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Activation of the Na^+-K^+ Pump in Frog Erythrocytes by Catecholamines and Phosphodiesterase Blockers

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ABSTRACT. K^+ and Na^+ influx into frog erythrocytes incubated in standard saline was studied using ^{86}Rb and ^{22}Na as tracers. 10 μM isoproterenol (ISP) produced a significant increase in K^+ influx for the first 15 min, which was sustained during the entire 60 min of cell incubation. Treatment of red cells with the phosphodiesterase (PDE) blockers theophylline (THEO, 1 and 5 mM) or 3-isobutyl-1-methylxanthine (IBMX, 0.5 mM) was also accompanied by an enhancement in K^+ influx. A distinct additive effect on K^+ influx into red cells was found when ISP and THEO or IBMX were added together. The increase in K^+ transport induced by ISP plus IBMX was totally abolished by pretreatment of red cells with 0.1 mM ouabain. The ouabain-sensitive K^+ influx in frog erythrocytes was elevated in the presence of ISP plus IBMX to 2.05 ± 0.45, as compared with the control level of 0.39 ± 0.11 mmol/L cells/hr ($P < 0.001$). Similar effects of ISP and IBMX on K^+ influx were observed after chloride was replaced by nitrate. A dose-related increase in K^+ influx into frog erythrocytes was observed at ISP concentrations of 10^{-8}–10^{-6} M, with a half-maximal stimulatory concentration of approximately 0.02 μM. The effects of ISP (10^{-8}–10^{-5} M) on K^+ transport were completely abolished with 10 μM of the β-adrenergic blocker propranolol, but α-adrenergic antagonists (phentolamine, prazosin, and yohimbine) did not alter the ISP-induced increase in K^+ influx. The drugs tested had no effect on ^{22}Na influx in frog red cells, but ISP produced a small decline (13%) in intracellular Na^+ concentration. Thus, our study indicates that catecholamines and PDE blockers enhance K^+ (^{86}Rb) transport in frog erythrocytes mediated by Na^+-K^+ pump activity. The frog erythrocyte membrane may serve as a convenient model to investigate the hormonal modulation of the Na^+-K^+ pump. BIOCHEM PHARMACOL 52;9:1347–1353, 1996. *Copyright © 1996 Elsevier Science Inc.*

KEY WORDS. erythrocyte membrane; catecholamines; potassium transport; Na^+-K^+-ATPase; phosphodiesterase blockers; frog

Hormone-sensitive ion transport systems have been described in many different biological tissues, including nucleated erythrocytes of fish, amphibia, and birds. A large number of studies have demonstrated the ability of catecholamines to activate Na/H exchange in fish erythrocytes and Na-K-2Cl cotransport in avian erythrocytes (see review [1, 2]). However, relatively little is known about the ion transport mechanisms and their hormonal regulation for amphibian red blood cells. These investigations have mainly characterized the contribution of ion transport systems to cell volume and intracellular pH regulation in the red cells of some amphibian species [1, 3–5]. Earlier studies [6, 7] revealed the presence of β-adrenoceptors in frog erythrocyte membranes and established that their stimulation by catecholamines was associated with an increase in intracellular cAMP† level. In contrast to the situation in fish erythrocytes, catecholamines are not considered to affect the red cell function of amphibia studied under physiological conditions. In the erythrocytes of *Rana pipiens*, catecholamines produced a significant increase in Na^+ and K^+ influxes only if PDE activity was inhibited [4]. The effect of catecholamines on frog red cells was accounted for by an activation of the Na/H exchanger because the hormone-stimulated Na^+ and K^+ fluxes were inhibited by amiloride [3]. Numerous works on many cell types have shown that catecholamines exhibit a direct stimulatory action on Na^+-K^+-ATPase (see reviews [8–11]). In a study on carp erythrocytes [12], it was shown that catecholamines activate both Na/H exchange and the Na^+-K^+ pump. Our previous work on erythrocytes of frog *Rana temporaria* [13] showed that K^+ influx is mediated by three pathways, including the Na^+-K^+ pump, K-Cl cotransport, and a diffusion leak. The present study was undertaken to ascertain the possible hormonal effects on ion transport mechanisms in the frog erythrocyte membrane. We have studied K^+ and

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† *Abbreviations:* ISP, isoproterenol; PDE, phosphodiesterase; THEO, theophylline; IBMX, 3-isobutyl-1-methylxanthine; NOR, norepinephrine; cAMP, adenosine 3′,5′-cyclic monophosphate.

