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Catecholamine release evoked by lithium from the perfused adrenal gland of the cat

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1 The effect of Li on catecholamine release by cat isolated retrogradely perfused adrenal gland was investigated. Replacement of Na (119 mM) by Li in the Krebs solution evoked a progressive increase in the spontaneous release of catecholamines that reached a maximum within 45 min and was Ca-dependent. This response was specific for Li, since sucrose or choline used as osmotic substitutes for Na, failed to increase the spontaneous release of catecholamines by the adrenal gland.

2 In glands perfused with Li-Krebs for 30 min a sharp secretory response was observed when Li was replaced by sucrose or choline; no such an effect was seen when Li was replaced by Na.

3 Partial replacement of Na by sucrose, in ouabain (10^{-4}M, 10 min) pretreated glands perfused with normal Krebs induced a sharp increase in the catecholamine output whilst replacement by Li produced a significantly lower response.

4 Reintroduction of Ca (2.5 mM, 2 min) in glands previously perfused with Ca-free, Mg-containing Li-Krebs, evoked a sharp increase in catecholamine release. No such an effect was seen when the glands were perfused with Ca-free normal, choline- or sucrose-Krebs.

5 The release of catecholamines evoked by Ca reintroduction in glands previously perfused with Ca-free Li-Krebs was directly dependent on the Li concentration and the length of time of the Li loading period.

6 In summary, our results indicate that Li accumulates in the cells and can partially substitute Na in the Na-Ca counter-transport system at the plasma membrane of the chromaffin cell.

Introduction

Lithium has been widely used as a Na substitute to investigate Na-dependent phenomena (Schou, 1976; Ehrlich & Diamond, 1980). It is well established that Li can passively penetrate into the cell, and also cross the membrane through voltage-dependent Na channels (Hille, 1970). Because Li is not a good substrate for the sodium pump (Keynes & Swan, 1959), the cation accumulates easily inside the cells. Recently, Li has also been demonstrated to participate in special counter-transport mechanisms in muscle, nerve, red blood cells and epithelial membranes, probably mimicking other physiological alkali cations, particularly Na (Ehrlich & Diamond, 1980).

In our laboratory we have previously characterized a Na-Ca exchange system that seems to modulate the rate of adrenomedullary catecholamine release; such a mechanism is activated by Na accumulation in glands treated with ouabain to inhibit Na pumping (García *et al.*, 1980; Esquerro *et al.*, 1980). Because Li substitutes for Na but is a poor substrate for the Na pump (Keynes & Swan, 1959), we thought that cat adrenal glands preperfused with Li could behave as ouabain-treated glands. The present investigation was undertaken to study the behaviour of such glands as far as their catecholamine secretory rate was concerned. Our results demonstrate that replacement of Na by Li causes an enhancement of the rate of catecholamine release, that could be mediated by the activation of a Li-Ca exchange mechanism.

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Methods

Perfusion of cat isolated adrenal glands

Cats of either sex weighing 2.5–4 kg were anaesthetized with ether followed by chloralose (40 to 60 mg kg^{-1} i.v.). Both cat adrenal glands were isolated

and prepared for retrograde perfusion with Krebs-bicarbonate solution at room temperature as previously described (García et al., 1980). The perfusion rate was adjusted to 1 ml min^{-1}.

Perfusion media

The normal Krebs-bicarbonate solution had the following composition (mM): NaCl 119, KCl 4.7, KH_2PO_4 1.2, $MgSO_4 7H_2O$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25 and glucose 11. This solution was equilibrated with 95% O_2 and 5% CO_2 the final pH being 7.4 to 7.5. Ca-free solutions were made up simply by deleting $CaCl_2$ from the perfusion medium without osmotic adjustments. No differences in the secretory response was observed when $CaCl_2$ was replaced by equimolar amounts of $MgCl_2$. When the Na concentration was reduced to 25 mM, the osmolarity of the solution was maintained with equivalent amounts of sucrose, choline chloride or LiCl. These modified perfusion media will be referred to in the text as sucrose-Krebs, choline-Krebs or Li-Krebs. When $[Na]_o$ was decreased to less than 25 mM, tris-hydroxymethyl-amino methane (10 mM), instead of bicarbonate, was used as a buffer. Under these circumstances the modified Krebs solution was bubbled with pure O_2. Potassium rich solution (59 mM) was prepared by the addition of KCl and reduction of NaCl to maintain isotonicity, and was perfused for a 2 min period at the end of all experiments in order to ascertain the functional viability of the glands.

