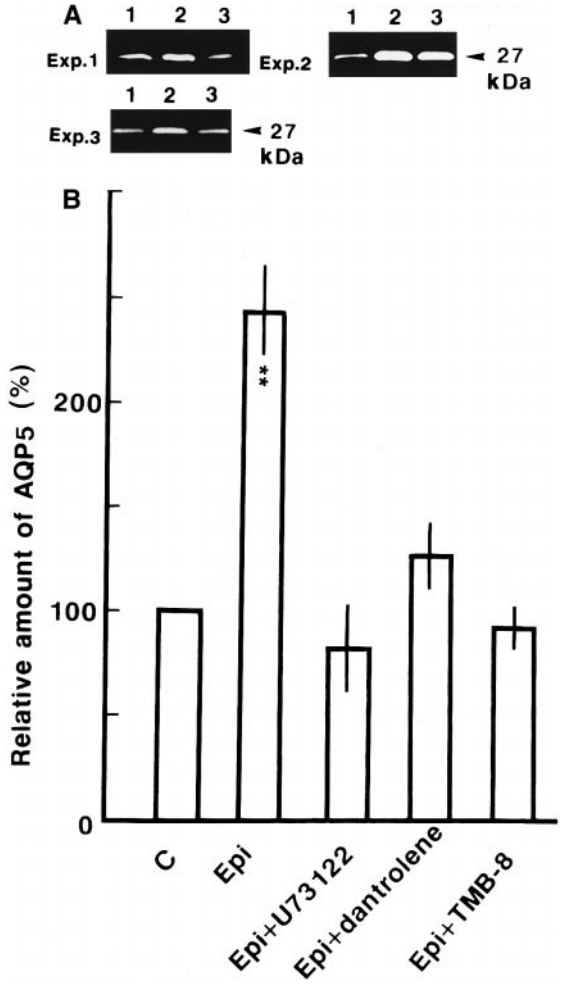

FIG. 4. Effects of U73122, dantrolene and TMB-8 on the epinephrine-induced increase in the amount of AQP5 in the APM. (A) Rat parotid tissue slices were pretreated for 30 min at 37°C in the absence (Exp. 1 to 3, lanes 1 and 2) or presence of 10 μM U73122 (Exp. 1, lane 3), 30 μM dantrolene (Exp. 2, lane 3) and 30 μM TMB-8 (Exp. 3, lane 3) and then incubated for 1 min at 37°C in the absence (Exp. 1 to 3, lane 1) or presence (Exp. 1 to 3, lanes 2 and 3) of 10 μM epinephrine in the continued absence or presence of U73122, dantrolene and TMB-8. The APM fraction (5 μg of protein) was then prepared and subjected to immunoblot analysis with antibodies to AQP5. (B) Immunoblots similar to those shown in (A) were subjected to densitometric analysis, and the amount of AQP5 was expressed as a percentage of the value for cells incubated in the absence of U73122, dantrolene and TMB-8. Data are means ± SE of three independent experiments. $^{**}p < 0.01$ versus the value for control cells.

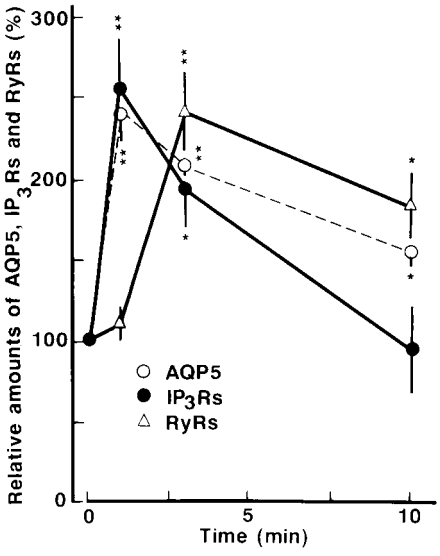

FIG. 5. Effect of epinephrine on the amounts of AQP5 in the APM, IP_3Rs in ERMs, and Ry3Rs in microsomal membranes. Rat parotid tissue slices were incubated for 0, 0.25, 1, 3, or 10 min at 37°C with 10 μM epinephrine, after which the APM fraction (5 μg of protein) was prepared and subjected to immunoblot analysis with antibodies to AQP5. ERMs (40 μg of protein) and microsomal membranes (40 μg of protein) were prepared and subjected to immunoblot analysis with antibodies to IP_3Rs and Ry3Rs. Then, immunoblots were subjected to densitometric analysis, and the amounts of AQP5, IP_3Rs and Ry3Rs were expressed as a percentage of the values for zero time. Data are means ± SE of three independent experiments. $*p < 0.05$, $**p < 0.01$ versus the values for zero time.

