Adrenergic Receptor-Mediated Cl^- Transport in Rabbit Corneal Endothelial Cells

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Received September 2, 1994 Accepted January 9, 1995

ABSTRACT—Adrenoceptor-mediated Cl^- transport in cultured rabbit corneal endothelium was examined using a Cl^- -sensitive fluorescent dye. The intracellular Cl^- concentration ($[\text{Cl}^-]_i$) in the endothelial cells was estimated to be about 30 mM. Noradrenaline (0.001–0.1 mM) transiently decreased the $[\text{Cl}^-]_i$ in a dose-dependent manner. Such a decrease in $[\text{Cl}^-]_i$ was completely antagonized by pretreatment with the α -adrenoceptor antagonist phentolamine (0.1 mM). The selective α_2 -adrenoceptor agonist UK 14304-18 (5-bromo-6-[(4*H*,5*H*-imidazol-2-yl)amino]quinoxaline, 0.1 mM) persistently decreased the $[\text{Cl}^-]_i$, but neither the α_1 -adrenoceptor agonist phenylephrine (0.1 mM) nor the β -adrenoceptor agonist isoproterenol (0.1 mM) had any effect. The α_2 -adrenoceptor agonist/antagonist yohimbine (0.1 mM) persistently and more strongly decreased the $[\text{Cl}^-]_i$ than UK 14304-18 did. The yohimbine-induced decrease in the $[\text{Cl}^-]_i$ was not further altered by UK 14304-18 or phenylephrine, but partly reversed by noradrenaline, isoproterenol and an adenylate cyclase activator, forskolin (0.1 mM). The yohimbine-induced decrease in $[\text{Cl}^-]_i$ was inhibited by the carbonic anhydrase inhibitor acetazolamide (1 mM), and $\text{Cl}^-/\text{HCO}_3^-$ exchange inhibitors, 4-acetamido-4'-isothiocyano-stilbene-2,2'-disulfonic acid and 4,4'-diisothiocyano-stilbene-2,2'-disulfonic acid, but not by the H^+ -ATPase inhibitor *N,N'*-dicyclohexylcarbodiimide. The forskolin-induced recovery in $[\text{Cl}^-]_i$ was inhibited by the $\text{Na}^+/\text{K}^+/\text{Cl}^-$ cotransport inhibitor bumetanide (0.1 mM), but not by the Cl^- channel blocker 5-nitro-2-(3-phenylpropylamino)-benzoic acid. These findings suggest that corneal endothelial cells extrude Cl^- upon α_2 -adrenoceptor stimulation and accumulate Cl^- upon β -adrenoceptor stimulation under low $[\text{Cl}^-]_i$ conditions, probably via acceleration of $\text{Cl}^-/\text{HCO}_3^-$ exchange and $\text{Na}^+/\text{K}^+/\text{Cl}^-$ cotransport, respectively.

Keywords: Corneal endothelium, Adrenergic receptor, $\text{Cl}^-/\text{HCO}_3^-$ exchanger, $\text{Na}^+/\text{K}^+/\text{Cl}^-$ cotransporter, Cl^- -sensitive fluorescent dye

Corneal endothelium, a monolayer of cells covering the posterior surface of the cornea, reportedly transports fluid and plays a role in the maintenance of corneal thickness and transparency (1–3). Transport of Cl^- as well as Na^+ and HCO_3^- across the corneal endothelium has been considered to be responsible for the fluid movement (2–4). In the epithelia covering the anterior surface of the cornea, Cl^- transport from the stroma to tears also contributes to maintenance of corneal hydration and transparency (1, 2, 4), and this transport is regulated by activation of adrenergic receptors (5, 6). In spite of the presence of adrenoceptors in corneal endothelia (7), the effects of adrenoceptor stimulation on Cl^- transport in the endo-

thelial cells have not been examined yet. The present report is the first to describe adrenoceptor-mediated corneal endothelial Cl^- transport using a Cl^- -sensitive fluorescent probe and cultured rabbit corneal endothelial cells.

