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Mechanism of Catecholamine Secretion Evoked by Lithium from the Isolated Perfused Rat Adrenal Gland@

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ABSTRACT

Lithium (Li) is known to be used not only during acute manic psychosis but also acute depressive phase in manic-depression. In the present study, it was attempted to investigate the effect of lithium on catecholamine (CA) secretion from the isolated perfused rat adrenal gland and to clarify the mechanism of its action.

Replacement of Na^+ (118.4 mM) by lithium in the normal Krebs-bicarbonate solution used to perfuse the gland produced gradually an increased response in the spontaneous catecholamine release, which was peaked at 30~60 min after its perfusion. Li-Krebs solution was perfused into an adrenal vein for 2 hours in every experiments. Li-Krebs-evoked CA secretory responses were depressed significantly under loading with Ca^{++}-free medium. This CA secretion evoked by lithium loading was also reduced markedly by the pretreatment with nicardipine (10^{-6} M), TMB-8 (10^{-5} M) and chlorisondamine (10^{-6} M) for 20 min, respectively, while was not affected by preloading with a pirenzepine (2×10^{-6} M)-containing Krebs. Na^+ pump inhibition by pretreatment with ouabain (10^{-4} M) for 20 min did make the marked depression in Li-evoked CA secretory responses. Moreover, Li-evoked CA release was also diminished markedly by preloading with tetrodotoxin (5×10^{-7} M)-containing Krebs for 20 min.

All these experimental results taken together suggest that lithium enhances CA secretion in a Ca^{++}-dependent fashion by its accumulation in the adrenomedullary chromaffin cells of the rat, and that this secretory effect may be meidated by a dual mechanism: (i) chromaffin cell depolarization and subsequent opening of voltage-sensitive Ca^{++} channels and (ii) activation of a $[Li]_i$-$[Ca]_o$ counter-transport system.

Key Words: Lithium, Chromaffin cell depolarization, Activation of a $[Li]_i$-$[Ca]_o$ counter-transport system

INTRODUCTION

Alkali metal ion Li^+ has diverse biochemical effects in a variety of cells including endocrine cells, neurones and muscles. It has been known that lithium is used not only during acute manic phase but also acute depressive phase in manic-depressive illness (Mendels *et al.*, 1972; Baron *et al.*, 1975; Worral *et al.*, 1979), and that lithium potentiates the action of antidepressants in therapy-resistant depression (de Montigny *et al.*, 1983; Heninger *et al.*, 1983; Terao *et al.*, 1990). Lithium has been also widely used as a Na^+ substitute to investigate Na^+-dependent phenomena (Schou, 1976; Ehrlich and Qiamond, 1980). Hille (1970) has found that lithium can passively penetrate into the cell, and also cross

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the membrane through voltage-dependent Na^+-channels. Because lithium sustitutes for Na^+ but is a poor substrate for the sodium pump (Keynes and Swan, 1959), the cation accumulates easily inside the cells. Ehrlich and Diamond (1980) have demonstrated that lithium participates in special counter-transport mechanisms in muscle, nerve, red blood cells and epithelial membranes, probably mimicking other physiological alkali cations, particularly Na^+. It has been also shown that lithium accumulates in the cells and can partially substitute Na^+ in the Na^+-Ca^{2+} counter-transport system at the plasma membrane of the chromaffin cell, resulting in releasing catecholamines (CA) from the perfused cat adrenal gland (Abajo *et al.*, 1987; Abajo *et al.*, 1991). More recently, Sanchez-Garcia and his coworkers (1994) have demonstrated that sodium pump plays a role on lithium accumulation and extrusion within chromaffin cells, on the extent $[Li]_i$-$[Ca]_o$ counter-transport mechanisms and therefore on the ability of the cation to release CA from the perfused cat adrenal gland. Torok (1991) has reported that the $[Na^+]_i$-$[Ca^{2+}]_o$ exchange plays a significant role in the "excitation-secretion coupling" of peripheral sympathetic nerves and adrenal medullary chromaffin cells.

In addition, lithium action has been widely studied in relation to alteration of adrenergic functions in the central nervous system. These studies showed that the CA content in the brain was unchanged (Corrodi *et al.*, 1969; Ho *et al.*, 1970) or increased (Casado *et al.*, 1989) by lithium treatment. Synthesis of dopamine was decreased (Friedman and Gershon, 1973), but the activity of tyrosine hydroxylase was increased (Segal *et al.*, 1975) by lithium. Recently, Terao and his colleagues (1992) have demonstrated that lithium treatment increases the synthesis and secretion of CA, and the activity of protein kinase C in cultured bovine adrenal medullary cells, suggesting that lithium may enhance the synthesis and secretion of CA in the brain. However, Otero Losada and Rubio (1992) showed that a single intracerebroventricular injection of lithium chloride diminished the monoamine content of the rat mediobasal hypothalamus by inhibiting tyrosine hydroxylase activity, resulting in inhibition of monoamine synthesis. Moreover, long-term treatment with lithium chloride to adult male albino rats produces the reduction in the levels of dopamine and norepinephrine as in that of 5-hydroxytryptamine in brain region (Ghoshdastidar and Poddar, 1990).

On the other hand, the other possibility of some mechanisms proposed for lithium-induced increase in hormone secretion is obtained from the fact that a blockade by intracellular lithium of K^+ channels leads to a membrane depolarization. This depolarization activates Ca^{2+} channels and influx of Ca^{2+}, thereby promoting hormone secretion (Kato and Suzuki, 1989; 1990; Koto *et al.*, 1991). In squid giant axon, internally perfused lithium blocks delayed rectifier K^+ current (Bezanilla and Armstrong, 1972). On the basis of these findings, it is thought that adrenal glands perfused with lithium-containing Krebs may behave as ouabain-treated glands. Therefore, the present study was undertaken to establish the mechanism of CA secretion evoked by lithium from the isolated perfused rat adrenal gland.

MATERIALS AND METHODS

Experimental animals

Mature male Sprague-Dawley rats, weighing 180-300 grams, were anesthetized with ether. The adrenal gland was isolated by the methods described previously (Wakade, 1981). The abdomen was opened by a midline incision, and the left adrenal gland and surrounding area were exposed by placing three hook retractors. The stomach, intestine and portion of the liver were not removed, but pushed over to the right side and covered by saline-soaked gauge pads and urine in bladder was removed in order to obtain enough working space for tying blood vessels and cannulations.