Collection of perfusate samples

After 1 h of initial perfusion with normal Krebs solution, collection of perfusate samples at 2 min intervals was initiated. The first two samples were collected to determine the spontaneous catecholamine output. Then, the appropriate modified Krebs solution containing LiCl, sucrose or choline chloride, in the presence or absence of Ca, was perfused through the gland and samples were collected at 2 min intervals, in iced assay tubes containing enough perchloric acid to give a final concentration of 0.05 N.

Catecholamine assay

The total catecholamine content of the samples (noradrenaline plus adrenaline) was determined according to the method of Anton & Sayre (1962), without further purification on alumina. The catecholamines present in each collection tube were expressed as $\mu g\,2\,min^{-1}$ perfusion period. Net catecholamine release was calculated by subtracting the basal spontaneous output from the evoked release, and was expressed as $\mu g\,2\,min^{-1}$ or $\mu g\,8\,min^{-1}$.

Statistical analysis

The data are expressed as means ± s.e.mean. Tests for significance were carried out by use of Student's t test. P values of less than 0.05 were considered significant.

Results

Catecholamine release evoked by Li from cat perfused adrenal glands

The spontaneous catecholamine release in glands perfused with Krebs-bicarbonate solution amounted to 45 ± 5 ng 2 min^{-1} ($n = 25$). Replacement of NaCl (119 mM) by LiCl (Li-Krebs) in equimolar amounts significantly increased the output of catecholamines. An increased release was already apparent after 10 min of perfusion, then it increased gradually and reached a maximum of 0.26 ± 0.04 $\mu g\,2\,min^{-1}$ ($n = 16$) at 35–45 min (Figure 1). From this time onwards the Li-evoked catecholamine secretory response progressively decreased, reaching basal values 120 min later. The catecholamine secretory effect described above was not apparent when sucrose or choline chloride, instead of LiCl, were used as osmotic substitutes for Na (Figure 1). It is interesting to note that the catecholamine secretory response induced by Li was abolished when Ca was deleted from the Li-Krebs used to perfuse the gland. The presence of mecamylamine (10^{-4} M) plus atropine (10^{-6} M) did not modify the Li-induced catecholamine release.

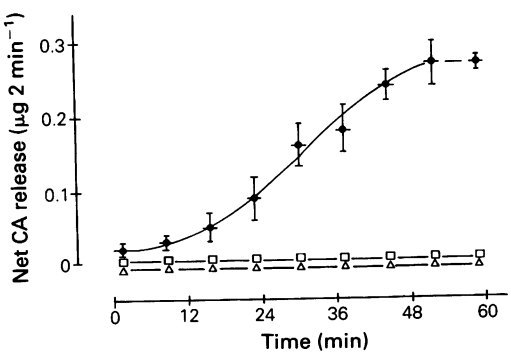

Figure 1 The effect of Na (119 mM) replacement by Li (Li-Krebs) on the spontaneous catecholamine (CA) release from perfused cat adrenal glands. Glands were first perfused with normal Krebs for a 60 min period. (●) Perfusion with Li-Krebs; (□) perfusion with sucrose-Krebs; (△) perfusion with choline-Krebs. Data are means from 16 experiments; vertical lines show s.e.mean. Horizontal bars represent a 2 min collection period.

The effect of replacement of Li by Na or sucrose in the perfusion fluid on the output of catecholamines induced by Li-Krebs

It is known that Li, like Na, can passively permeate the cell but as Li is a poor substrate for the Na-K activated ATPase (Keynes & Swan, 1959), its intracellular concentration will progressively increase. If the chromaffin cell membrane could not distinguish between Na and Li (Lastowecka & Trifaro, 1974), the possibility exists that Li could move out of the cell in exhange for Ca using the sodium site in the Na-Ca counter-transport system. If this were indeed the case, then the removal of Li, once it has been sufficiently accumulated inside the cell, would immediately invert its electrochemical gradient and, therefore, should induce an increase in the catecholamine secretory response.