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Na^+ influx responses to ISP, norepinephrine, and the PDE blockers THEO and IBMX. The results indicate that both catecholamines and PDE blockers directly stimulate the Na^+-K^+ pump in the frog erythrocyte membrane. The catecholamine effect on the Na^+-K^+ pump is similar to that described in skeletal muscle, cardiomyocytes, and renal cells [8–11].

MATERIALS AND METHODS

Animals

The experiments were carried out on frogs, *Rana temporaria*, from December to April. Animals were kept at 2–4°C in aquaria with a small amount of tap water, periodically replaced with dechlorinated tap water.

Preparation of Cell Suspension

Frogs were immobilized by the destruction of the spinal cord and blood was obtained from the heart. The blood was pooled in the heparinized tubes and immediately sedimented by centrifugation (2700 g for 5 min at 4°C). The supernatant was aspirated and the upper layer of white cells removed. Erythrocytes were washed 3 times with cold standard saline. Washed red cells were suspended at a haematocrit of 30–40% in standard saline. The cell suspension was kept at 20°C for 60 min in a thermostatted bath before K^+ and Na^+ influx measuring experiments were performed.

Solutions and Chemicals

The standard saline contained (mM): 103 NaCl, 2.7 KCl, 1 $MgCl_2$, 10 Tris-HCl and 10 glucose (pH 7.6 at 20°C). For Cl^--free assays, Cl^--salts were replaced by NO_3^- salts.

Ouabain, isoproterenol, propranolol, phentolamine, prazosin, yohimbine, and theophylline were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.); 1-Norepinephrine bitartrate and 3-isobutyl-1-methylxanthine (IBMX) were from Serva (Heidelberg, Germany). A fresh aqueous solution of 1 mM ISP was prepared for each experiment. ^{86}Rb and ^{22}Na were obtained from the ISOTOP company (Moscow, Russia).

K^+ influx measurement

K^+ influx was determined from the uptake of ^{86}Rb [13]. To study the time-course of K^+ influx, the cell suspension was added to 3 mL incubation medium with or without ISP (final haematocrit of 1–3%). After 5 min preincubation at 20°C, ^{86}Rb was added to the cell suspension (0.2–0.7 MBq/mL medium) and samples were taken at several time intervals. The samples were injected into 10 mL ice-cold saline and immediately centrifuged (2700 g for 1 min at 4°C). A small amount of the supernatant was taken for the determination of medium radioactivity. Red cells were washed twice with the same solution, then lysed in 1 mL of 5% trichloracetic acid and centrifuged after 15 min. The radioactivity of the cell lysates and media was measured by a liquid scintillation spectrometer using the Cerenkov effect. In another series of experiments, aliquots of the cell suspension (30–40%) were added to several tubes containing 1 mL of standard saline in the absence or presence of various agents. Red cells were preincubated with ouabain for 30 min before ISP addition. After 5 min, ^{86}Rb was added and its uptake for 60 min measured. Because ^{86}Rb uptake was shown to be linear with time up to 60 min, unidirectional K^+ influx was calculated as follows: $A_{RBC} \cdot C_K/A_M\, t$ where: A_{RBC} and A_M are the radioactivity of 1 mL of packed cells and 1 mL of medium, respectively; C_K is the K^+ concentration in the medium; and t is the time of incubation. All experiments were performed at 20°C.

For Na^+ influx measurements, the cells were incubated for 60 min in standard saline containing 2 MBq ^{22}Na per mL. ^{22}Na radioactivity was measured with a gamma scintillation counter. To determine the cell concentrations of Na^+ and K^+, the red cells were washed with solution containing 76 mM $MgCl_2$ and 10 mM Tris-HCl (pH 7.6 at 4°C). The cell ion concentrations were measured by a flame photometer.

Statistics

All values are listed as means ± SEM. Statistical significance was assessed using Student's paired *t* test. A value of $P < 0.05$ was considered to be significant.

RESULTS

The Effect of Isoproterenol (ISP) on the Time-Course of ^{86}Rb Uptake

A series of experiments was carried out to estimate the optimal time required for determination of unidirectional K^+ (^{86}Rb) influx. Figure 1 demonstrates that ^{86}Rb uptake by the frog red cells was linear over a 60-min period of cell incubation in the absence and presence of 10 μM ISP. A significant increase in K^+ uptake was evident during the first 15 min of cell incubation with ISP as estimated from paired statistical analysis. The difference in K^+ uptake for 15 min between ISP-treated and control cells averaged 0.31 ± 0.045 mmol/L cells ($P < 0.001$). The enhanced rate of K^+ uptake by frog red cells incubated with ISP (31%) was sustained over the 60 min of cell incubation. Thus, it is apparent that exposure of the frog erythrocytes to ISP was associated with a rapid activation of K^+ transport, and that the ^{86}Rb uptake for 60 min reflected an unidirectional K^+ influx into frog red blood cells. In further experiments, the 60-min ^{86}Rb uptake was measured to determine K^+ influx in the frog erythrocytes.