The left renal vein for perfusion of the adrenal gland was cannulated and the tip of the cannula remained near the junction of renal and adrenal veins. All other blood vessels including branches of adrenal vein (if any), vena cava and aorta were ligated.

A small slit was made into the adrenal cortex

just opposite entrance of adrenal vein. Perfusion of the gland was started, making sure that no leakage was present, and the perfusion fluid escaped only from the slit made in adrenal cortex. Then the adrenal gland, along with ligated blood vessels and the cannula, was carefully removed from the animal and placed on a platform of a leucite chamber. The chamber was continuously circulated with water heated at 37 ±1℃

Perfusion of adrenal gland

The adrenal glands were perfused by means of a ISCO pump (WIZ Co.) at a rate of 0.4 ml/min. The perfusion was carried out with Krebs-bicarbonate solution of following composition (mM): NaCl, 118.4; KCl, 4.7; $CaCl_2$, 2.5; $MgCl_2$, 1.18; $NaHCO_3$, 25; KH_2PO_4, 1.2; glucose, 11.7. The solution was constantly bubbled with 95 % O_2+5% CO_2 and the final pH of the solution was maintained at 7.4 to 7.5. The solution contained disodium EDTA (10 ug/ml) and ascorbic acid (100 ug/ml) to prevent oxidation of catecholamine.

Drug administration

The Krebs-bicarbonate solution replaced Na (118.4 mM) by Li^+ (Li-Krebs) or single injection of ACh (5.32×10^{-3} M) in a volume of 0.05 ml were made into perfusion stream via a three way stopcock. In the preliminary experiments, it was found that upon administration of the ACh, secretory response to Ach returned to preinjection level in about 4 min. Generally, the adrenal glands were perfused with normal Krebs solution for about one hour before the experimental protocols are initiated.

Collection of perfusate

As a rule, prior to each stimulation with Li-Krebs or ACh, samples were collected (4 min) to determine the spontaneous secretion of CA ("background sample"). Immediately after the collection of the "background sample", collection of the perfusate was continued in another tube as soon as the perfusion medium containing the lithium or ACh reached the adrenal gland. The perfusate for Li-Krebs was collected for 120 min at 15 min intervals and that for ACh was collected for 4 min. The amounts secreted in the "background sample" have been subtracted from those secreted from the "stimulated sample" to obtain the net secretion value of CA, which is shown in all of the figures.

To study the effect of a test agent on the spontaneous and drug-evoked secretion, the adrenal gland was perfused with Krebs solution containing the agent for 20-30 min, then the perfusates was collected for a specific time period ("background sample"), and then the medium was changed to the one containing the test agent and the perfusates were collected for the same period as that of the control period. The adrenal perfusate was collected in chilled tubes.

Measurement of catecholamines

CA content of perfusate was measured directly by the fluorometric method of Anton and Sayre (1962) without the intermediate purification alumina for the reasons described earlier (Wakade, 1981), using fluorospectrophotometer (Shimadzu Co., Japan). A volume of 0.2 ml of the perfusate was used for the reaction.

The CA content in the perfusate of glands stimulated by ACh or Li-Krebs was high enough to obtain readings several-fold greater than the reading of control samples (unstimulated). The sample blanks were also lowest for perfusates of stimulated and non-stimulated samples.

The content of CA in the perfusate was expressed in terms of epinephrine (base) equivalents.

Drugs and their sources

The following drugs were used: Lithium chloride, acetylcholine chloride, norepinephrine bitartrate, nicardipine hydrochloride and 3.4.5-trimethoxy benzoic acid 8-(diethylamino) octylester (TMB-8), tetrodotoxin and ouabain octahydrate were purchased from Sigma Chemical Co., U.S.A., pirenzepine 2HCl from Shinpoong Pharmaceutical Manufac., Co., Korea, and chlorisondamine chloride from Ciba Co., U.S.A..

Drugs were dissolved in distilled water (stock) and added to the normal Krebs solution as required. Concentrations of all drugs used are expressed in terms of molar base.

Statistical analysis

The statistical significance between groups was determined by utilizing the Student's paired t-test. A P-value of less than 0.05 was considered to represent statistical significant changes unless specifically noted in the text. Values given in the text refer to means with standard errors of the mean (S.E.M.).

The statistical analysis of the present experimental results was made by computer program of statistics described previously by Tallarida and Murray (1987).

RESULTS

Catecholamine secretion evoked by lithium from the rat adrenal glands

The spontaneous secretion of CA from the isolated perfused rat adrenal glands reached a constant level about one hour after the start of perfusion with normal Krebs-bicarbonate solution. The basal release of CA amounted to 62 + 5 ng for 2 min from 20 adrenal glands. Replacement of NaCl (118.4 mM) by lithium chloride (Li-Krebs) in equimolar amounts in the normal Krebs-bicarbonate solution used to perfuse the adrenal gland for 2 hours significantly produced progressive increased output of CA.

As shown in Fig. 1, the amounts of CA secreted into the perfusate which was collected for 2 hrs at 15 min intervals were 639±101 ng (0~15 min), 1390±145 ng (15~30 min), 1691±129 ng (30~45 min), 1438±133 ng (45~60 min), 1182±119 ng (60~75 min), 906±110 (75~90 min), 896±105 (90~105 min) and 703±93 (105~120 min) from 12 rat adrenal glands, respectively. Thus, the increased secretion was already apparent after a few minutes of perfusion, then it increased gradually and reached a maximun during 30 - 60 min periods. From this time onwards the Li-Krebs-evoked CA release progressively decreased, reaching similar level to 1st period (0~15 min) 2 hours later. The present experimental results are identical to those obtained previously from the perfused cat adrenal gland (Abajo *et al.*, 1987; Abajo *et al.*, 1991; Sanchez-Garcia *et al.*, 1994). In order to examine the tachyphylaxis to releasing effect of CA evoked by lithium, Li-Krebs was perfused into the adrenal gland for 2 hours twice consecutively at 60 min intervals. As shown in Fig. 2, there was no statistical difference in amounts of CA secreted by Li-Krebs between 1st and 2nd periods from 8 experiments. Tachyphylaxis to releasing effect of CA evoked by lithium was not observed in the present investigation.