In order to explore this possibility in a group of paired experiments, both adrenal glands were perfused with Li-Krebs and 45 min later, at the maximum of the catecholamine secretory response, Li was removed. In gland (a) Li was replaced by isoosmotic amounts of sucrose, whereas in gland (b) normal Krebs was perfused, in both cases for 2 min. The results of a typical experiment are given in Figure 2, and show that in the presence of sucrose, the net catecholamine release increased by $0.93 \pm 0.06\,\mu g\,2\,min^{-1}$ ($n = 7$) over the previous plateau value. This increment was significantly higher ($P < 0.001$) than that found in glands previously perfused with Li-Krebs when the perfusion fluid was replaced by Krebs containing normal amounts of NaCl. In this case, the increment was $0.08 \pm 0.03\,\mu g\,2\,min^{-1}$ ($n = 7$). The catecholamine secretory effect induced by substitution of sucrose for Li was dependent on extracellular Ca and cannot be explained by a direct effect of sucrose. In fact, the replacement of 119 mM Na in the normal Krebs by equiosmolar amounts of sucrose, did not cause any change in the catecholamine output, as shown in Figure 1. Similar results were obtained when choline chloride, instead of sucrose, was used to replace Li.

The effects of replacement of Na by Li or sucrose in ouabain pretreated glands perfused with normal Krebs

Since Na prevented the catecholamine secretory response evoked by Li removal in glands preperfused with Li-Krebs, it was of interest to determine if Li could prevent in a similar way the catecholamine release evoked by Na removal in ouabain poisoned glands. The results are shown in Figure 3. Both adrenal glands (a and b) were initially perfused with normal Krebs, and pretreated with a pulse of ouabain (10^{-4} M, 10 min). Thirty min later the catecholamine output amounted to $0.79 \pm 0.13\,\mu g\,2\,min^{-1}$ ($n = 22$). The replacement of Na by sucrose in the perfusion

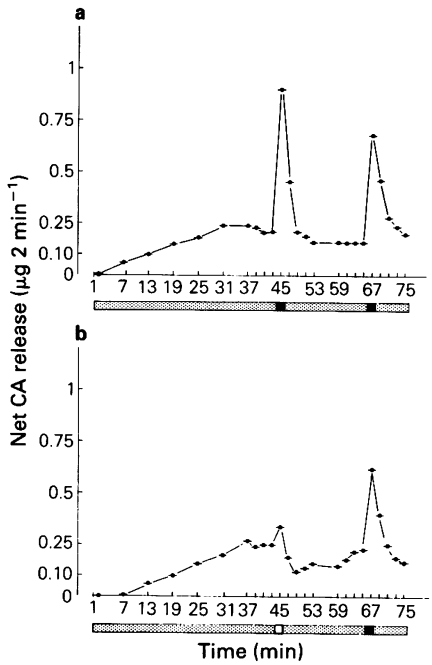

Figure 2 The effect of replacement of Li by Na or sucrose in the perfusion fluid on the catecholamine (CA) release induced by Li-Krebs. Both adrenal glands, from the same animal, were initially perfused with Li-Krebs for a 45 min period. Then Li was replaced by sucrose in gland (a) and by Na in gland (b), in both cases for a 2 min period. Twenty min later, Li was replaced by sucrose (for 2 min) in both glands. Samples were collected at 2 min intervals. The horizontal bars indicate 2 min collection period. The date were selected from one out of seven similar experiments. Stippled bar represents infusion with Li-Krebs; solid bar, sucrose-Krebs; open bar, normal-Krebs.

fluid (for a 2 min period) provoked a sharp increase ($4.03 \pm 0.64\,\mu g\,2\,min^{-1}$, $n = 12$) in the catecholamine secretion, which was several times greater than, and statistically different ($P < 0.001$) from that seen when Li was used to replace Na ($0.93 \pm 0.11\,\mu g\,2\,min^{-1}$, $n = 12$). Note that in this group of experiments Na 134 mM, instead of 119 mM, was replaced by sucrose or Li in order to enhance the $[Na]_i$–$[Ca]_o$ exchange. The remaining $[Na]_o$ was 10 mM.

