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CATECHOLAMINE SECRETORY RESPONSE TO CALCIUM REINTRODUCTION IN THE PERFUSED CAT ADRENAL GLAND TREATED WITH OUABAIN

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Abstract—The effect of external $[Na]_o$, internal sodium $[Na]_i$ and external calcium $[Ca]_o$ concentrations on the secretion of catecholamines (CA) from the retrogradely perfused and ouabain-treated cat adrenal gland was investigated. CA release in response to a pulse of ouabain was $[Ca]_o$ dependent. Inhibition of the sodium pump with the glycoside allowed the loading of the chromaffin cells with different Na concentrations in the absence of $[Ca]_o$. $[Ca]_o$ reintroduction evoked a CA secretory response which was directly proportional to the loading Na concentration and therefore to the internal concentration of this ion $[Na]_i$. The secretory response evoked by $[Ca]_o$ in a Na-loaded gland was inversely related to $[Na]_o$ at the moment of $[Ca]_o$ reintroduction, secretion being higher at lower $[Na]_o$. Half-time ($t_{1/2}$) for the decline from the peak secretory response after $[Ca]_o$ introduction was related to the $[Na]_o$ at the time of $[Ca]_o$ introduction. However, the rate of decay of CA secretion was independent of $[Na]_i$ at the time of $[Ca]_o$ application. Addition of $[Ca]_o$ to a ouabain-treated gland during perfusion with 140 mM K- and CA-free solution resulted in a secretory response which was much greater than that obtained in untreated glands. The data imply that ouabain-induced secretion was not mediated only by cell depolarization. It is concluded that the secretory response to $[Ca]_o$ in a ouabain-treated gland is the consequence of a rise of intracellular ionized Ca levels, presumably resulting from the activation of an internal Na-dependent Ca influx system located in the chromaffin cell membrane.

Cardiac glycosides induce the release of catecholamines (CA) from the perfused bovine adrenal gland [1], guinea pig vas deferens [2–4], rabbit heart [5], cat spleen [6–8] and cat adrenal gland [9].

García *et al.* [9] have recently explained the ouabain-induced CA secretory response from the perfused cat adrenal gland in terms of redistribution of monovalent cations in the chromaffin cells, secondary to inhibition by the glycoside of the sodium pump. Such ionic redistribution would lead to a reduction of the electrochemical gradient for Na; a rise in internal Na concentration $[Na]_i$ would reduce the Na-dependent Ca efflux and increase Ca uptake in exchange for Na loss resulting in an increase of intracellular ionized $[Ca]_i$ and the CA secretory response (see Ref. 10).

If this explanation of the action of ouabain is correct, then manipulation of the intra- and extracellular [Na] by inhibiting the sodium pump with ouabain or changing [Na] in the perfusing solution, respectively, should lead to changes in intracellular $[Ca]_i$ and therefore of CA release. The overall aim of the present work was to explore further the existence of the Na–Ca exchange mechanism in the chromaffin cell of the cat adrenal gland, and its relationship to the CA secretory mechanism.

METHODS

Both cat adrenal glands were isolated and prepared for retrograde perfusion with Krebs–bicarbonate solution at room temperature, as previously described [9]. The perfusion rate was about 1 ml/min.

Perfusion media. The normal Krebs–bicarbonate solution had the following composition (mM): NaCl, 119; KCl, 4.7; $CaCl_2$; 2.5; $MgSO_4.7H_2O$, 1.2; $NaHCO_3$, 25; KH_2PO_4, 1.2; Glucose, 11. This solution was equilibrated with 95% O_2 and 5% CO_2, the final pH being 7.4–7.5. Ca-free Krebs solution (0 Ca) was made up by removing $CaCl_2$; no osmotic adjustments were made. Twenty-five millimolar Na Krebs solution (25 Na) was prepared by substituting NaCl by iso-osmolar amounts of sucrose or choline chloride; zero Na solution (0 Na) was made by substituting NaCl and $NaHCO_3$ with sucrose and adding 10 mM tris-hydroxymethyl amino-methane (tris). The pH was adjusted to 7.4 after equilibration with 95% O_2–5% CO_2. High Na Krebs solution (263 mM Na) was made by simply adding 119 mM NaCl to normal Krebs solution. K-rich solution (140 mM) was prepared by the addition of K_2SO_4 with or without reduction of the corresponding amount of NaCl to maintain isotonicity.

Design of experiments and collection of perfusate samples. After 40 min of initial perfusion of the glands with Krebs solution, samples were collected for 2 min each in chilled tubes to determine the resting secretion of CA. Then the gland was perfused with ouabain (10^{-4}M) in Ca-free solution for 10 min and then in 0 Ca solution containing different concentrations of Na for 50 additional min. This perfusion time (60 min) will be referred to as the Na-loading period. After this perfusion period, during which no significant CA output was observed, Ca

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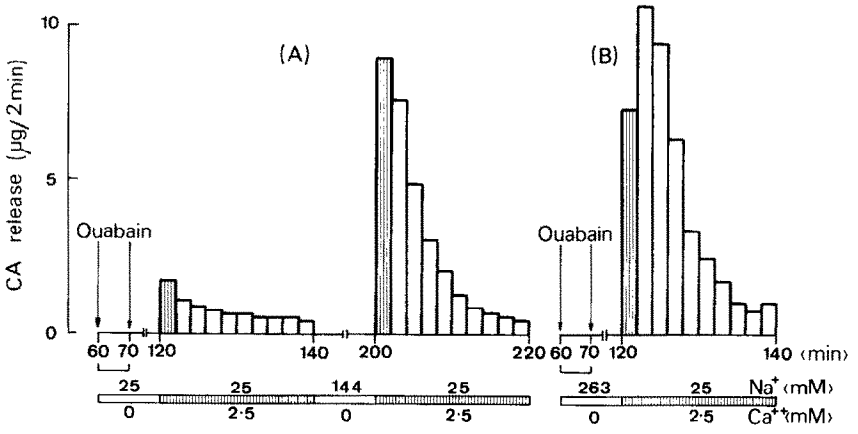