Influence of Ca^{++}-free medium on Li-Krebs-evoked CA secretion

Since it has been reported that calcium plays an indispensible role as the coupler in the stimulus-secretion coupling in the exocytotic secretion of CA and other neurohumoral transmitters (Douglas and Rubin, 1961; 1963; Rubin,

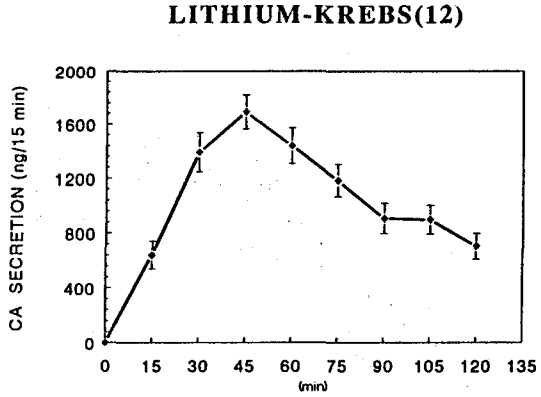

Fig. 1. Time course of Li-Krebs-evoked catecholamine (CA) secretion from the isolated perfused rat adrenal gland. CA secretion was induced by a continuous perfusion of Li-Krebs for 120 min after perfusion with normal Krebs solution for one hour prior to initiation of the experimental protocol. Numeral in the parenthesis indicates number of experimental rat adrenal glands. Vertical bar on each dot represents the standard error of the mean (S.E.M.). Ordinate: the amounts of CA secreted from the adrenal gland in ng. Abscissa: sampling time (min) during the perfusion of Li-Krebs solution. All data were statistically significant as compared the baseline secretion with that of each period. The perfusate was collected for 120 minutes at 15 min intervals.

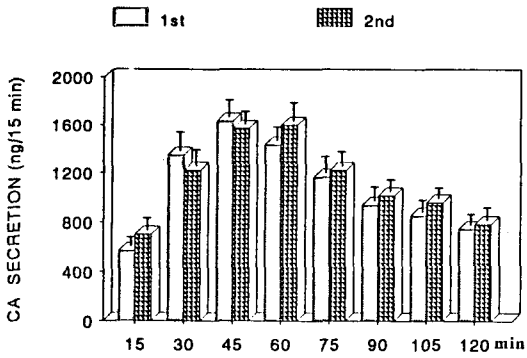

Fig. 2. Time course of repetitive perfusion of Li-Krebs on catecholamine secretion from the isolated perfused rat adrenal gland. Li-Krebs solution was perfused twice successively for 120 min after washing out with normal Krebs for 60 min between 1st and 2nd perfusion. There was no statistical difference in amounts of catecholamines secreted by Li-Krebs between 1st and 2nd perfusion. Other legends are the same as in Fig. 1.

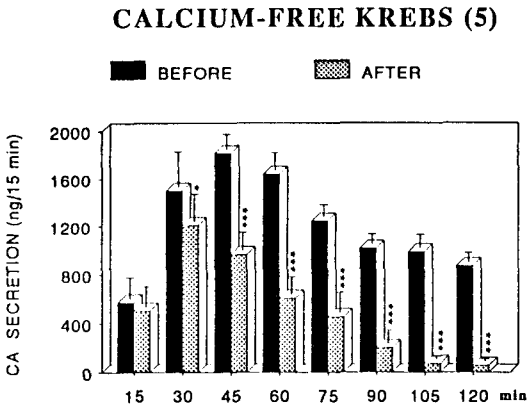

Fig. 3. Influence of perfusion of Ca^{++}-free medium on Li-Krebs-evoked catecholamine secretion from the rat adrenal gland. Ca^{++}-free Krebs was perfused for 30 min before initiation of perfusion with Li-Krebs solution after obtaining the control response. "BEFORE" and "AFTER" represent catecholamine secretion evoked by Li-Krebs before and after preloading with Ca^{++}-free medium. Other legends are as in Fig. 1. *: $P<0.05$, ***: $P<0.01$

1982; William, 1981), it is likely considered of particular to investigate the role of calcium in CA secretion evoked by Li-Krebs from the perfused rat adrenal gland. To do so, the adrenal gland was preloaded with calcium-free Krebs solution 30 min before the perfusion of Li-Krebs was initiated after obtaining the control release of CA by Li-Krebs. In the absence of extracellular calcium, Ca^{++}-free Li-Krebs-evoked CA releasing responses were greatly attenuated to 500±169 ng (0~15 min, ns), 1209±230 ng (15~30 min, $P<0.05$), 965±152 ng (30~45 min, $P<0.01$), 605±141 ng (45~60 min, $P<0.01$), 453±166 ng (60~75 min, $P<0.01$), 203±109 ng (75~90 min, $P<0.01$), 73±29 ng (90~105 min, $P<0.01$) and 58±22 ng (105~120 min, $P<0.01$), respectively from 5 glands as compared with each corresponding control release of 570±176 ng (0~15 min), 1500±295 ng (15~30 min), 1814±125 (30~45 min), 1639±146 ng (45~60 min), 1244±105 ng (60~75 min), 1017±86 (75~90 min), 988±111 ng (90~105 min) and 872±78 ng (105~120 min). As shown in Fig. 3, when calcium was deleted from the Li-Krebs used to perfuse the gland, it was interesting to note that CA secretory response evoked by lithium was significantly abolished.

Influence of nicardipine on Li-Krebs-evoked CA secretion

In order to explore the effect of nicardipine, a dihydropyridine derivative and L-type Ca^{++} channel blocker (Gilman *et al.*, 1991), on Li-Krebs-evoked CA secretion, nicardipine (10^{-6} M) was preloaded into the adrenal gland for 30 min before the introduction of Li-Krebs. In the presence of nicardipine, Li-Krebs-evoked CA releasing responses for 120 min amounted to 455±176 ng (0~15 min, ns), 562±110 ng (15~30 min, $P<0.05$), 484±141 ng (30~45 min, $P<0.01$), 271±97 ng (45~60 min, $P<0.01$), 174±106 ng (60~75 min, $P<0.01$), 19±12 ng (75~90 min, $P<0.01$), 10±1 ng (90~105 min, $P<0.01$) and 10±1 ng (105~120 min, $P<0.01$) from 6 experiments, respectively as compard to their control secretory responses of 570±133 ng (0~15 min), 1143±158 ng (15~30 min), 1192±115 ng (30~45 min), 1152±166 ng (45~60), 823±109 ng (60~75 min), 591±94 ng (75~90 min), 591±81 ng