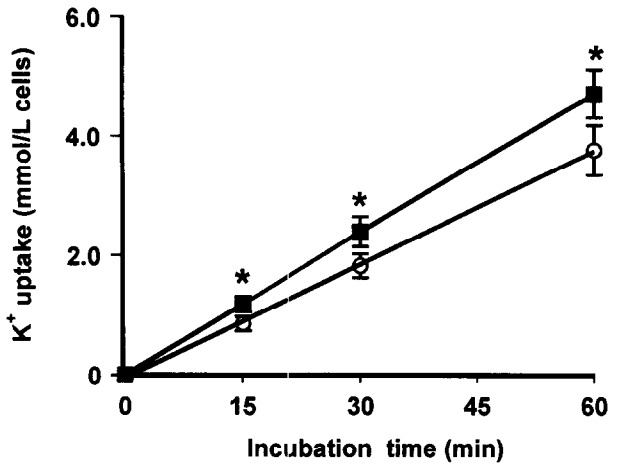

FIG. 1. Effect of isoproterenol (ISP) on time-course of K^+ uptake. Frog erythrocytes were preincubated in standard saline without (○) and with 10 μM ISP (■) for 5 min. Then, at zero time, ^{86}Rb was added to the cell suspension and its uptake determined for various time intervals. Data are means ± SEM for 6 separate experiments. Lines are drawn according to a linear regression. *$P < 0.001$ compared to control (paired t test).

Comparison of ISP and Norepinephrine (NOR) Effect on K^+ Influx

In paired experiments, frog red blood cells were treated with 10 μM ISP or NOR and with a combination of the two. Figure 2 shows that both drugs produced a significant increase in the K^+ influx into frog erythrocytes. The K^+ influx induced by ISP was significantly greater than that stimulated by NOR (1.07 ± 0.22 vs 0.58 ± 0.18 mmol/L/hr, $P < 0.02$). When both agents were added together to the incubation medium, the stimulation of K^+ influx (1.06 ± 0.24 mmol/L/hr) did not differ from that produced by ISP alone. Thus, the effects of ISP and NOR on K^+ influx in the frog red cells were not additive. The ISP concentration of 10 μM used in our experiments appears to be optimal, because its increase up to 50 μM was not associated with a further enhancement in K^+ influx (data not shown). In this series of experiments, the effect of ISP on the total K^+ influx in frog erythrocytes (39%) was in good agreement with the results obtained in the first series of experiments (Fig. 1).

Effect of Theophylline (THEO) on K^+ Influx

There is substantial evidence that the effects of catecholamines in many cell types are mediated via cyclic adenosine 3′,5′-monophosphate (cAMP)-dependent mechanisms. The following experiments were designed to verify whether or not the blockade of phosphodiesterase activity with THEO influences K^+ transport in frog erythrocytes. Figure 3 illustrates the comparative effects on K^+ influx of 10 μM ISP and THEO at concentrations of 1 and 5 mM. The inhibition of phosphodiesterase activity with THEO was associated with a significant increase in K^+ transport into frog red cells. Moreover, the change in K^+ influx produced by 5 mM THEO (57 ± 6%) was significantly greater than that caused by 1 mM THEO (32 ± 3%). In all experiments, maximal increments in K^+ influx were observed when ISP and THEO were added together to the incubation medium. In both series of experiments, the activation of K^+ influx achieved by the combination of the drugs (1.62 ± 0.23 and 2.00 ± 0.21 mmol/L/hr) was not different from the sum of individual effects of ISP (0.77 ± 0.11 and 0.87 ± 0.18 mmol/L/hr) and THEO (0.62 ± 0.15 and 1.17 ± 0.08 mmol/L/hr). These data indicate that ISP and THEO exerted an additive activating influence on K^+ transport into the frog erythrocytes. Because the effect of THEO on K^+ influx was enhanced with increasing concentration of the drug, we carried out additional experiments using a more selective phosphodiesterase blocker, IBMX.