The effect of preperfusion of glands with Li-Krebs on the secretory reponse evoked by Ca reintroduction

It has been demonstrated (García *et al.*, 1980; 1981; Esquerro *et al.*, 1980) that calcium reintroduction in ouabain pretreated glands perfused with Ca-free Mg-containing Krebs solution evokes a vigorous increase

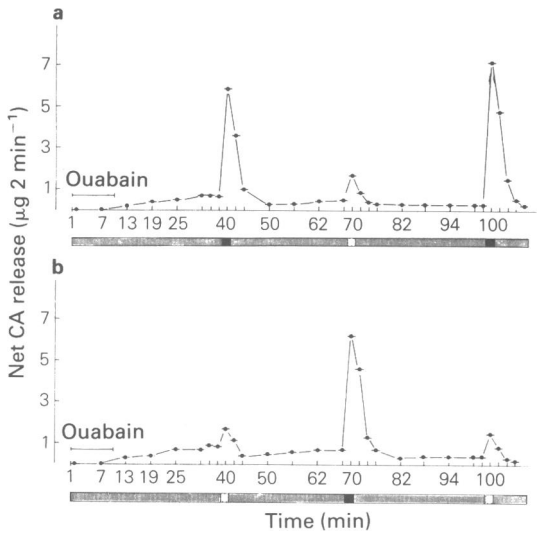

Figure 3 Differences in catecholamine (CA) output obtained during replacement of Na (134 mM) by iso-osmotic amounts of Li or sucrose in ouabain pretreated (10^{-4} M, 10 min) glands perfused with normal Krebs. Thirty min after ouabain pretreatment, in gland (a) Na (134 mM) was replaced by sucrose, Li and sucrose, for 2 min, at 40, 70 and 100 min, respectively, whereas in gland (b) Na was replaced by Li, sucrose, and Li, also for a 2 min period, at the same time intervals. Horizontal bars indicate 2 min collection periods. The figure shows the results of a typical experiment out of four. Note that the scale for catecholamine release is different from that used in Figure 2. Open bar represents infusion with normal Krebs; solid bar, sucrose-Krebs; stippled bar, Li-Krebs.

in spontaneous catecholamine release. Intracellular accumulation of Li would lead to an ionic distribution similar, in a sense, to that obtained by inhibition of the sodium pump in adrenal glands perfused with normal Krebs, the only difference being that the prevalent intracellular monovalent cation, in this particular case, is Li instead of Na.

Therefore, it seemed of interest to investigate the effect of Ca reintroduction on the spontaneous catecholamine release in glands previously perfused with Ca-free, Mg-containing Li-Krebs. Perfusions with Ca-free, Mg-containing normal, sucrose- or choline-Krebs were used as controls. Calcium (2.5 mM, 2 min) was reintroduced in all cases after 60 min of perfusing the glands with the different solutions. All experiments (see Figure 4) ended with an additional 60 min perfusion period with Ca-free, Mg-containing Li-Krebs solution, followed by Ca reintroduction (2.5 mM, 2 min). The results indicate that perfusing the adrenal glands with Ca-free normal (a), choline (b)-, or sucrose (c)-Krebs for 60 min failed to increase catecholamine release when the perfusion media was changed to 2.5 mM Ca, sucrose-Krebs. However, perfusion of the glands with Ca-free Li-Krebs (d) induced a dramatic catecholamine release, when the perfusion solution was changed to Ca-containing sucrose-Krebs. The presence of mecamylamine (10^{-4} M) plus atropine (10^{-6} M) did not modify this secretory response.