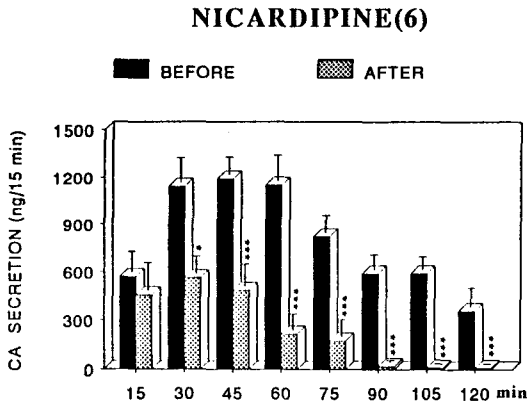

Fig. 4. Influence of nicardipine on Li-Krebs-evoked catecholamine secretion from the rat adrenal gland. Nicardipine (10-6 M) was perfused for 30 min prior to initiation of perfusion with Li-Krebs solution after obtaining the control response. Other legends are as in Fig. 1 and 3. *: P<0.05, ***: P<0.01

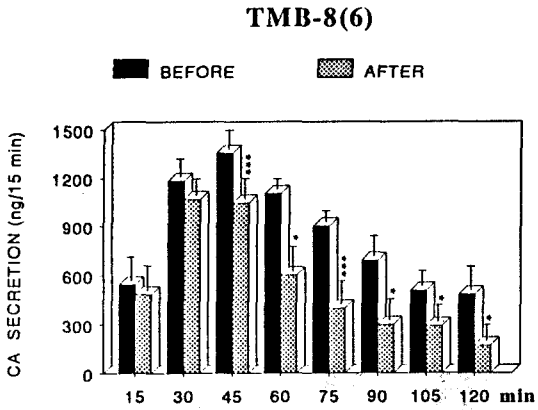

Fig. 5. Influence of TMB-8 on Li-Krebs-evoked catecholamine secretion from the rat adrenal gland. TMB-8 (10^{-5} M) was perfused for 30 min before initiation of perfusion with Li-Krebs solution after obtaining the control response. Other legends are as in Fig. 1 and 3. *: P<0.05, ***: P<0.01

(90~105 min) and 358±119 ng (105~120 min). Fig. 5 shows that Li-Krebs-evoked CA secretory responses are clearly blocked by nicardipine-treatment. In 5 experiments, ACh-induced CA secretion under the presence of nicardipine was also depressed to 294±55 ng (P<0.01) for 4 min as compard to the corresponding control response of 906±96 ng/4 min as shown in Fig. 10.

Influence of TMB-8 on Li-Krebs-evoked CA secretion

Since it has been known that TMB-8 inhibits caffeine-evoked CA release from the rat adrenal gland (Lim *et al.*, 1991) and caffeine-induced $^{45}Ca^{2+}$ release from a sarcoplasmic reticulum of skeletal muscle (Chiou and Malagodi, 1975), and inhibit cholinergic receptor stimulation and membrane depolarization -mediated CA secretory responses (Lim *et al.*, 1992; Nakazato *et al.*, 1988), it is of interest to explore the effects of TMB-8 on Li-Krebs-evoked CA release from the rat adrenal gland. The secretory responses of CA induced by Li-Krebs in the presence of TMB-8 (10^{-5} M) were markedly inhibited to 484±152 ng (0~15 min, ns), 1068±102 ng (15~30 min, ns), 1046±129 ng (30~45 min, P<0.01), 601±147 ng (45~60 min, P<0.05), 397±143 ng (60~75, P<0.01), 300±129 ng (75~90 min, P<0.05), 291±102 ng (90~105 min, P<0.05) and 174±100 ng (105~120 min, P<0.05) from 6 glands, respectively as compared to their control responses of 543±144 ng (0~15 min), 1182±114 ng (15~30 min), 1356±117 ng (30~45 min), 1104±67 ng (45~60 min), 901±69 ng (60~75 min), 688±126 ng (75~90 min), 506±97 ng (90~105 min) and 484±146 ng (105~120 min). Fig. 5 shows that TMB-8 depresses Li-Krebs-evoked CA secretion. In the presence of TMB-8, ACh-induced CA release was decreased to 421±53 ng/4 min (P<0.01) as compared to the corresponding control release of 640±54 ng/4 min from 12 rat adrenal glands (Fig. 10).

Influences of pirenzepine on Li-Krebs-evoked CA secretion

It has been found that pirenzepine is a selective M1-muscarinic receptor antagonist (Doods *et al.*, 1978; Hammer *et al.*, 1988). Thus, it would be of interest to test the effect of pirenzepine on CA secretion induced by Li-Krebs. In the present investigation, the CA release by Li-Krebs was evoked from the adrenal gland preloaded with 2×10^{-6} M pirenzepine for 30

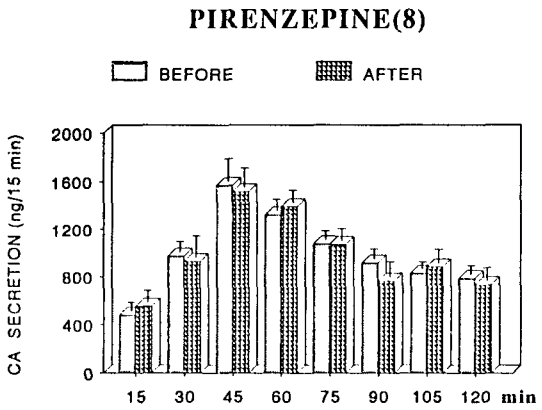

Fig. 6. Influence of pirenzepine on Li-Krebs-evoked catecholamine secretion from the rat adrenal gland. Pirenzepine (2×10^{-6} M) was perfused for 30 min before initiation of perfusion with Li-Krebs solution after obtaining the control response. There was no statistical difference between amounts of catecholamines secreted by Li-Krebs before and after preloading with pirenzepine. Other legends are as in Fig. 1 and 3.