Effects of IBMX, Ouabain, and Cl^- Removal on K^+ Influx

In a previous work [13] we showed that K^+ transport across the frog erythrocyte membrane is mediated by at least 3 mechanisms, including the Na^+-K^+ pump, K-Cl cotransport, and a diffusion leak. To ascertain which ion transport pathways are activated by catecholamines, experiments were performed using ouabain to inhibit the Na^+-K^+ pump and replacing Cl^- with NO_3^- to eliminate K-Cl cotransport. Figure 4A and B presents the results of these experiments. At concentration of 0.5 mM, the phosphodiesterase blocker IBMX caused a significant stimulation of K^+ influx into frog red cells, similar to that produced by 5 mM THEO. Again, the combination of ISP and IBMX produced an additional increase in K^+ influx as compared with the addition to the incubation medium of each agent alone. The increments in K^+ influx induced by ISP, IBMX, and

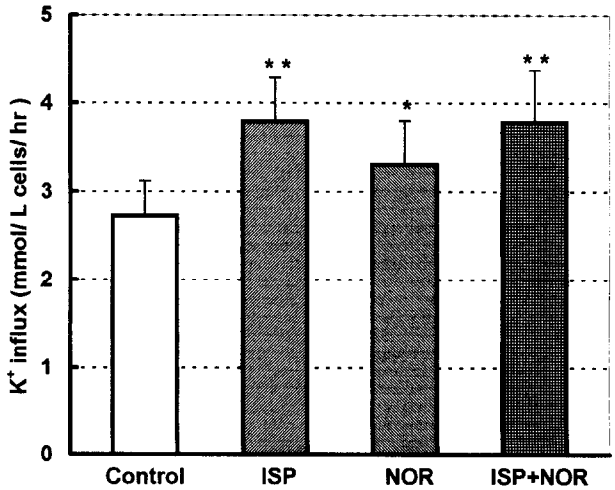

FIG. 2. The effects of isoproterenol (ISP) and norepinephrine (NOR) on K^+ influx. Frog red cells were preincubated in standard saline for 5 min in the absence and presence of 10 μM ISP, 10 μM NOR, and ISP plus NOR. The K^+ influx was calculated from ^{86}Rb uptake for 60 min. Values represent the means ± SEM of 7 independent experiments. *$P < 0.05$, **$P < 0.01$ compared to control (paired t test).