MATERIALS AND METHODS

Materials

N-(6-Methoxyquinolyl) acetoethyl ester (MQAE) was synthesized by the method of Verkman et al. (8). UK 14304-18 (5-bromo-6-[(4*H*,5*H*-imidazol-2-yl)amino]quinoxaline; a gift from Pfizer Central Research, Aichi) was dissolved in ethanol. Yohimbine, forskolin (Sigma Chemical Co., St. Louis, MO, USA), KT5720 ((8*R**,9*S**,11*S**)-

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(—) - 9 - *n* - hexyloxycarbonyl - 9 - hydroxy - 8 - methyl - 2,3,9,10-tetrahydro-8,11-epoxy-1*H*,8*H*,11*H*-2,7*b*,11*a*-triazadibenzo[*a,g*]cycloocta[*c,d,e*]trinden-1-one; Kyowa Medex Co., Tokyo) and *N,N'*-dicyclohexylcarbodiimide (DCCD, Sigma) were dissolved in dimethylsulfoxide (Sigma). The drugs were applied to the cells so that the final concentrations of ethanol and dimethylsulfoxide were below 0.05% and 0.1%, respectively. Acetazolamide (Sigma), bumetanide (Sigma), 5-nitro-2-(3-phenylpropyl-amino)-benzoic acid (NPPB; a gift from Hoechst AG, Frankfurt, Germany), 4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonic acid (SITS; Aldrich Chemical Co., Milwaukee, WI, USA) and 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS, Aldrich) were dissolved in distilled water on the day of the experiments. *N*-2-Hydroxyethyl-piperazine-*N'*-2-ethanesulfonic acid (HEPES, Sig-

ma), and other chemicals used were of the highest grade commercially available.

Cell culture

Endothelial cells isolated from albino rabbit corneas were cultured as described by Madsen et al. (9). After the animals were anesthetized with ether, the cornea was immediately cut out along the limbs of the eye and placed in cold Dulbecco's modified Eagle Medium (DMEM). The endothelial cells with pieces of Desmet's membrane were then isolated using a pair of fine scissors and a pair of tweezers; and they were then cultured on glass coverslips in DMEM (pH 7.30 ± 0.05) containing 5% fetal calf serum, 5% horse serum, 10 mM HEPES, penicillin-G (100 IU/ml) and streptomycin (100 μ g/ml). The endothelial cells cultured for 10–14 days at 37 °C in 5% CO₂ formed a

Fig. 1. A light microscopic photograph (A) and the fluorescence images (B) of cultured rabbit corneal endothelial cells loaded with MQAE. Scale bar, 100 μ m.

confluent monolayer. After removing the pieces of Desmet's membrane using a fine forceps, confluent cultures on the coverslips were washed three times with Krebs-HEPES buffer solution (pH 7.4 ± 0.05) of the following composition: 128 mM NaCl, 2.5 mM KCl, 2.7 mM CaCl₂, 1 mM MgSO₄, 1 mM Na₂HPO₄, 20 mM HEPES and 16 mM glucose.

Fluorescence microscopic observation and photometry

The cells were exposed to the Krebs-HEPES buffer containing 5 mM of a Cl⁻-sensitive fluorescent dye, MQAE, for 1 hr at 37°C, and then they were washed more than five times with the dye-free solution. The intracellular MQAE fluorescence intensity was monitored and calibrated as previously described (10–13). In brief, fluorescence intensity of MQAE in a single cell was measured in a cell-chamber warmed at 32°C using an inverted fluorescence microscope system (Olympus, Tokyo; Interdeck, Osaka; a 50 W xenon lamp, a 355–365 nm band pass excitation filter, a 455 nm dichroic mirror, a 450–460 nm band pass emission filter, and a silicon-intensified target video camera). The intracellular Cl⁻ concentration index ($[Cl^-]_i$ index), as a parameter for $[Cl^-]_i$, was determined by dividing the initial MQAE fluorescence by that at each incubation time. Test drugs other than SITS and DIDS were applied by total replacement of the cell-chamber solution (Krebs-HEPES buffer) with that containing a definite concentration of the drugs. SITS and DIDS were applied into the culture media 18 hr before the experiments, and the cells were washed thoroughly to minimize the fluorescence of the reagents during the fluorometry. No other direct interaction was observed between MQAE and the drugs as measured in the cell-free system.