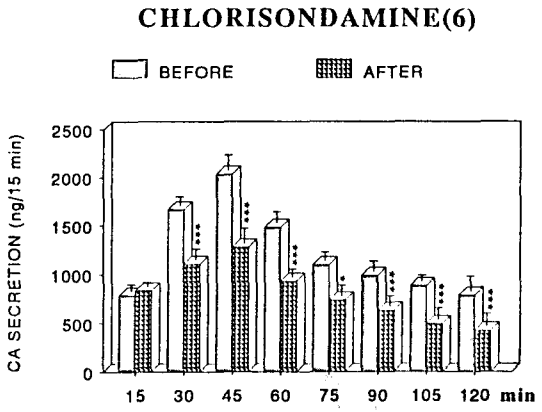

Fig. 7. Influence of chlorisondamine on Li-Krebs-evoked catecholamine secretion from the rat adrenal gland. Chlorisondamine (10-6 M) was perfused for 30 min before initiation of perfusion with Li-Krebs solution after obtaining the control response. Other legends are as in Fig. 1 and 3. *: $P<0.05$, ***: $P<0.01$

min after the control release of it was obtained. In 8 glands Li-Krebs-evoked CA secretory responses under the presence of pirenzepine were still preferentially released. There was no statistical difference in amounts of CA secretion evoked by Li-Krebs between before and after the M1-muscarinic blockade as shown in Fig. 6. However, ACh-induced CA secretion in the absence of pirenzepine was greatly depressed to 417±77 ng/4 min ($P<0.01$) from 7 experiments as compared to the corresponding control response of 679±81 ng/4 min (Fig. 10).

Influence of chlorisondamine on Li-Krebs-evoked CA secretion

In order to explore the effect of chlorisondamine, a selective nicotinic receptor antagonist (Gilman *et al.*, 1991), on Li-Krebs-evoked CA secretion, the rat adrenal gland was perfused with chlorisondamine (10^{-6} M) for 20 min before the introduction. In the presence of chlorisondamine effect, the CA secretory responses evoked by Li-Krebs were greatly inhibited to 833±36 ng (0~15 min, ns), 1104±115 ng (15~30 min, $P<0.01$), 1279±152 ng (30~45 min, $P<0.01$), 930±84 ng (45~60 min, $P<0.01$), 736±113 ng (60~75 min, $P<0.01$), 630±110 ng (75~90 min, $P<0.01$), 494±120 ng (90~105 min, $P<0.01$) and 426±131 ng (105~120 min, $P<0.01$) from 6 adrenal glands, respectively as compared to their corresponding control responses of 785±72 ng (0~15 min), 1666±93 ng (15~30 min), 2034±155 ng (30~45 min), 1482±117 ng (45~60 min), 1095±92 ng (60~75 min), 988±107 ng (75~90 min), 882±69 ng (90~105 min) and 785±154 ng (105~120 min) as shown in Fig. 7. ACh-induced CA secretion under the presence of nicotinic blockade was greatly attenuated to 297±75 ng/4 min ($P<0.01$) from 11 experiments as compared to the control release of 789±39 ng/4 min (Fig. 10).

Influence of ouabain on Li-Krebs-evoked CA secretion

Since ouabain actions have been mainly explained by an increase in Na^+-dependent Ca^{2+} influx resulting from the inhibition of Na^+-K^+ pump (Garcia *et al.*, 1980; 1981a; Sorimachi *et al.*, 1981), it would be exciting to test the effect of ouabain on Li-Krebs-evoked CA secretion was also infused sequentially for 2 hours at 60 min intervals before and after exposure to ouabain (10^{-4} M) for 30 min. As illustrated in Fig.

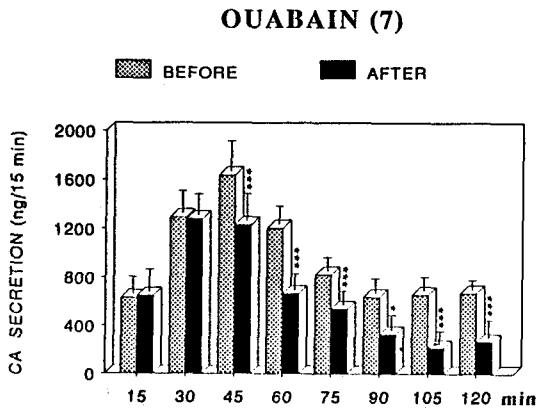

Fig. 8. Influence of ouabain on Li-Krebs-evoked secretory responses of catecholamines from the rat adrenal gland. Ouabain (10-4 M) was perfused for 30 min before initiation of perfusion with Li-Krebs solution after obtaining the control response. Other legends are the same as in Fig. 1 and 3. *: $P<0.05$, ***: $P<0.01$

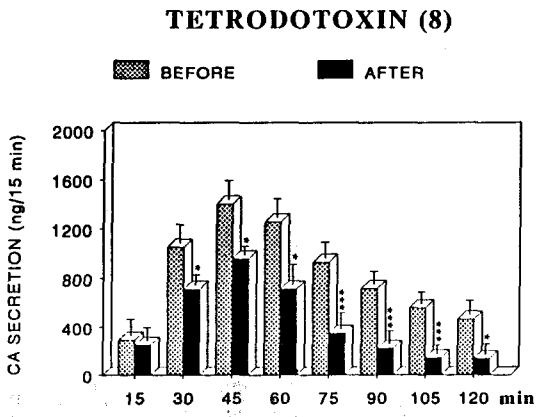

Fig. 9. Influence of tetrodotoxin on Li-Krebs-evoked secretory resposes of catecholamines from the rat adrenal gland. Tetrodotoxin (5×10^{-7} M) was perfused for 30 min prior to initiation of perfusion with Li-Krebs solution after obtaining the control secretory response. Other legends are as in Fig. 1 and 3. *: $P<0.05$, ***: $P<0.01$

8, Li-Krebs-evoked CA secretory responses in the presence of ouabain amounted to 647±186 ng (0~15 min, ns), 1287±174 ng (15~30 min, ns), 1228±222 ng (30~45 min, $P<0.01$), 664±131 ng (45~60 min, $P<0.01$), 538±115 ng (60~75 min, $P<0.01$), 330±126 ng (75~90 min, $P<0.05$), 219±106 ng (90~105 min, $P<0.01$) and 271±143 ng (105~120 min, $P<0.01$) from 7 experiments, respectively compared to the corresponding control responses of 629±143 ng (0~15 min), 1292±184 ng (15~30 min), 1637±245 ng (30~45 min), 1201±150 ng (45~60 min), 823±110 ng (60~75 min), 639±123 ng (75~90 min), 656±114 ng (90~105 min) and 672±78 ng (105~120 min). In 13 glands, ACh-evoked CA release under the presence of ouabain effect was weakened to 484±121 ng/4 min ($P<0.05$) as compared to its control of 828±52 ng/4 min (Fig. 10).