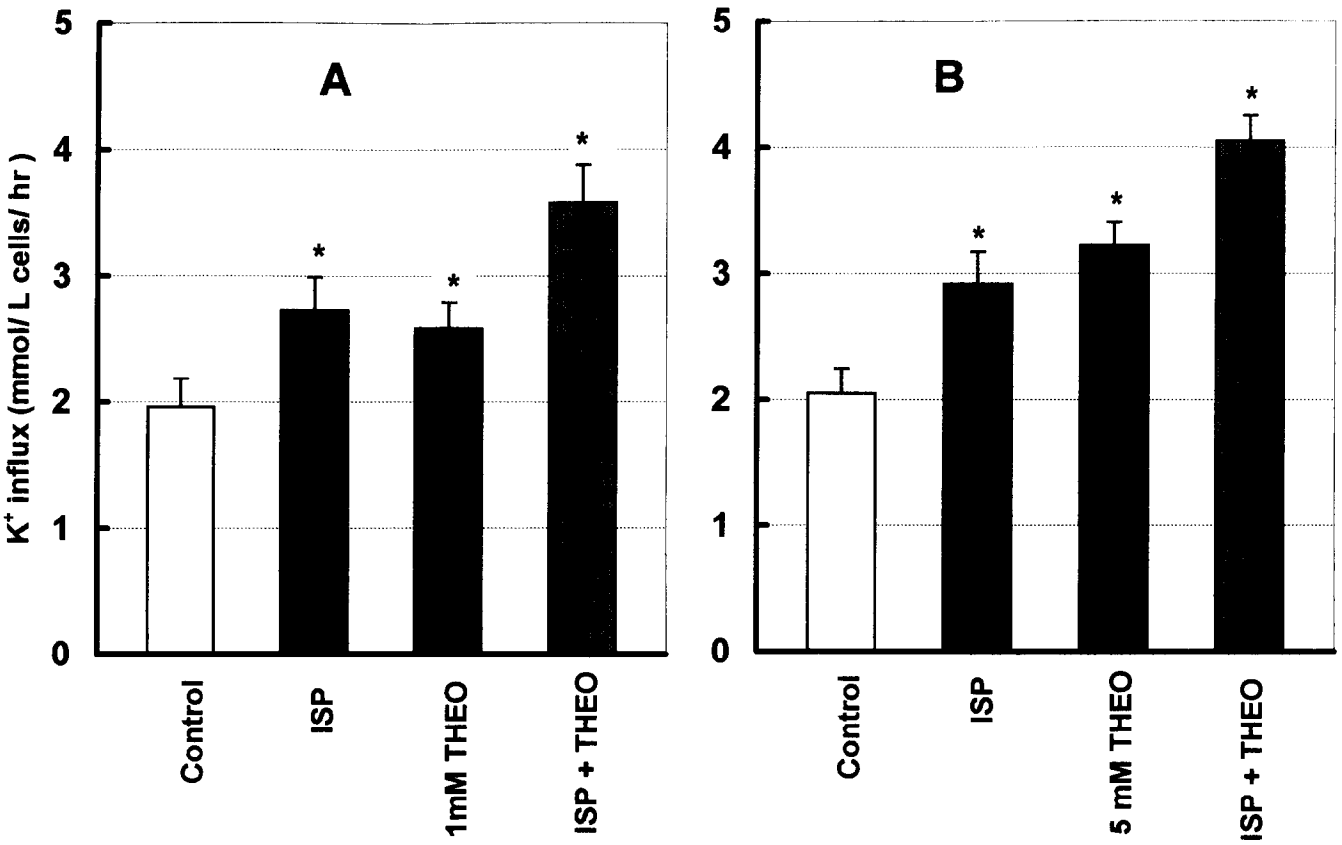

FIG. 3. Stimulation of K^+ influx by isoproterenol (ISP) and/or theophylline (THEO). Frog erythrocytes were preincubated for 5 min in standard saline containing ISP (10 μM) and/or THEO [1 mM (A); 5 mM (B)]. The K^+ influx was then determined using ^{86}Rb uptake for 60 min. Results are means ± SEM of 6 separate experiments. *P < 0.01 (paired t test).

ISP plus IBMX were 0.67 ± 0.13, 0.87 ± 0.27 and 1.68 ± 0.39 mmol/L/hr, respectively. The total K^+ influx in frog erythrocytes was decreased by an average of 64% after NO_3^- was substituted for Cl^- in the incubation media. The replacement of Cl^- with NO_3^- did not alter the ISP- and/or IBMX-induced influxes of K^+ in frog erythrocytes. From the data presented in Fig. 4A and B, it is quite evident that the K^+ influx mediated by the K-Cl cotransporter in the frog erythrocyte membrane was unaffected by ISP and IBMX treatment. On the other hand, the addition of 0.1 mM ouabain to the incubation medium completely abolished the K^+ influx produced by the combination of ISP and IBMX. The residual components of K^+ influx in the presence of 0.1 mM ouabain were 1.72 ± 0.14 mmol/l/hr for control cells and 1.75 ± 0.15 mmol/L/hr for ISP plus IBMX-treated cells incubated in standard saline. In NO_3^- media, the residual components of K^+ influx in the presence of ouabain were 0.036 ± 0.004 and 0.037 ± 0.003 mmol/L/hr for control cells and ISP plus IBMX-treated cells, respectively. The treatment of the frog erythrocytes with ISP plus IBMX caused an increase in the ouabain-sensitive K^+ influx from a control value of 0.39 ± 0.11 to 2.05 ± 0.45 mmol/L/hr in Cl^- media and from 0.73 ± 0.18 to 2.45 ± 0.45 mmol/L/hr in NO_3^- media. Thus, the results clearly indicate that the effect of ISP and IBMX on K^+ transport was associated only with an activation of the Na^+-K^+ pump in the frog erythrocyte membrane.

Effects of Increasing ISP Concentrations and Adrenergic Blockers

The effects of raising external ISP concentrations ranging from 10^{-9} to 10^{-5} M on K^+ transport in frog erythrocytes were examined in the presence and absence of 10^{-5} M propranolol, a β-adrenergic blocker. To determine more accurately the influence of these agents on K^+ influx, the experiments were performed on frog red cells incubated in nitrate media, in which approximately 95% of the total K^+ transport into the cells occurs via the Na-K-pump [13]. Moreover, in preliminary studies, it was found that 10 μM propranolol can stimulate the K^+ influx in frog erythrocytes incubated in standard chloride medium. As shown in Fig. 5A, ISP at a concentration of 10^{-9} M did not cause any significant activation of K^+ transport in frog red cells. A significant increase in K^+ influx above control levels (36%) was observed at an ISP concentration of 10^{-8} M. Maximal stimulation of K^+ transport was seen with ISP at 10^{-6}–10^{-5} M, and half-maximal stimulatory concentration was approximately $2 \cdot 10^{-7}$ M. In paired measurements, addition of 10^{-5} M propranolol to cell suspensions entirely abolished the ISP-induced effects on K^+ influx (Fig. 5B). Propranolol alone did not significantly alter K^+ transport in frog red cells. The treatment of frog red cells with α-adrenergic antagonists (phentolamine, prazosin, and yohimbine) had no appreciable effect on the ISP-stimulated response in K^+

