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INFLUENCE OF ISOPROTERENOL ON NET POTASSIUM UPTAKE IN WHOLE PIGEON ERYTHROCYTES IN VITRO

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Summary

Isoproterenol increases net uptake of potassium in whole pigeon erythrocytes in vitro; effect of 10^{-5} M isoproterenol is blocked by 10^{-4} M propranolol. Pentifylline, a potent inhibitor of cAMP-phosphodiesterase, significantly amplifies effect of isoproterenol, indicating that isoproterenol-effect is mediated by cAMP. cAMP alone has no direct influence on net potassium uptake, while dibuturyl-cAMP has a very weak effect. Isoproterenol-effects are also mediated by the cell membrane protein-phosphorylation.

Introduction

The effects of adrenergic agonists on tissue electrolyte transport are not well characterized and the mechanisms involved are poorly understood / 1, 2 /, although it appears that the endocrine regulation of electrolyte transport is of universal importance / 3 /. The elegant work of Hamprecht and coworkers / 4, 5, 6 / on the stimulation of adenylate-cyclase by hormones in nerve cell cultures, has motivated us to investigate a direct influence of adrenergic effectors on potassium transport in cell cultures. We have chosen avian /pigeon/ erythrocytes as a model cell-culture, as they are simple, easy to obtain in pure state and possess developed adrenergic receptors / 7 /.

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First report that norepinephrine causes an increased potassium uptake in avian erythrocytes is due to Orskov / 8 /. Later, Riddick et al. / 9 / showed that norepinephrine reduced the rate of loss of potassium from duck erythrocytes in vitro; after a period of incubation without catecholamine, addition of norepinephrine causes the reaccumulation of potassium. Our present study deals with the influence of pure β-adrenergic agonist isoproterenol on net potassium uptake in whole pigeon erythrocytes in vitro, and the mechanism of this hormonally regulated transport across the cell membrane.

Materials and Methods

Pigeon erythrocytes were obtained from the blood of domestic pigeons, purchased from local town breeders. Citrated blood was centrifuged, and erythrocytes washed three times in a cold Krebs-Ringer solution. Net potassium uptake in whole erythrocytes was measured by the following experimental protocol / 2 /.

Washed erythrocytes were suspended in Krebs-Ringer solution /plus 10 mM glucose/, and incubated at 40°C; after 90 min suspension was centrifuged and erythrocytes resuspended in the same medium at the concentration of 3%. Then, the effectors were added, and immediately thereafter the flux of potassium was started by raising its concentration by 20 mM /by addition of small aliquots of concentrated KCl/; the solutions were kept at 40°C. After exactly 20 min, erythrocytes were sedimented in a high-speed centrifuge, supernatant discarded and packed red cells extracted with 2 M trichloracetic acid. Potassium concentration in TCA-extracts was determined in an Atomic Absorbtion Spectrophotometer / Perkin-Elmer Corp., Norwalk, Conn., U.S.A./.

1-Hexyl-3,7-dimethyl-xanthine /pentifylline/ and its analog 1-/5'-oxohexyl/-3-methyl-7-propyl-xanthine, were obtained from Hoechst AG, Wiesbaden /Germany/. cAMP, dibutyryl-cAMP, dl-isoproterenol and dl-propranolol were purchased from Sigma Chemie GmbH München, Taufkirchen /Germany/.

Each experiment presented in this work was repeated 2 - 4 times, but always the results of a single representative experiment are presented. Each treatment, shown in Table I, was performed in 4 repetitions, and the results statistically evaluated according to the Student's t-test / 10 /.

Results and Discussion

Pigeon erythrocytes were preincubated in an isotonic Krebs-Ringer medium /with 10 mM glucose/ for 90 min at 40°C.

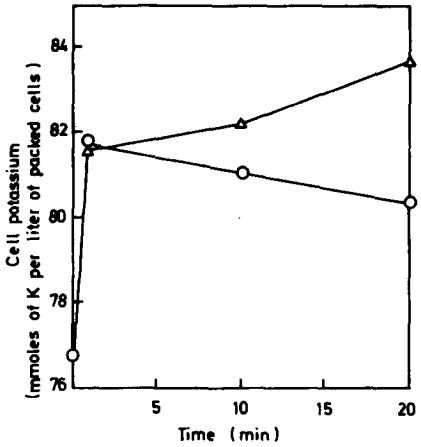

Figure 1. Influence of isoproterenol on the rate of net potassium uptake in whole pigeon erythrocytes. Experimental protocol is described in the section Material and Methods; the samples were incubated in the absence / o /, and in the presence / Δ / of 10^{-5} M isoproeterenol.

Net potassium uptake was started by raising its concentration by 20 mM; immediately thereafter, a rapid uptake of potassium takes place, followed by a steady-state of net transport during next 20 min /Figure 1/.

This steady-state of net transport is significantly influenced by the presence of 10^{-5} M isoproterenol in the medium, effecting an increased net potassium uptake /Figure 1/; for this reason, a fixed period of 20 min was chosen for all subsequent experiments /Table I/ in order to estimate the influence of isoproterenol and other effectors on net potassium uptake in pigeon erythrocytes.