Influence of tetrodotoxin on Li-Krebs-evoked CA secretion

It is found that in cultured bovine adrenal medulla cells, veratridine-induced influx of $^{22}Na^{+}$ through tetrodotoxin-sensitive voltage-dependent Na^{+} channels and subsequent accumulation of Na^{+} in the cells are causally involved in the regulation of $^{45}Ca^{2+}$ influx and CA secretion (Wada *et al.*, 1984; 1985; 1985). It is of interest to explore the influence of tetrodotoxin on Li-Krebs-evoked CA secretion. In 8 experiments as shown in Fig. 9, the secretory responses of CA evoked by Li-Krebs under the presence of tetrodotoxin (5×10^{-7} M) were greatly inhibited to 254±112 ng (0~15 min, ns), 712±85 ng (15~30 min, $P<0.05$), 959±71 ng (30~45 min, $P<0.05$), 712±167 ng (45~60 min, $P<0.05$), 349±136 ng (60~75 min, $P<0.01$), 225±111 ng (75~90 min, $P<0.01$), 145±72 ng (90~105 min, $P<0.01$) and 138±89 ng (105~120 min, $P<0.05$), respectively as compared to their control releasing responses of 298±138 ng (0~15 min), 1054±147 ng (15~30 min), 1402±159 ng (30~45 min), 1257±155 ng (45~60 min), 930±129 ng (60~75 min), 719±105 ng (75~90 min), 560±97 ng (90~105 min) and 465±121 ng (105~120 min). ACh-induced CA release in the presence of tetrodotoxin was also attenuated to 375±77 ng/4 min ($P<0.05$) as compared to 537±32 ng/4 min from 8 glands (Fig. 10).

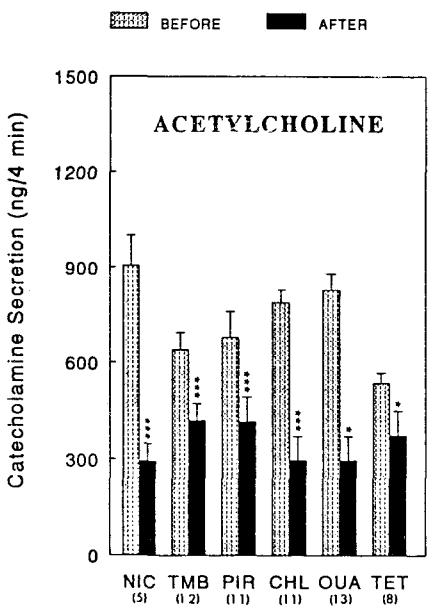

Fig. 10. Influence of nicardipine, TMB-8, pirenzepine, chlorisondamine, ouabain and tetrodotoxin on ACh-evoked catecholamine secretion from the rat adrenal gland. Nicardipine (NIC, 10^{-6} M), TMB-8 (TMB, 10^{-5} M), chlorisondamine (CHL, 10^{-6} M), pirenzepine (PIR, 2×10^{-6} M), ouabain (OUA, 10^{-4} M), tetrodotoxin (TET, 5×10^{-7} M) were perfused, respectively, 30 min before injection of ACh (5.32×10^{-3} M) after obtaining the corresponding control secretory response. Other legends are as in Fig. 1 and 3. *: $P<0.05$, ***: $P<0.01$

DISCUSSION

In the present study, it has been demonstrated that the replacement of Na^+ (118.4 mM) by lithium in the normal Krebs-bicarbonate solution produces a progressively increased response of the spontaneous CA secretion in a Ca^{2+}-dependent fashion by its accumulation in the adrenomedullary chromaffin cells of the rat. This secretory effect seems to be exerted by a dual mechanism: (i) chromaffin cell depolarization and subsequent opening of voltage-sensitive Ca^{2+} channels and (ii) activation of a $[Li]_i$-$[Ca]_o$ counter-transport system.

Generally, in the exocytotic secretion of CA and other neurohumoral transmitters, calcium plays an indispensable role as the coupler in the stimulus-secretion coupling (Douglas and Rubin, 1961; 1963; Rubin, 1982; William, 1981). Stimulation of the cell membrane causes a transient increase of membrane permeability to Ca^{2+} (Ca^{2+} influx) which is necessary for the initiation of the secretion. In adrenal medulla cells the physiological secretagogue ACh (Feldberg *et al.*, 1934) binds to its membrane receptor, resulting in the increased influx of Ca^{2+} (Douglas and Poisner, 1961; 1962) which occurs mainly through voltage-dependent Ca^{2+} chanels and additionally through receptor-associated Ca^{2+} channels (Holz *et al.*, 1983; Kilpatrick *et al.*, 1982; Schneider *et al.*, 1981). In the present investigation, the CA secretory response induced by perfusing the glands with Li-Krebs solution gradually increased, reaching a maximum within 30~60 min and slowly declined approaching basal (resting) levels after 2 hours. This response was Ca^{2+}-dependent exocytosis due to membrane depolarization-induced Ca^{2+}-influx because Li-Krebs-evoked CA release was greatly inhibited in the presence of nicardipine, a blocker of L-type Ca^{2+}-channel (Gliman *et al.*, 1991) or in the Ca^{2+}-free medium.

Moreover, this secretory effect of CA evoked by Li-Krebs was also depressed by TMB-8, which is known to inhibit caffeine-evoked CA release from the rat adrenal gland (Lim *et al.*, 1991) and caffeine-induced $^{45}Ca^{2+}$ release from a sarcoplasmic reticulum of skeletal muscle (Chiou and Malagodi, 1975), and to inhibit cholinergic receptor stimulation and membrane depolarization-mediated CA secretory responses (Lim *et al.*, 1992; Nakazato *et al.*, 1988). In support of this idea, it has been found that inhibitors of voltage-dependent Ca^{2+} channels such as magnesium (Douglas and Rubin, 1963; Lastowecka and Trifaro, 1974; Rubin, 1982; Schneider *et al.*, 1981; Wada *et al.*, 1985) and Ca^{2+} antagonists (Cena *et al.*, 1983; Kilpatrick *et al.*, 1982; Rubin, 1982; Schneider *et al.*, 1981) suppress ACh- as well as depolarization-induced Ca^{2+} influx. Wada and his coworkers (1985) have shown that magnesium inhibits the influx of $^{45}Ca^{2+}$ and CA secretion caused by carbachol,

veratridine and high K^+.