Data analyses

The data are expressed as means $\pm$ S.E., statistically analyzed by Student's *t*-test for paired or unpaired comparison, and the difference between mean values with a probability level of $P < 0.05$ was considered to be significant.

RESULTS

Cultured rabbit corneal endothelial cells formed a monolayer within 2 weeks, and such cells were evenly loaded with the Cl⁻-sensitive fluorescent probe MQAE (Fig. 1). The fluorescence was calibrated for the Cl⁻ concentration using calibration media as described in the legend of Fig. 2. Under these conditions, $[Cl^-]_i$ is assumed to be equal to the concentration of Cl⁻ in the calibration media (10–13). The $[Cl^-]_i$ index increased as Cl⁻-concentrations in the calibration media were logarithmically increased ($[Cl^-]_i$ index/log $[Cl^-] = 0.283$

Fig. 2. Calibration of MQAE fluorescence for Cl⁻ concentration in rabbit corneal endothelial cells. Cells were perfused with calibration solutions containing the various concentrations of Cl⁻ shown on the abscissa (1–135 mM), 135 mM K⁺, 5 μ M nigericin, 10 μ M valinomycin and 10 μ M tributyltin. Under these conditions, $[Cl^-]_i$ is equal to the Cl⁻ concentration in the calibration solution (ref. 10). $[Cl^-]_i$ index: the ratio of initial MQAE fluorescence against that after perfusion with the calibration solutions. Each point with bars represents the mean $\pm$ S.E. of 6 experiments.

Fig. 3. Effects of adrenoceptor agonists on the intracellular Cl⁻ concentrations ($[Cl^-]_i$) in corneal endothelial cells. $[Cl^-]_i$ index: the ratio of initial MQAE fluorescence to that at each incubation time. At the time indicated by the arrow, each agonist was added. ○: none, ●: 0.1 mM noradrenaline, ▲: 0.1 mM phenylephrine, ▼: 0.1 mM UK 14304-18, □: 0.1 mM isoproterenol. Each point represents the mean $\pm$ S.E. from 3–7 experiments. * $P < 0.05$, vs corresponding control (none) at the same incubation time.

Table 1. Effects of noradrenaline on the $[Cl^-]_i$ level in corneal endothelial cells

Treatment	$[Cl^-]_i$ index	(n)
Control	1.01 ± 0.04	(4)
Noradrenaline (1 μ M)	0.94 ± 0.06	(3)
Noradrenaline (10 μ M)	0.89 ± 0.07	(4)
Noradrenaline (0.1 mM)	$0.80 \pm 0.05^*$	(5)
Phentolamine (0.1 mM)	0.99 ± 0.05	(2)
Phentolamine (0.1 mM) + Noradrenaline (0.1 mM)	1.02 ± 0.004	(4)

$[Cl^-]_i$ index was determined at 15 sec after the drug application. $[Cl^-]_i$ index: the ratio of the initial MQAE fluorescence to the fluorescence after drug application. Values are means $\pm$ S.E. n: the number of experiments. * $P < 0.05$, compared with the control.

± 0.09 ; the r value of the slopes = 0.907 ± 0.106 ; means $\pm$ S.E. of 6 experiments), indicating that the $[Cl^-]_i$ index is useful as a parameter of $[Cl^-]_i$ in the corneal endothelia (Fig. 2). From the values of Cl^- concentration at $[Cl^-]_i$ index = 1, the apparent $[Cl^-]_i$ of cultured corneal endothelium was estimated to be 30.1 ± 1.6 mM ($n = 6$).