Experiment 1 /Table I/ shows a significant influence of isoproterenol in the concentration range of 10^{-4} M to 10^{-6} M, which is typical for hormone-receptor interaction / 4, 5, 6 /. In order to ascertain that isoproterenol, a β-adrenergic agonist, binds to β-receptors of cell membrane, we have investigated the blocking effect of a pure β-antagonist /propranolol/

TABLE I

Influence of isoproterenol on net potassium uptake in whole pigeon erythrocytes in vitro

Exp.	Treatment	Cell potassium ± S.D.	Statistically significant at:
1.	Control	100.0[a] ± 1.9	reference
	+ 10^{-4}M iprot	106.4 ± 2.5	$P < 0.01$
	+ 10^{-5}M iprot	109.5 ± 1.2	$P < 0.005$
	+ 10^{-6}M iprot	109.5 ± 0.3	$P < 0.005$
2.	Control	100.0[b] ± 0.1	reference
	+ 10^{-5}M iprot	101.8 ± 1.0	$P < 0.05$
	+ 10^{-5}M iprot + 10^{-4}M propran	101.5 ± 2.5	N.S.
	+ 10^{-5}M iprot + 10^{-3}M propran	99.9 ± 1.5	N.S.
3.	Control	100.0[c] ± 1.7	reference
	+ 10^{-3}M pentifylline	100.8 ± 0.7	N.S.
	+ 10^{-5}M iprot	102.8 ± 0.8	$P < 0.05$
	+ iprot + pentifylline	105.1 ± 0.8	$P < 0.005$
4.	Control + 10^{-3}M pentifylline	100.0[d] ± 1.4	reference
	Control + 10^{-5}M cAMP + + 10^{-3}M pentifylline	100.4 ± 1.4	N.S.
5.	Control + 10^{-5}M DBcAMP	100.0[e] ± 0.8	reference
	Control + 10^{-5}M DBcAMP + + 10^{-3}M pentifylline	101.6 ± 1.2	$P < 0.075$

Abbreviations: iprot = dl-isoproterenol; propran = dl-propranolol; DBcAMP = dibutyryl-cAMP.
Cell potassium: /in mmoles of potassium per liter of packed cells/ a = 98.3, b = 86.1, d = 83.2, d = 84.2 and e = 83.8.

on the isoproterenol-effect. Experiment 2 /Table I/ shows that already a 10-fold molar excess of antagonist over agonist significantly blocks β-adrenergic effect of the latter; this assures that the effect of isoproterenol is mediated via

β-receptors. A similar effect of isoproterenol on sodium transport in whole turkey erythrocytes was observed earlier by Gardner et al. / 3 /; it was suggested by these authors that the effect of isoproterenol is mediated by cAMP, a suggestion that led us to check the effect of cAMP on potassium transport of pigeon erythrocytes.

Experiment 3 /Table I/ shows an effect of pentifylline, a potent inhibitor of cAMP-phosphodiesterase / 11 /, on isoproterenol-induced net potassium uptake. 10^{-3} M pentifylline significantly amplifies effect of isoproterenol; since pentifylline increases intracellular concentrations of cAMP / 12 /, this indicates that isoproterenol-effect is, at least partially, mediated by cAMP. Similar effect as pentifylline /1-hexyl-3,5-dimethyl-xanthine/ has its analog: 1-/5'-oxohexyl/--3-methyl-7-propyl-xanthine.

Experiment 4 /Table I/ shows a direct influence of cAMP upon net potassium uptake; cAMP, added directly to external medium, has no effect on net potassium uptake, even in the presence of pentifylline. This is probably explained by the fact that cAMP does not penetrate the cell membrane / 13 /. When dibutyryl-cAMP, a derivative of cAMP that penetrates the cell membrane, is added to the medium, a very weak influence on net potassium uptake is observed /Experiment 5, Table I/. The addition of pentifylline has a much stronger influence on isoproterenol-induced net potassium uptake /Experiment 5/, then has the addition of dibutyryl-cAMP to the external medium /Experiment 5/; this indicates that the isoproterenol-effect is probably mediated by the locally generated cAMP.

Greengard / 14 / reported an isoproterenol-dependent

phosphorylation of a specific protein in the membrane of turkey erythrocytes by $H_3{}^{32}PO_4$; isoproterenol effect is completely abolished by excess propranolol. Our preliminary investigations indicate that the endogenous phosphorylation of membrane in whole pigeon erythrocytes by /γ-^{32}P/ATP is influenced by the presence of cAMP, orthophosphate and isoproterenol / 15 /. These observations indicate that isoproterenol-effect might be mediated, inter alia, by membrane protein phosphorylation.

In conclusion, it appears that the mechanism of activation of net potassium uptake in avian erythrocytes by isoproterenol is rather complex. Net potassium transport of a whole cell is a sum of active influx, active efflux and a passive transport of potassium across the cell membrane. It remains to establish the influence of isoproterenol on each of these components of the net flux, in turn; our laboratory is currently working in that direction.

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