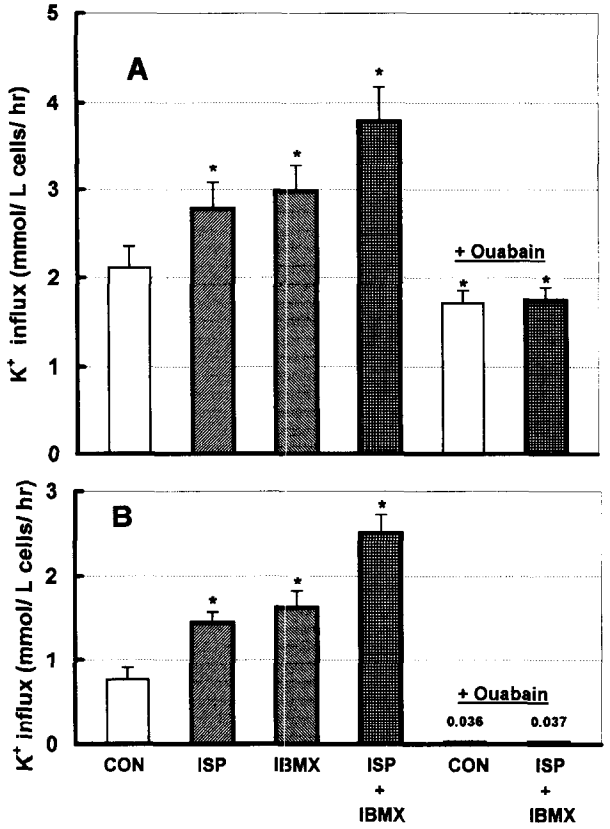

FIG. 4. The effects of isoproterenol (ISP) and 3-isobutyl-1-methylxanthine (IBMX) on K^+ influx in the presence and absence of ouabain in chloride (A) and nitrate (B) media. (A) Frog erythrocytes were preincubated in standard saline with or without 0.1 mM ouabain for 30 min and with or without 10 μM ISP and 0.5 mM IBMX for 5 min. Then ^{86}Rb was added and the suspensions incubated for 60 min to determine K^+ influx. (B) The frog erythrocytes were washed and incubated in saline containing nitrate salts instead of chloride salts. Values are means ± SEM for 5 (A) and 6 (B) separate experiments. *$P < 0.01$ compared to control in the absence of agents.

influx (data not shown). Propranolol, at a concentration of 100 μM, caused a nonspecific action on the frog erythrocyte membrane associated with a partial hemolysis.

Effect of ISP on Na^+ Influx and Intracellular Ion Concentration

Many studies on nucleated erythrocytes of fish and birds have demonstrated the effect of catecholamines on Na^+ transport due to an activation of the Na/H exchanger and the Na-K-Cl cotransporter [1, 2, 12]. The stimulatory action of the hormones on Na^+ influx has also been shown for erythrocytes of frog *Rana pipiens* [3, 4]. Catecholamines may indirectly activate the Na^+-K^+ pump by increasing Na^+ influx and intracellular Na^+ concentration. Therefore, Na^+ transport in the frog red cells and intracellular Na^+ concentration were examined using ^{22}Na as a tracer to measure Na^+ influx, and flame photometry to estimate cellular Na^+ and K^+ concentration. The Na^+ influx calculated from a 60-min ^{22}Na uptake in control red cells (1.37 ± 0.12 mmol/L/hr) did not change in the presence of 10 μM ISP (1.34 ± 0.15 mmol/L/hr). Treatment of the red cells with 0.5 mM IBMX or with a combination of ISP and IBMX was associated with a small decrease in Na^+ influx (1.06 ± 0.09 and 1.08 ± 0.13 mmol/L/hr, respectively), but the change was not statistically significant.

The K^+ concentration was the same in red cells incubated in standard saline for 60 min in the absence (99.0 ± 4.9 mmol/L cells) or the presence of 10 μM ISP (99.2 ± 6.2 mmol/L cells). A small reduction in the Na^+ concentration in the presence of ISP was observed (10.7 ± 0.7 mmol/L/hr) as compared to control values (12.3 ± 0.7 mmol/L/hr), which was statistically significant with $P < 0.02$ in a paired t test (four duplicate experiments).