The secretory response to Ca reintroduction as a function of the length of time of the Li loading period

As shown in Figure 4, Ca reintroduction evokes a vigorous catecholamine secretion from a gland preperfused with Ca-free Li-Krebs solution for a 60 min period. In this group of experiments the secretory response to Ca reintroduction (2.5 mM, 2 min) was always obtained in sucrose-Krebs, in order to maximize Li output. The glands were initially perfused with Li-Krebs, and the only variable was the duration of perfusion with Ca-free Li-Krebs (Li-loading period): 5, 15, 30, 60 and 120 min. The results (Figure 5) indicate that catecholamine secretion evoked by Ca reintroduction is directly related to the perfusion time during the Li loading period up to 30 min; lengthening of the Li loading period to 60 or 120 min did not enhance further the secretory response.

The secretory response to Ca reintroduction as a function of the concentration of Li during the Li-loading period

In these experiments the secretory response to Ca reintroduction was also obtained in sucrose-Krebs and the only variable was the $[Li]_o$ (25, 60, 119 and 238 mM) during the 30 min Li loading period. The results are shown in Figure 6 and indicate that the secretory response to Ca reintroduction is directly dependent on the $[Li]_o$ during the Li-loading period.

Effect of Na, Li and sucrose during Ca reintroduction on the output of catecholamines

Since in all previous experiments Ca was reintroduced in sucrose-Krebs, it seemed of interest to explore the effect of the presence of Na and Li during Ca reintroduction on the catecholamine secretory response, in adrenal glands previously perfused with Li-Krebs.

Paired glands were perfused with Ca-free, Mg-containing Li-Krebs. One hour later Ca was reintroduced every 20 min for a 30 s period (see Figure 7). In the gland used as control Ca was always reintroduced in normal Krebs, whereas in the other Ca reintroduction in S_3 and S_4 was carried out in Li- and sucrose-Krebs, respectively. Results are expressed as a percentage of the catecholamine secretory response obtained during