epinephrine and that of Ry3Rs peaking at 3 min. While, incubation of tissue slices with 10 μM epinephrine for various times revealed that the amount of AQP5 in the APM increased rapidly. The effect was statistically significant at 15 s (147.7 ± 3.2% of control) and maximal at 1 min (233.8 ± 21.0% of control), after which the amount of AQP5 in the APM gradually returned toward control values. However, even at 10 min, the amount of AQP5 in the APM was significantly higher than that of control. These observations suggest that IP_3Rs and Ry3Rs contribute sequentially to the increase in $[Ca^{2+}]i$ induced by epinephrine and then lead to increase the amount of AQP5 in APM.

Effects of cytochalasin D and tubulozole-C on the epinephrine-induced trafficking of AQP5. It has been proposed that membrane traffic in polarized epithelial cells requires both actin filaments and microtubules. We therefore tested the effects of cytochalasin D, an actin-disrupting agent, and of tubulozole-C, a microtubule-depolymerizing agent, on the epinephrine-induced trafficking of AQP5 (22). Both agents inhibited the epinephrine-induced increase in the abundance of AQP5 in the APM in a dose-dependent manner (Fig. 6); at a concentration of 5 μM, cytochalasin D inhibited the effect of epinephrine by 80% and tubulozole-C inhibited it completely. These results indicate that both actin filaments and microtubules are important for the epinephrine-induced trafficking of AQP5 in rat parotid cells.

DISCUSSION

We have shown that incubation of rat parotid tissue with 10 μM epinephrine results in a rapid and transient increase in the amount of AQP5 in the APM fraction that is maximal after 1 min (Figs. 1 and 5).