DISCUSSION

The results of our study provide the first demonstration that the Na^+-K^+ pump in the frog erythrocyte membrane is directly activated by catecholamines. In 34 paired experiments, the K^+ influx in frog erythrocytes incubated in standard saline was enhanced from 2.78 ± 0.20 mmol/L/hr in controls to 3.67 ± 0.23 mmol/L/hr in the presence of 10 μM ISP ($P < 0.001$). The observed increase in K^+ transport in

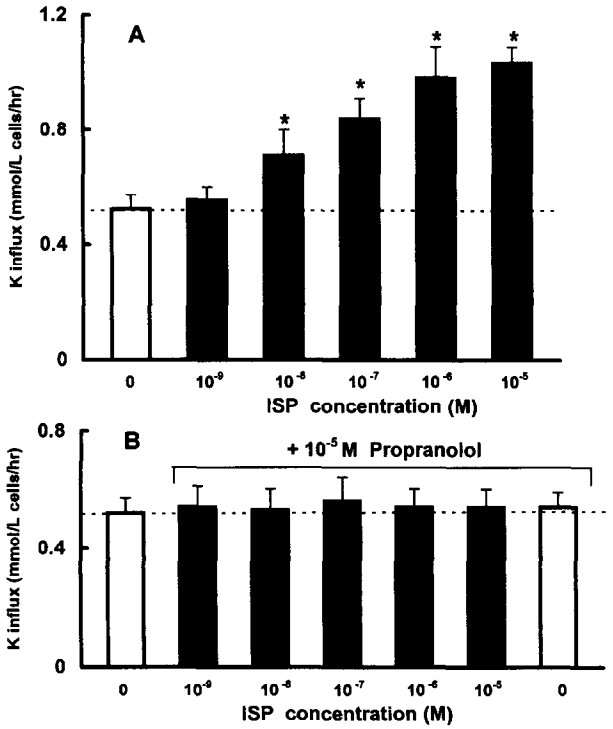

FIG. 5. Concentration-dependent effects of isoproterenol (ISP) on K^+ influx. The frog erythrocytes were preincubated for 5 min in NO_3^- media containing different concentrations of ISP in the absence (A) or presence of 10^{-5} M propranolol (B). Then, ^{86}Rb was added and its uptake measured for 60 min. The broken lines show control levels in the absence of the drugs. Values are means ± SEM for 5 separate experiments. *$P < 0.001$ compared to control level.

the presence of ISP could be due to an activation of adenylate cyclase and an intracellular accumulation of cAMP, which has been well demonstrated in amphibian erythrocytes [6, 7]. Therefore, inhibition of PDE activity would be expected to cause an elevation in cAMP concentration in red cells. Our present results clearly demonstrate that the blockade of PDE activity by THEO (Fig. 3A, B) and IBMX (Fig. 4A, B) resulted in the stimulation of K^+ transport in the frog red cells. When ISP and PDE blockers were added together to the incubation medium, an evident additive effect on K^+ influx was observed in all 3 series of experiments. The summarizing effects of ISP and PDE blockers on K^+ influx were completely abolished after pretreatment of the red cells with 0.1 mM ouabain, indicating that the increment in K^+ transport is solely mediated via the ouabain-sensitive Na^+-K^+ pump. This conclusion is supported by the data of experiments in which Cl^- medium was replaced by NO_3^- medium. It was evident that the stimulatory action of ISP and PDE blockers on K^+ transport was not associated with changing K^+ movement through the K-Cl cotransporter. This action of catecholamines on active K^+ transport was relatively rapid and sustained over the 60 min of cell incubation. It is most likely that ISP causes an immediate switching of Na^+-K^+ pump functional activity in the frog red cells. It should be noted that the magnitude of the active K^+ influx in the red cells varied appreciably, ranging from 0.14 to 0.67 mmol/L/hr in Cl^- media and from 0.37 to 0.98 mmol/L/hr in NO_3^- media. There was no significant difference between the average values of the ouabain-sensitive K^+ influxes in both media. The wide variability in active K^+ transport is probably due to regulatory effects of intracellular messengers on the Na-K pump. Under the conditions of our assays, we were unable to find any changes in Na^+ transport in the frog red cells incubated in the presence of 10 μM ISP. Therefore, the small reduction in intracellular Na^+ concentration induced by ISP appears to be accounted for by the activation of the Na^+-K^+-pump.