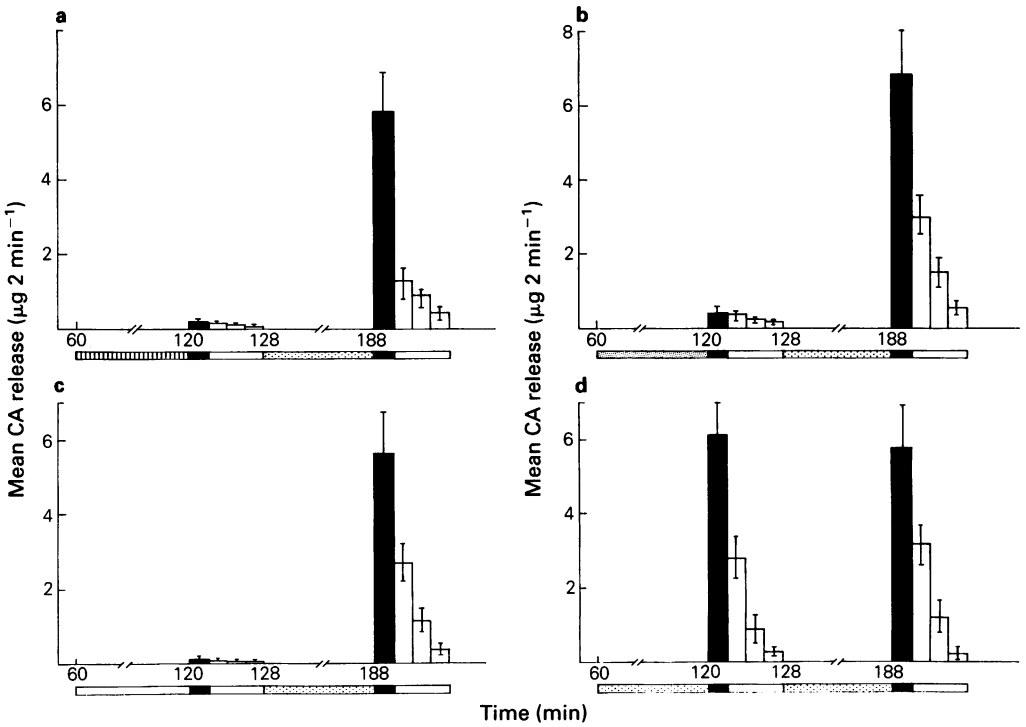

Figure 4 Differences in catecholamine (CA) output evoked by Ca reintroduction in adrenal glands previously perfused with Ca-free Mg- containing normal, sucrose-, choline- or Li-Krebs solution. Glands were initially perfused with (a) Ca-free normal Krebs (hatched bar), (b) Ca-free choline-Krebs (close stippled bar), (c) Ca-free sucrose-Krebs (open bar), and (d) Ca-free Li-Krebs (spaced-stippled bar) for a 60 min period. Then Ca (2.5 mM) was reintroduced as sucrose-Krebs (solid bar) for a 2 min period. In all four cases the experiment ended with one additional 60 min perfusion period with Ca-free Li-Krebs, followed by reintroduction of Ca. Each column represents the mean from 6 experiments and vertical lines show s.e.mean.

the second Ca reintroduction period (S_2). A statistically significant difference was found between the catecholamine secretion evoked by Ca reintroduction in Li (S_3)- and sucrose (S_4)-Krebs when compared with their respective controls in normal Krebs ($P < 0.01$). Differences in catecholamine secretion evoked by Ca reintroduction in Li (S_3)- and sucrose (S_4)-Krebs were also statistically significant ($P < 0.05$).

Discussion

In the present study the effect of Li on the spontaneous catecholamine output of perfused cat adrenal glands has been examined. The results of our experiments show three main findings: (1) Partial replacement of Na by Li, but not by sucrose or choline, in the Krebs solution increases the spontaneous catecholamine release and this secretory response is strictly dependent on Ca. (2) Replacement of Li by sucrose or choline, but not by Na, in glands previously perfused with Li-Krebs, evokes a sharp increase in the catecholamine output. (3) Adrenal glands perfused with Ca-free Li-Krebs, but not with Ca-free normal, sucrose- or choline-Krebs, show a vigorous secretory response when Ca is reintroduced.

The catecholamine secretory response obtained by perfusing the glands with Li-Krebs gradually increased reaching a maximum within 45 min. This response was Ca-dependent. Since neither mecamylamine, a ganglionic blocking agent, nor atropine modified the secretory response, it is likely that the Li effect is due to a direct action on chromaffin cells and not to the release of acetylcholine from presynaptic cholinergic nerve terminals present in the adrenal medulla. The fact that no such an increase in spontaneous catecholamine release was seen when Na was replaced by sucrose or choline, under identical

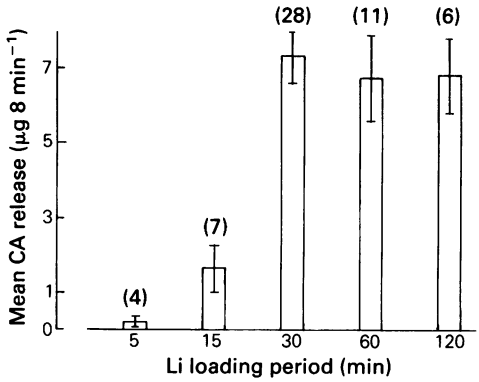

Figure 5 The effect of the length of time of the Li loading period on the catecholamine (CA) secretory response evoked by Ca reintroduction. Glands were perfused with Ca-free Mg-containing Li-Krebs for different periods of time (5, 15, 30, 60, 120 min). At the end of the perfusion period, Ca (2.5 mM, 2 min) was reintroduced. Each column represents the mean and vertical lines show s.e.mean. The numbers in parentheses represent the number of experiments. Other conditions were the same as those described in Figure 4 legend.