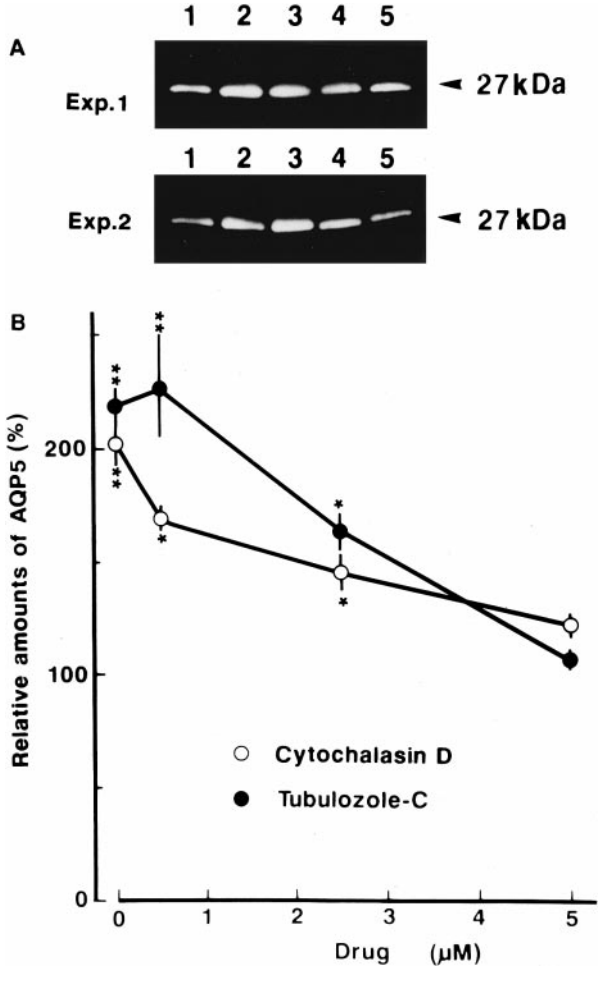

FIG. 6. Effects of cytochalasin D and tubulozole-C on the epinephrine-induced increase in the amount of AQP5 in the APM. (A) Rat parotid tissue slices were pretreated for 30 min at 37°C in the absence (Exp. 1 and 2, lanes 1 and 2) or presence of cytochalasin D at the concentration of 0.5, 2.5 and 5.0 μM (Exp. 1, lanes 3 to 5, respectively), tubuluzole-C at the concentration of 0.5, 2.5 and 5.0 μM (Exp. 2, lanes 3 to 5, respectively) and then incubated for 1 min at 37°C in the absence (Exp. 1 and 2, lane 1) or presence (Exp. 1 and 2, lanes 2 to 5) of 10 μM epinephrine in the continued absence or presence of cytochalasin D or tubulozole-C. The APM fraction (5 μg of protein) was then prepared and subjected to immunoblot analysis with antibodies to AQP5. (B) Immunoblots similar to those shown in (A) were subjected to densitometric analysis, and the amount of AQP5 was expressed as a percentage of the value for control cells. Data are means ± SE of three independent experiments. $*p < 0.05$, $**p < 0.01$ versus the value for zero time.

Conversely, prolongation of such treatment with epinephrine for longer than 1 min decreased gradually the amount of AQP5 in the APM, but still maintained the elevated level of its amount in the APM after 10 min of the reaction with the agonist (Fig. 5). The identification of the specific adrenergic receptor subtype involved in epinephrine-induced trafficking of AQP5 between the APM and ICMs in rat parotid tissue by using specific antagonists directed against α- and β-adrenergic receptors showed that epinephrine acted at α-adrenergic receptors and induced the trafficking of AQP5 between the APM and ICMs in parotid glands (Fig. 2). This finding was supported by the result that the treatment of the tissues with phenylephrine, an α_1-adrenergic agonist, induced marked increase in the amount of AQP5 in the APM (Fig. 3). Thus, this effect of epinephrine was shown to be mediated by α_1-adrenoceptors.

Trafficking from ICMs to the plasma membrane has previously been demonstrated for AQP1 in the rat bile duct cells and for AQP2 in rat renal collecting duct cells in response to secretin (23) and vasopressin (24, 25), respectively. The vasopressin-induced trafficking of AQP2 is mediated by binding of the hormone to its receptor in the BLM of collecting duct cells, dissociation of the heterotrimetic G protein Gs, activation of adenylate cyclase, and an increase in the intracellular concentration of cAMP (26, 27). The resulting phosphorylation of AQP2 located in ICMs by protein kinase A then triggers vesicle exocytosis. As the result, AQP2 is inserted into the plasma membrane.

Treatment of rat parotid tissue with U73122, TMB-8 or dantrolene completely inhibited the epinephrine-induced increase in the amount of AQP5 in the APM (Fig. 4). These observations suggest that the interaction of epinephrine with α_1-adrenoceptor activates PLC-β and subsequent increase the release of Ca^{2+} from intracellular stores. Two distinct classes of channels that mediate the release of Ca^{2+} from intracellular stores have been identified: One is sensitive to IP_3, which is produced by PLC-β-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate (4, 11, 28), and the other is sensitive to ryanodine (29). In general, IP_3-gated channels might release Ca^{2+} which would in turn induce Ca^{2+} release from the Ca^{2+}-induced Ca^{2+} release pathway through RyR (30). In the present study, incubation of rat parotid tissue with epinephrine resulted in increases in the abundance of IP_3Rs and Ry3Rs in the fraction that were maximal at 1 and 3 min, respectively (Fig. 5). It is suggested by these findings that the initial phase of increase in the $[Ca^{2+}]$i is mediated by activation of IP_3Rs, whereas the later phase is associated with Ry3Rs, contributing sequentially to the epinephrine-induced trafficking of AQP5 to the APM. In addition, it seems that this contribution of IP_3Rs is larger than that of Ry3Rs.

It has been suggested that membrane traffic in polarized epithelial cells requires both actin filament and microtubules (31). Consistent with this hypothesis, we have now shown that cytochalasin D and tubulozole-C each inhibited the trafficking of AQP5 to the APM by 80 to 100% at the concentration of 5 μM (Fig. 6). However, Ribeiro *et al.* (32) suggested that cytoskeletal disruption also affects alters the spatial relation between PLC and IP_3Rs, and thereby impairs PLC-dependent Ca^{2+} signaling. The precise role of the cytoskeleton in the epinephrine-induced trafficking of AQP5 therefore requires further investigation.

In summary, epinephrine acts at α_1-adrenergic receptors in rat parotid tissue and induces the rapid trafficking of AQP5 from ICMs to the APM via elevation of $[Ca^{2+}]$i in the tissue. In this process, the cytoskeleton may play an important role.

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