Noradrenaline (0.1 mM) reduced the $[Cl^-]_i$ with the maximum response at about 15 sec after the drug administration (Fig. 3). Among the adrenoceptor agonists, neither phenylephrine, an α_1 -adrenoceptor agonist, nor isoproterenol, a β -adrenoceptor agonist, affected the $[Cl^-]_i$, but UK 14304-18, an α_2 -adrenoceptor agonist, decreased it. The effect of noradrenaline was dose-dependent and completely inhibited by the α -adrenoceptor blocker phentolamine (Table 1). These findings suggested that noradrenaline reduces $[Cl^-]_i$ through α_2 -adrenocep-

Table 2. Effects of forskolin and KT5720 on the $[Cl^-]_i$ level in yohimbine-treated corneal endothelial cells

Treatment	$[Cl^-]_i$ index	(n)
Control	1.08 ± 0.12	(4)
Forskolin (0.1 mM)	1.13 ± 0.03	(3)
Yohimbine (0.1 mM)	$0.72 \pm 0.07^*$	(5)
Yohimbine + Forskolin	0.99 ± 0.11	(3)
KT5720 (50 nM) + Yohimbine	$0.71 \pm 0.10^*$	(5)
KT5720 + Yohimbine - Forskolin	$0.72 \pm 0.11^*$	(5)

Values are means $\pm$ S.E. n: the number of experiments. * $P < 0.05$, vs control. $[Cl^-]_i$ index was determined at a definite time after the application of drugs (forskolin-1 min, yohimbine-3 min, yohimbine-3 min, followed by yohimbine + forskolin-1 min). KT5720 was applied to the cells 5 min before the treatment with yohimbine.

tor stimulation.

Compared with the response to UK 14304-18, a decrease in $[Cl^-]_i$ by noradrenaline was transient as if the amine stimulated another mechanism once $[Cl^-]_i$ was decreased. To test this possibility, the effects of adrenoceptor agonists on the $[Cl^-]_i$ that had been lowered with yohimbine were examined (Fig. 4). Yohimbine, which stimulates α_2 -adrenoceptor with a blocking effect against intrinsic or exogenously applied α_2 -agonists, markedly decreased the $[Cl^-]_i$ to a plateau level in 2 min. Under such conditions, phenylephrine and UK 14304-18 did not change the $[Cl^-]_i$ level, but noradrenaline and isoproterenol increased it, suggesting that β -adrenoceptor stimulation is responsible for the recovery of $[Cl^-]_i$. Stimulation of β -adrenoceptor by noradrenaline reportedly increases the cyclic AMP level of cultured corneal endothelial cells

Fig. 4. Effects of adrenoceptor agonists on the intracellular Cl^- concentrations ($[Cl^-]_i$) in corneal endothelial cells in the presence of 0.1 mM yohimbine. $[Cl^-]_i$ index: the ratio of initial MQAE fluorescence to that at each incubation time. At the time indicated by the single arrow, 0.1 mM yohimbine was added; and thereafter, the adrenoceptor agonists were added at the time indicated by the double arrow. ◇: none, ●: 0.1 mM yohimbine, ○: 0.1 mM noradrenaline, ▼: 0.1 mM UK 14304-18, ▲: 0.1 mM phenylephrine, □: 0.1 mM isoproterenol. Each point represents mean $\pm$ S.E. from 3–7 experiments. * $P < 0.05$, vs corresponding value with yohimbine alone at the same incubation time.

Table 3. Effects of acetazolamide and bumetanide on yohimbine- and forskolin-induced changes in the [Cl⁻]_i level in corneal endothelial cells

Pretreatment		[Cl ⁻] _i index		(n)
		Yohimbine ^a	Yohimbine + Forskolin ^b	
Control	(none)	0.68 ± 0.05	0.92 ± 0.04 (^a 10, ^b 5)	
Acetazolamide	(1 mM)	0.98 ± 0.03**	0.99 ± 0.05 (6)	
Bumetanide	(0.1 mM)	0.78 ± 0.01	0.77 ± 0.05* (4)	

Values are means ± S.E. n: the number of experiments. *P < 0.05, **P < 0.01, vs control. [Cl⁻]_i index was determined at a definite time after the application of drugs (^a: yohimbine-3 min, ^b: yohimbine-3 min, followed by yohimbine + forskolin-1 min). Pretreatment of cells with each drug was performed during the 5 min before the application of yohimbine.