Lithium, a monovalent cation that shares many features with Na^+ (Schou, 1976) is a poor substrate for the Na^+ pump (Keynes and Swan, 1959) and passively permeates the membrane, and easily accumulates inside the cells. Therefore, it could be felt that lithium like sodium passively permeates membrane through voltage-sensitive Na^+ channels, resulting in depolarization along with subsequent opening of voltage-sensitive Ca^{2+} channels. In the present work, the finding that Li-Krebs-evoked CA secretory responses were markedly depressed by preloading with tetrodotoxin, a highly selective inhibitor of voltage-dependent Na^+ channels, supports this hypothesis of lithium. In support of these facts, it has been known that, in cultured bovine adrenal medulla cells, veratridine-induced influx of $^{22}Na^+$ through tetrodotoxin-sensitive voltage-dependent Na channels and subsequent accumulation of Na^+ in the cells are causally involved in the regulation of $^{45}Ca^{2+}$ influx and CA secretion (Wada *et al.*, 1984; 1985). Moreover, veratridine-induced influx of $^{45}Ca^{2+}$ and CA secretion are found to be completely abolished by tetrodotoxin and also not to be observed in Na^+-free medium (Wada *et al.*, 1985). These results indicate that veratridine-induced Ca^{2+} influx does not occur without the influx of Na^+ through voltage-dependent Na^+ channels. In the isolated adrenal medulla cell, veratridine was reported to cause membrane depolarization (Knight and Whitaker, 1978; Knight and Baker, 1983), which was dependent on Na^+ in the medium. This influx of Na^+ caused by veratridine is probably responsible for membrane depolarization. Wada and his coworkers (1985) showed in cultured bovine adrenal medulla cells that ouabain, an inhibitor of Na^+, K^+-adenosine triphosphatase potentiated the veratidine-induced intracellular accumulation of $^{22}Na^+$, while diphenylhydantoin, an anticonvulsant known to reduce intracellular Na^+, abolished the effect of ouabain, and that the modulation of $^{22}Na^+$ accumulation by these drugs is causally involved in the alterations of $^{45}Ca^{2+}$ influx and CA secretion. In the light of these facts, it is likely that accumulation of lithium instead of Na^+ within in the rat adrenomedullary chromaffin cells and subsequent depolarization of cell membrane activate voltage-dependent Ca^{2+} channels through which Ca^{2+} enters into the cells and triggers the exocytotic secretion of CA. Moreover, Richelson (1977) also found that lithium ion entry even at low concentration (1 to 5 mM) into an electrically active adrenergic clone of mouse neuroblastoma cells was stimulated by veratridine; and this stimulation was blocked by tetrodotoxin. These data provide biochemical evidence that lithium ions enter by way of the sodium channel which may be a major pathway for entry of this ion into electrically active cells, and it is also confirmed that lithium enters chromaffin cells by way of Na^+ channel.

It is also known that there exists in the adrenal medulla, a Na^+-Ca^{2+} counter-transport mechanism (Garcia *et al.*, 1980; Esquerro *et al.*, 1980; Aunis and Garcia, 1981; Torok, 1991) similar to that described in the giant axon of the squid (Baker *et al.*, 1969; Baker, 1972; Blaustein, 1974). This Na^+-Ca^{2+} exchange system might be involved in the control of the intracellular concentration of ionized Ca^{2+} levels and, therefore, in the modulation of CA release by chromaffin cells. Procedures leading to an increase in the ratio $[Na]_i/[Na]_o$, such as Na^+ deprivation or ouabain treatment, will activate this system favoring the entry of Ca^{2+} into the cell, in exchange for internal Na^+ (Garcia *et al.*, 1980; Nishimura *et al.*, 1981; Nishimura and Sorimachi, 1984; Sorimachi and Nishimura, 1984). Then the elevated intracellular $[Ca^{2+}]$ will result in a parallel increase of CA output by the gland. Furthermore, it is also found that the intracellular lithium accumulation is the critical factor for the secretory response evoked by this ion; i. e. in a similar manner that Na^+ accumulation is for CA release evoked by ouabain (Esquerro *et al.*, 1980; Garcia *et al.*, 1980; 1981). The close similarity between the secretory response induced by both lithium and ouabain suggests that a $[Li]_i$-$[Ca]_o$ exchange could be the mechanism involved in the CA release evoked by lithium. It has been demonstrated that Na^+-dependent Ca^{2+} movements in cardiac sarcolemmal vescles are inhibited when Na^+ is present on the same site of the membrane as Ca^{2+}, a finding consistent with a competitive antagonism between Na^+ and Ca^{2+} for a com-