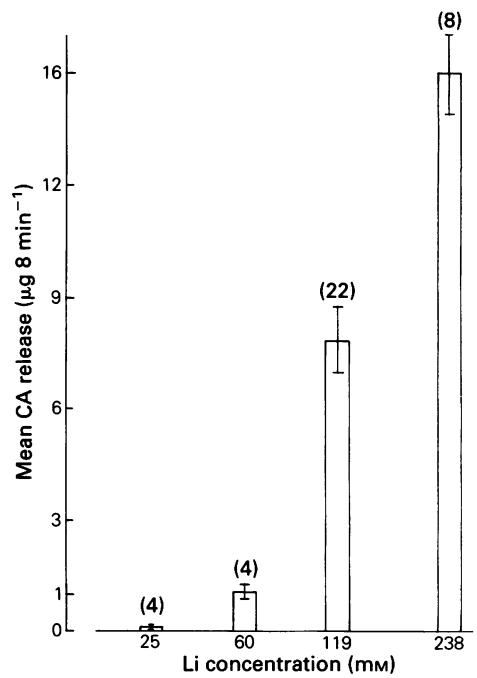

Figure 6 The effect of Li concentration on the catecholamine (CA) secretory response induced by Ca reintroduction. Glands were perfused for a 30 min period with Ca-free Mg-containing Li-Krebs solution with different amounts of Li (25, 60, 119 and 238 mM). At the end of the perfusion period Ca was reintroduced. Each column represents the mean and vertical lines show s.e.mean. The numbers in parentheses represent the number of experiments. Other conditions were the same as those described in Figure 4 legend.

experimental conditions, excluded the possibility that the simple reduction of extracellular [Na] could be the cause of such a secretory event. In fact, all modified perfusion solutions, independent of the osmotic agent used to replace Na, contained the same final amount of Na (25 mM).

It is well known that the presence of Ca is a critical requirement for the secretion of catecholamines in the adrenal medulla (Douglas & Rubin, 1961; 1963). It is also known that there exists in the adrenal medulla, a Na-Ca counter-transport mechanism (García *et al.*, 1980; Esquerro *et al.*, 1980; Aunis & García, 1981) similar to that described in the giant axon of the squid (Baker *et al.*, 1969; Baker, 1972). This Na-Ca exchange system might be involved in the control of the intracellular concentration of ionized Ca levels and, therefore, in the modulation of catecholamine 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 favouring the entry of Ca into the cell, in exchange for internal Na (García *et al.*, 1980; Nishimura *et al.*, 1981; Nishimura & Sorimachi, 1984; Sorimachi & Nishimura, 1984). Then the elevated intracellular [Ca] will result in a parallel increase of catecholamine output by the gland.

Even though catecholamine release evoked by Li-Krebs is of lesser magnitude than that induced by ouabain, the time course in both cases is similar. It increases progressively, reaches a maximum at 35–45 min and then slowly declines approaching resting levels after 120 min. In addition, the fact that catecholamine release induced by Ca reintroduction in glands previously perfused with Ca-free, Mg-containing Li-Krebs is dependent on the length of the Li loading period and on the concentration of Li in the perfusion fluid indicates that the intracellular Li accumulation is the critical factor for the secretory response evoked by this ion; i.e. in a similar manner that Na accumulation is for catecholamine release evoked by ouabain (Esquerro *et al.*, 1980; García *et al.*, 1980; 1981). The close similarity between the secretory response induced by both Li and ouabain suggests that a $[Li]_i-[Ca]_o$ exchange could be the mechanism involved in the catecholamine release evoked by Li.

It has been demonstrated that Na-dependent Ca movements in cardiac sarcolemmal vesicles are inhibited when Na is present on the same side of the membrane as Ca, a finding consistent with a com-

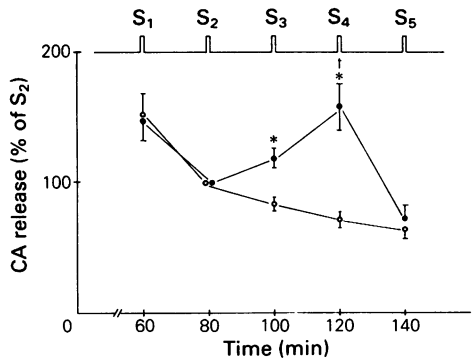

Figure 7 Differences in the catecholamine (CA) output evoked by Ca reintroduction in normal, Li- or sucrose-Krebs. Paired glands were perfused with Ca-free, Mg-containing Li-Krebs. After one hour of perfusion Ca was reintroduced for a 30 s period every 20 min as indicated in the figure (S_1, S_2, S_3, S_4, S_5). In the control gland (○) Ca was always reintroduced in normal Krebs. However in the experimental gland (●) Ca reintroduction was carried out in Normal Krebs in S_1, S_2 and S_5 periods and in Li- and sucrose-Krebs in S_3 and S_4, respectively. Results are expressed as percentage of the CA output obtained in S_2. Data are means, with vertical lines indicating s.e.means, from 4 paired experiments. * $P<0.01$ as compared to the control. † $P<0.05$ as compared to Li-Krebs.