(7). As shown in Table 2, the adenylate cyclase activator forskolin also reversed the [Cl⁻]_i of the endothelial cells after the treatment with yohimbine without significant effects before the treatment. Furthermore, KT5720 (50 nM), an inhibitor of cyclic AMP-dependent protein kinase, blocked such a recovery in the [Cl⁻]_i.

To characterize the Cl⁻ transporters involved in the mechanisms of adrenoceptor-mediated changes in [Cl⁻]_i, the effects of inhibitors of ion transport on the yohimbine- and forskolin-induced changes in [Cl⁻]_i were examined (Table 3). Acetazolamide (1 mM), which inhibits carbonic anhydrase activity and thereby reduces intracellular production of H⁺ and HCO₃⁻, blocked the yohimbine-induced decrease in [Cl⁻]_i, suggesting that H⁺- or HCO₃⁻-requiring Cl⁻ extrusion was stimulated by yohimbine. On the other hand, bumetanide (0.1 mM), an inhibitor of Na⁺/K⁺/Cl⁻ co-transporter, completely abolished the forskolin-induced recovery in [Cl⁻]_i. The Cl⁻ channel

blocker NPPB (0.1 mM) slightly reduced the resting [Cl⁻]_i level ([Cl⁻]_i index = 0.83 ± 0.06, n = 3, P < 0.05 vs control = 1.0). In the presence of NPPB, yohimbine further decreased the [Cl⁻]_i level ([Cl⁻]_i index = 0.51 ± 0.03, n = 3, P < 0.01 vs NPPB alone), and forskolin recovered it ([Cl⁻]_i = 0.75 ± 0.03, n = 3, P < 0.01 vs NPPB + yohimbine). To further characterize the yohimbine-induced change in [Cl⁻]_i, the effects of an inhibitor of H⁺-ATPase, DCCD (0.1 mM), and inhibitors of Cl⁻/HCO₃⁻ exchanger, SITS (1 mM) and DIDS (1 mM), were examined (Table 4). The stilbene derivatives, but not DCCD, significantly reduced the response to yohimbine, suggesting that Cl⁻/HCO₃⁻ exchange was stimulated by this reagent.

DISCUSSION

A noninvasive method using Cl⁻-sensitive probes (8, 10–13) has now circumvented methodological difficulties due to the fragile monolayer arrangement of corneal endothelial cells in the study of corneal endothelial Cl⁻ transport. This is the first report describing adrenergic receptor-mediated changes in [Cl⁻]_i in corneal endothelial cells. A decrease in [Cl⁻]_i caused by noradrenaline or α₂-adrenergic agonist suggests that the corneal endothelial cells extrude intracellular Cl⁻ by α₂-adrenergic receptor stimulation. The decrease in [Cl⁻]_i was reversed by β-agonists and forskolin as well as noradrenaline, suggesting that the corneal endothelial cells with lowered [Cl⁻]_i accumulate Cl⁻ by means of activation of adenylate cyclase coupled with β-adrenoceptor stimulation. α₂-Adrenoceptor stimulation reportedly not only reduces cellular cyclic AMP levels, but also sensitizes cyclic AMP-producing stimuli with unknown mechanisms in HT29 human colonic adenocarcinoma cells (14). Since noradrenaline as well as isoproterenol elevated [Cl⁻]_i only after α₂-adrenoceptor stimulation (Table 2 and Fig. 3), the inwardly directed Cl⁻ transport of the cells may also be related to facilitated stimulation of adenylate cyclase. Cl⁻ levels reportedly modulate intracellular signaling via affecting hormone-sensitive G protein in various types of cells (15–17). Since the dissociation of GDP from the α-subunit of G protein is accelerated at lower concentrations of Cl⁻ (16), stimulation of α₂-adrenoceptors in corneal endothelial cells may sensitize the G protein-linked cyclic AMP-producing stimuli, at least in part, through this mechanism. Furthermore, forskolin also increased the level of [Cl⁻]_i only after the α₂-adrenoceptor stimulation, and the increase in [Cl⁻]_i appeared to be mediated by cyclic AMP-dependent protein kinase (Table 2). Therefore, forskolin and/or the kinase may also be more effective at the lower [Cl⁻]_i. A transient decrease by noradrenaline in [Cl⁻]_i is probably due to an α₂-adrenoceptor-