The results of the present study provide evidence that β-adrenergic receptors are involved in the catecholamine action on the frog erythrocyte membrane. This conclusion is grounded on the ability of the β-adrenergic antagonist, propranolol, to inhibit the ISP-induced K^+ influx and the lack of inhibitory effects of α-adrenergic blockers. NOR at the same concentration (10 μM) was less potent (by 37%) than ISP, and the combination of NOR and ISP elicited the same effect or K^+ transport as did ISP alone (Fig. 2). The existence of β-adrenergic receptors has been well documented in the erythrocyte membrane of frog *Rana pipiens* [6, 7] and *Rana catesbiana* [14]. The β-adrenergic receptors have been shown to exhibit more affinity for ISP than for NOR [15]. Catecholamine effects on fish and avian red cells are also mediated via β-adrenergic receptors and are blocked by propranolol [2, 16]. The estimated concentration of ISP ($\sim 2 \cdot 10^{-7}$ M) for half-maximal activation of K^+ influx in frog erythrocytes in the present study is similar to that reported for activation of Na^+ influx in trout erythrocytes [17].

A large number of studies have demonstrated the ability of catecholamines to activate different ion transport mechanisms in many cell types, including erythrocytes [2, 8–10]. In erythrocytes of frog *Rana pipiens*, ISP was found to cause a several-fold increase in Na^+ and K^+ fluxes, but only when PDE activity was blocked by IBMX [4]. These effects of ISP plus IBMX on the frog erythrocytes were mimicked by a combination of cAMP and IBMX. Amiloride inhibited the cAMP-stimulated Na^+ and K^+ fluxes in a dose-dependent manner, indicating that the K^+ component may be due to an activation of the Na^+-K^+ pump after Na^+ entry [3]. It is well known that catecholamines increase cAMP levels in fish and avian erythrocytes, resulting in an activation of Na/H exchange and Na-K-Cl cotransport mechanisms, respectively [1, 2]. In carp erythrocytes, ISP induced a stimulation of both Na/H exchange and Na^+-K^+ pump activity [12]. In many other types of cells, numerous studies have demonstrated the stimulatory effects of catecholamines on the Na^+-K^+ pump [8–11, 18]. Most of the detailed studies on the effect of catecholamines on the Na^+-K^+ pump have been done with frog and rat skeletal muscle and mammalian renal tubules. The catecholamine-induced activation of the Na^+-K^+ pump in frog erythrocyte membrane is very similar to that described for skeletal muscle. An increase in Na^+-K^+ pump activity in skeletal and smooth muscle cells [19–23], as well as in the frog red cells in our study occurs within a few min of hormone treatment and is associated with a decrease in intracellular Na^+ concentration. Similar stimulation of the Na^+-K^+ pump by NOR and by permeable analogue cAMP has recently been described for rat and rabbit renal cortical tubules [24, 25]. Moreover, there is some evidence for the stimulatory effect of catecholamines on the Na^+-K^+ pump in leucocytes [26], macrophages [27], liver cells [28] and cardiomyocytes [29, 30].

It is noteworthy that each PDE blocker, in combination with ISP, produced an evident additive effect on the K^+ influx in the frog erythrocytes. Such an additive action of catecholamines and PDE inhibitors on ion transport has not been found in erythrocytes of other species. Rudolph and Greengard [4] demonstrated a significant increase in Na^+ and K^+ influxes in erythrocytes of frog *Rana pipiens* after simultaneous addition of ISP and IBMX, but only small changes in the ion fluxes were observed in the presence of each drug alone. The activation by epinephrine of ^{22}Na efflux from skeletal muscle was also potentiated in the presence of THEO, but THEO itself did not cause a stimulation of Na^+ transport [21]. The precise sequence of events leading to adrenergic activation of the Na^+-K^+ pump remains unclear. It has been suggested that stimulation of cAMP-dependent protein kinase (PKA) by cAMP, or of protein kinase C by elevation of diacylglycerol may directly lead to such hormonal pump regulation in smooth muscle [23], hepatocytes [28], renal cells [31], nerve tissue [11], and

oocytes [32]. On the basis of our data, we cannot exclude the possibility that the effects of ISP and PDE blockers on the Na^+-K^+ pump are mediated via different intracellular messengers.

In summary, the data presented in our paper clearly indicate that both catecholamines (ISP and, with less potency, norepinephrine) and PDE blockers (THEO and IBMX) stimulate the K^+ (^{86}Rb) transport in the frog red cells mediated by the Na^+-K^+ pump. These effects of ISP and PDE blockers are strictly additive and they possibly occur via different mechanisms. In addition to the Na^+-K^+ pump, catecholamines are also known to exert a modulatory influence on various ion transport processes in many cell types. Other than the Na^+-K^+ pump, the frog erythrocyte membrane contains only the K-Cl cotransporter, which may be eliminated by removing Cl^- [13]. Therefore, the red blood cells of frog *Rana temporaria* are quite a convenient model to examine the hormonal modulation of the Na^+-K^+ pump.

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