petitive antagonism between Na and Ca for a common divalent site (Reeves & Sutko, 1983). On these grounds, the catecholamine secretory response induced by replacing Na by sucrose in ouabain pretreated glands, previously perfused with normal Krebs, could be interpreted as a consequence of the instantaneous inversion of the electrochemical gradient for Na, which will activate the $[Na]_i$–$[Ca]_o$ exchange, and also as the lack of competition between Na and Ca for the carrier (Figure 3). The catecholamine secretory response evoked by substitution of sucrose for Li in adrenal glands preperfused with Li-Krebs could be interpreted in a similar manner. On the other hand when Li, instead of sucrose, was used to replace Na in ouabain poisoned glands perfused with normal Krebs (Figure 3), or Na was employed as a Li substitute in Li-Krebs perfused glands (Figure 2), the increase in catecholamine output was considerably less than that found when sucrose was used as an osmotic substitute. These findings are consistent with the idea that Li shares, to some extent, the functional properties of Na in the Na-Ca counter-transport system present in the membrane of chromaffin cells.

Our results also show that replacement of Na by Li in ouabain-treated glands, perfused with normal Krebs, does not totally prevent the increase in catecholamine release evoked by decreasing the $[Na]_o$. This observation may indicate that the affinity of Li for the carrier is less than that of Na. The fact that Ca reintroduction in Li-Krebs causes a greater catecholamine release than in normal Krebs (see Figure 7) also supports this suggestion. These results agree well with those found by Nishimura & Sorimachi (1984); they demonstrated that Li can partially replace Na in maintaining ^{45}Ca efflux. Moreover, in the squid axon Li can replace Na at low values of internal ionized Ca (Mullins, 1976). However, Li failed to maintain ^{45}Ca efflux in adrenomedullary slices (Rink, 1977). The reason for this discrepancy is unknown.

The secretory response evoked by Li could also be due to entry of Ca via a voltage-dependent channel. In fact Li is likely to depolarize the chromaffin cell by altering the distribution of intracellular monovalent cations, as ouabain does (Esquerro *et al.*, 1980). However, against a main role of voltage-dependent Ca channels in the secretory effect of Li is the fact that catecholamine release induced by activation of voltage-dependent Ca channels is not sustained in chromaffin cells (Baker & Rink, 1975), whereas both ouabain- and Li-evoked catecholamine release are long-lasting with little desensitization. Additionally, the increase of catecholamine release obtained when Li is deleted from the Li-Krebs solution and replaced by sucrose, as shown in Figure 2, is difficult to attribute to membrane depolarization alone as it is prevented by the presence of Na in the same experiment. Also the finding that the differences in the magnitude of secretory response evoked by Ca reintroduction depend on the presence of Na, Li or sucrose in the Krebs (Figure 7) does not support the hypothesis that activation of voltage-dependent Ca channels is the only mechanism involved, unless Na and Li could interfere with Ca^{2+} entry through channels with a different efficacy.

In summary, our results demonstrate that Li causes an increase in the rate of catecholamine release and suggest that the main mechanism for this response could involve Li-Ca exchange. To explain such a finding the following sequence of events is postulated: (1) Li passively permeates the membrane and due to the fact that it is not a good substrate for Na-K-ATPase, it accumulates in the cell: (2) Li partially replaces Na in the Na-Ca counter-transport system present in the membrane of chromaffin cells and (3) Li activates a Li-Ca exchange mechanism leading to an increase of $[Ca]_i$ that subsequently promotes catecholamine secretion.

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