Table 4. Effects of several drugs on yohimbine-induced changes in the [Cl⁻]_i level in corneal endothelial cells

Treatment	[Cl ⁻] _i index	(n)
Control	1.03 ± 0.01	(9)
Yohimbine (0.1 mM)	0.56 ± 0.05	(9)
DCCD (0.1 mM) + Yohimbine	0.63 ± 0.04	(3)
SITS (1 mM) + Yohimbine	0.77 ± 0.03**	(8)
DIDS (1 mM) + Yohimbine	0.75 ± 0.04**	(6)

Values are means ± S.E. n: the number of experiments. **P < 0.01, vs yohimbine. [Cl⁻]_i index was determined at 1 min after the application of yohimbine. Pretreatment of cells with DCCD was performed during the 5 min before the application of yohimbine. SITS and DIDS were added to the culture media 18 hr before the experiments, and the cells were washed thoroughly before the experiments to minimize the fluorescence of the reagents.

mediated decrease in $[Cl^-]_i$ followed by a facilitated β -adrenoceptor-mediated Cl^- accumulation.

The yohimbine-induced decrease in $[Cl^-]_i$ was blocked by pretreatment of the cells with acetazolamide (Table 3) and stilbene derivatives (Table 4), suggesting that the response to yohimbine is mediated by outwardly directed Cl^- transport through Cl^-/HCO_3^- exchange. Since the cells responded to yohimbine and forskolin in the presence of NPPB, facilitation or blockade of Cl^- channels is not likely involved in the mechanisms of these responses. The NPPB-induced decrease in the resting $[Cl^-]_i$ level suggests that passive Cl^- entry through Cl^- channels and probably Cl^- extrusion are working in corneal endothelial cells. The forskolin-induced increase in the $[Cl^-]_i$ level was inhibited by inhibitors of $Na^+/K^+/Cl^-$ cotransporter and cyclic AMP-dependent protein kinase, bumetanide and KT5720, respectively (Tables 2 and 3). The immunopurified shark rectal gland $Na^+/K^+/Cl^-$ cotransporter is reportedly phosphorylated when activated by forskolin (18). Furthermore, the cDNA for the cotransporter has been recently cloned, and the predicted amino acid sequence includes regulatory phosphoacceptor residues (19). Thus, β -adrenoceptor stimulation or activation by forskolin of the rabbit corneal endothelial cells may also accelerate $Na^+/K^+/Cl^-$ cotransport through direct phosphorylation of the cotransporter protein.

In frog corneal epithelium, short-circuit current studies showed that the Cl^- transport from the aqueous to the tear side is enhanced by α_1 - and β -adrenoceptor stimulation, and it is reduced by α_2 -adrenoceptor stimulation (6). In the present experiments, α_1 -adrenoceptor stimulation appeared not to affect endothelial Cl^- transport. Thus the corneal epithelia and endothelia seem to have distinct types of adrenoceptors to function bidirectionally in Cl^- transport.

In clinical cases, topically applied adrenaline induces toxic changes including swelling in corneal endothelial cells (20). This adrenaline-induced toxicity may thereby be related to the accumulation of Cl^- through excessive β -adrenoceptor stimulation in the endothelial cells under the possible stimulation of α_2 -adrenoceptor by endogenous catecholamines.

Acknowledgments

This study was supported by grants from the Ministry of Education, Science and Culture, Japan, Yamanouchi Foundation for Research on Metabolic Disorders, Uehara Memorial Foundation and the Alumni Association of Kansai Medical University (Katano Grant). The authors thank Kansai Medical University Laboratory Research Center and Laboratory Animal Center for providing experimental instruments and animals, respectively.