mon divalent site (Reeves and Sutko, 1983). These facts are consistent with the idea that lithium shares, to some extent, the functional properties of Na^+ in the Na^+-Ca^{2+} counter-transport system present in the membrane of chromaffin cells. In support of these ideas, Artalejo and Garcia (1986) have proposed that ouabain enhances the spontaneous rates of CA secretion by a dual mechanism; (i) chromaffin cell depolarization which subsequently would open voltage-dependent Ca^{2+} channels and (ii) activation of a Na^+-Ca^{2+} exchange system in reverse mode. Moreover, Abajo and his collaborators (1987) have also demonstrated that lithium accumulates in cells and can partially substitute Na^+ in the Na^+-Ca^{2+} counter-transport system at the plasma membrane of the cat adrenomedullary chromaffin cells. Recently, Abajo and his coworker (1991) thought that cat adrenal glands perfused with lithium-containing Krebs would behave as ouabain-treated glands. More recent studies indicate that the Na^+-pump plays a role on lithium accumulation and extrusion from the chromaffin cells of the perfused cat adrenal gland, on the extent $[Li]_i$-$[Ca]_o$ counter-transport mechanisms and therefore on the ability of cation to release CA (Sanchez-Garcia *et al.*, 1994). The present experimental result that Li-Krebs-evoked CA secretion was suppressed by ouabain-treatment suggests strongly that the CA secretory effect evoked by Li-Krebs may be mediated through the same mechanism with that by ouabain. Ouabain is also known to release CA from the perfused cat adrenal gland by a calcium-dependent exocytotic mechanism (Garcia *et al.*, 1980). The secretory effect of ouabain is not secondary to the release of ACh from the cholinergic nerve terminals present in the adrenal gland, but due to a direct action on the chromaffin cell itself. In addition, the results suggest that this action is exerted through redistribution of monovalent actions secondary to the inhibition by the glycoside of the sodium pump. Such monovalent cation redistribution may cause a rise of intracellular ionized Ca^{2+} levels through the activation of internal Na^+-dependent Ca^{2+} influx system probably located in the chromaffin cell membrane. Besides it has been well found that cardiac glycosides increase both spontaneous and evoked CA secretions from the perfused adrenal glands of various species (Banks, 1967; 1970; Garcia *et al.*, 1981b; Wakade 1981; Nakazato *et al.*, 1986) and isolated adrenal chromaffin cells (Aunis and Garcia, 1981; Sorimachi *et al.*, 1981; Pocock, 1983a, b). In the present experiment, the fact that ouabain-pretreatment depressed markedly Li-Krebs-evoked CA secretory responses in the rat adrenal gland indicates that the secretory effect of CA evoked by lithium is very similar to that of ouabain.

On the other hand, Li-Krebs-evoked CA secretion was significantly depressed by the pretreatment with chlorisondamine, selective nicotinic receptor antagonist (Gilman *et al.*, 1991). This finding suggests that the secretory effect of Li-Krebs is considerably associated with stimulation of nicotinic receptor in the adrenal medulla, since it has been known that the activation of nicotinic receptors stimulates CA release by increasing Ca^{2+} channels in both perfused rat adrenal glands (Wakade and Wakade, 1983) and bovine isolated adrenal chromaffin cells (Kilpatrick *et al.*, 1981; 1982; Knight and Kesteven, 1983). Although it was not attempted in the present study, in terms of the findings observed in studies on ouabain effects (Nakazato *et al.*, 1986), it could be suggested that lithium enhances CA secretion evoked by ACh and high K^+ by increasing the rate of Ca^{2+} influx through the ACh receptor-linked Ca^{2+} channels on adrenal chromaffin cells as a result of the inhibition of the Na^+, K^+-pump. In support of these findings, Cena and his coworkers (1983) observed in cultured bovine adrenal medulla cells that nicotine-induced release of radioactivity from [^{3}H] norepinephrine-prelabeled cells was abolished when the cells were preincubated in Na^+-free medium. Similar observations were reported in isolated bovine and guinea-pig adrenal medulla cells (Lemaire *et al.*, 1981; Role *et al.*, 1981). Wada and his colleagues (1985) also observed that decrease of carbachol-induced secretion of CA in Na^+-free medium was attributed to the suppression of $^{45}Ca^{2+}$ influx, and that the inhibitory effects of Na^+ removal were not deleterious, since its inhibitory action was reversible.

However, since pirenzepine, a muscarinic M_1-

receptor antagonist, failed to modify the secretory effect of CA evoked by Li-Krebs, it is likely that lithium effect is not due to the release of ACh by activation of M_1-muscarinic receptors from presynaptic cholinergic nerve terminals present in the adrenal medulla.

In conclusion, the present experimental results taken together suggest strongly that lithium produces an increased rate of spontaneous CA secretion from the isolated pefused rat adrenal gland, and this secretory effect of lithium seems to be exerted by the following postulated sequence of events; i) lithium passively diffuses the membrane through voltage-sensitive Na^+ channel and it accumulates in the chromaffin cells since it is a poor substrate for Na^+, K^+-ATPase, resulting in chromaffin cell depolarization and subsequent opening of voltage-operated calcium channels; ii) lithium partially replaces Na^+ in the Na^+-Ca^{2+} countertransport system present in the membrane of chromaffin cells, resulting in activation of a reverse Li^+-Ca^{2+} exchange mechanism leading to an increase of $[Ca]_i$ that subsequently promotes CA release.

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=국문초록=

흰쥐 적출관류부신에서 리튬에 의한 카테콜아민 분비작용의 기전

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리튬(Lithium)은 임상에서 조울병 치료에 이용되고 있다. 본 연구는 흰쥐 적출 관류부신 으로부터 catecholamine (CA) 분비에 대한 리튬의 작용을 검색 하고 그 기전을 규명하고자 하여 얻어진 결과는 다음과 같다.

정상 Krebs-bicarbonate 용액내의 Na^+ (118.4 mM)을 리튬으로 대치하여 관류하였을때 CA 분비는 점차적인 증가를 나타내었으며, 30~60분에서 최대 분비작용을 나타내었다. Li-Krebs액은 모든 실험에서 부신정맥을 통해서 2시간동안 관류하였다. Li-Krebs에 의한 CA 분비반응은 Ca^{++}-free Krebs액으로 전처치한 상태에서 유의하게 억제되었다. 이와같은 Li-Krebs액에 의한 CA 분비작용은 nicardipine (10^{-6} M), TMB-8 (10^{-5} M) 및 chlorisondamine (10^{-6} M) 등을 20 분간 각각 전처치 하였을때 현저히 감약되었으나 pirenzepine (2×10^{-6} M)에 의해서는 별다른 영향을 받지 않았다. Na^+ pump 억제제인 ouabain (10^{-4} M)으로 20 분간 전처치한 후 Li-Krebs에 의한 CA 유리작용은 뚜렷이 억제되었다. 더우기 tetrodotoxin (5×10^{-7} M)으로 20 분간 전처치 하였을때도 Li-Krebs에 의한 CA 분비반응은 현저히 감약되었다.

이상과 같은 실험결과를 종합하여 보면, 리튬은 흰쥐 부신수질의 크롬 친화성 세포내에 축적됨으로써 칼슘의존성의 CA 분비작용을 일으키며, 이러한 분비작용은 i) 크롬친화성 세포의 탈분극과 이어서 voltage-sensitive 칼슘채널의 개방과 ii) $[Li]_i$-$[Ca]_o$ counter-transport system의 활성화를 통한 두가지 작용기전에 의해서 매개되는 것으로 생각된다.