REFERENCES

- 1 Zadunaisky JA and Lande MA: Active chloride transport and control of corneal transparency. *Am J Physiol* **221**, 1837–1844 (1971)
- 2 Waring GO III, Bourne WM, Edelhauser HF and Kenyon KR: The corneal endothelium – Normal and pathologic structure and function. *Ophthalmology* **89**, 531–590 (1982)
- 3 Winkler BS, Riley MV, Peters MI and Williams FJ: Chloride is required for fluid transport by the rabbit corneal endothelium. *Am J Physiol* **262**, C1167–C1174 (1992)
- 4 Wiederholt M, Jentsch TJ and Keller SK: Electrogenic sodium-bicarbonate symport in cultured corneal endothelial cells. *Pflugers Arch Eur J Physiol* **405**, S167–S171 (1985)
- 5 Montoreano R, Candia OA and Cook P: α - and β -adrenergic receptors in regulation of ionic transport in frog cornea. *Am J Physiol* **230**, 1487–1493 (1976)
- 6 Chu T-C and Candia OA: Role of α_1 - and α_2 -adrenergic receptors in Cl^- transport across frog corneal epithelium. *Am J Physiol* **255**, C724–C730 (1988)
- 7 Chao W-T and Walkenbach RJ: Desensitization of β -adrenergic receptors in corneal endothelium. *J Ocul Pharmacol* **1**, 263–268 (1985)
- 8 Verkman AS, Sellers MC, Chao AC, Leung T and Ketcham R: Synthesis and characterization of improved chloride-sensitive fluorescent indicators for biological applications. *Anal Biochem* **178**, 355–361 (1989)
- 9 Madsen K, Schenholm M, Jahnke G and Tengblad A: Hyaluronate binding to intact corneas and cultured endothelial cells. *Invest Ophthalmol Vis Sci* **30**, 2132–2137 (1989)
- 10 Krapf R, Berry CA and Verkman AS: Estimation of intracellular chloride activity in isolated perfused rabbit proximal convoluted tubules using a fluorescent indicator. *Biophys J* **53**, 955–962 (1988)
- 11 Inoue M, Hara M, Zeng X-T, Hirose T, Ohnishi S, Yasukura T, Uriu T, Omori K, Minato A and Inagaki C: An ATP-driven Cl^- pump regulates Cl^- concentration in rat hippocampal neurons. *Neurosci Lett* **134**, 75–78 (1991)
- 12 Hara M, Inoue M, Yasukura T, Ohnishi S, Mikami Y and Inagaki C: Uneven distribution of intracellular Cl^- in rat hippocampal neurons. *Neurosci Lett* **143**, 135–138 (1992)
- 13 Ohnishi S, Hara M, Inoue M, Yamashita T, Kumazawa T, Minato A and Inagaki C: Delayed shortening and shrinkage of cochlear outer hair cells. *Am J Physiol* **263**, C1088–C1095 (1992)
- 14 Jones SB, Toews ML, Turner JT and Bylund DB: α_2 -Adrenergic receptor-mediated sensitization of forskolin-stimulated cyclic AMP production. *Proc Natl Acad Sci USA* **84**, 1294–1298 (1987)
- 15 Deterre P, Gozlan H and Bockaert J: GTP-dependent anion-sensitive adenylate cyclase in snail ganglia potentiation of neurotransmitter effects. *J Biol Chem* **258**, 1467–1473 (1983)
- 16 Higashijima T, Ferguson KM and Sternweis PC: Regulation of hormone-sensitive GTP-dependent regulatory proteins by chloride. *J Biol Chem* **262**, 3597–3602 (1987)
- 17 Nakajima T, Sugimoto T and Kurachi Y: Intracellular chloride inhibits the GTPase activity of Gk. *FASEB J* **6**, A96 (1992)
- 18 Lytle C and Forbush B III: The Na-K-Cl cotransporter protein of shark rectal gland. II. Regulation by direct phosphorylation. *J Biol Chem* **267**, 25438–25443 (1992)
- 19 Xu J-C, Lytle C, Zhu T-T, Payne JA, Benz E Jr and Forbush B III: Molecular cloning and functional expression of the bumetanide-sensitive Na-K-Cl cotransporter. *Proc Natl Acad Sci USA* **91**, 2201–2205 (1994)
- 20 Waltman SR, Yarian D, Hart W Jr and Becker B: Corneal endothelial changes with long-term topical epinephrine therapy. *Arch Ophthalmol* **95**, 1357–1358 (1977)