Fig. 1. Catecholamine (CA) secretory response to Ca. Adrenal glands were initially perfused with normal Krebs solution for 40 min and then with 25 mM Na–Ca free solution (A); or with 263 mM Na–Ca free solution (B) for 20 min. Ouabain (10^{-4}M) was then infused. The secretary response to Ca was obtained during perfusion of the gland with a solution containing 25 mM Na and 2.5 mM Ca. Data in (A) and (B) are obtained from the left and right glands of the same cat. The figure represents a typical result from five similar experiments.

was reintroduced in the presence of different [Na] and perfusate samples were continuously collected for 2 min each.

CA assay. Total CA content of the samples (noradrenaline plus adrenaline) were determined according to Anton and Sayre [11] without further purification on alumina. CA values are expressed as μg/2 min or μg/20 min perfusion period.

RESULTS

We have previously shown that a 10 min pulse of ouabain (10^{-4}M) causes a significant release of CA from the retrogradely perfused cat adrenal gland. Calcium introduction to a ouabain-treated gland perfused with CA-free but Mg-containing solution greatly potentiated CA release [9]. The following experiments were designed to examine the characteristics of the secretory response to Ca in a ouabain-treated gland and its relationship with Na ions.

The secretory response to Ca *as a function of* $(Na)_o$ *during the* Na-*loading period*. Ca introduction evokes a vigorous CA secretion from a gland previously treated with ouabain and perfused in 0-Ca for 1 hr. In these experiments the secretory response to Ca was always obtained in 25 mM Na and the only variable was the $[Na]_o$ during the Na-loading period. The secretory response to Ca seems to be directly dependent on the $[Na]_o$ during the Na-loading period but independent of this cation concentration during the Ca-introduction period. Thus, when Na concentration during the loading period was 25 mM, the CA release on Ca introduction in 25 mM Na was only 7.47 ± 0.11 μg/20 min (N = 5, Fig. 1). Release was markedly enhanced when $[Na]_o$ during the loading period was 144 mM (29.82 ± 1.60 μg/20 min, N = 5, $P < 0.001$). In fact, the total amount of CA released in response to a Ca-introduction period of 20 min was directly proportional to the $[Na]_o$ present during the Na-loading period (Fig. 2).

It is worth noting that $[Na]_o$ has an interesting effect on the secretory response to Ca, CA release being much more potentiated at lower than higher Na concentraions. If $[Na]_o$ was maintained constant at 144 mM during loading period, but varied from 25 to 263 mM during the Ca introduction period, the CA secretory response was substantially greater at lower $[Na]_o$; at 25, 144 and 263 mM Na, CA secretion was 28.9, 15.2 and 10 μg/20 min, respectively. Hypertonic Krebs solution made with sucrose neither caused release of CA nor influenced the secretory response to K or Ca.

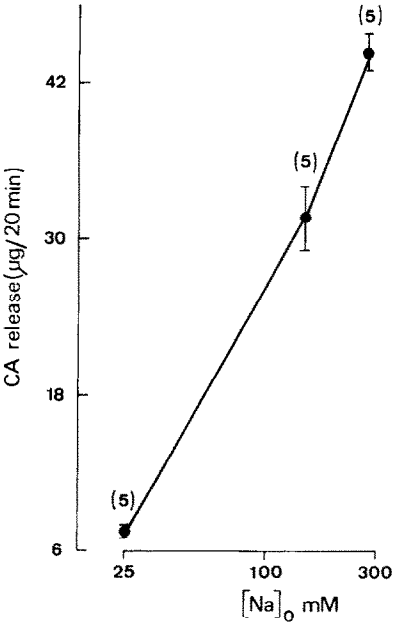

Fig. 2. Catecholamine (CA) secretory response to Ca in ouabain-treated glands. Experimental design as in Fig. 1. Data (ordinate) were calculated by adding the CA released in ten 2-min samples collected after Ca introduction. Abscissa shows the loading concentration of Na in a logarithmic scale. Vertical lines are S.E. of the means. The number of experiments are shown in parentheses.

Time course of the secretory response to Ca *introduction.* Figure 1 also shows that the secretory response to Ca reached a maximum during the first 2–4 min and declined during the following 20 min at a rate which depended on $[Na]_o$ present during the Ca introduction period (Fig. 3). In four experiments the half-time ($t_{1/2}$) for the decline from peak CA release during Ca introduction in the presence of three different Na concentrations was measured. Glands were loaded in 144 mM Na. In 0 Na the $t_{1/2}$ was 2.77 min, in 25 mM Na it was 4.95 min; in 263 mM Na two phases were observed, a first fast one with a $t_{1/2}$ of 6.3 min and a second slow one with a $t_{1/2}$ of 23.1 min. Therefore the secretory response fades more slowly when higher Na concentrations were present during the Ca introduction period.

The rate of decline from peak CA release does not, however, seem to be dependent on the $[Na]_o$ during the loading period. Two ouabain-treated glands from the same animal were loaded in 263 and 144 mM Na and CA secretion was evoked by reintroduction of Ca in 25 mM Na (sucrose). The $t_{1/2}$ for the decline was identical in both glands (3.85 min).

The effect of K*-depolarization on the secretory response to* Ca. Maintained depolarization of bovine adrenal medullary cells with high K produces a phasic release of CA which results from activation and subsequent inactivation of Ca entry [12].

Exposure of normal cat adrenal glands to 140 mM K solution in which Ca has been replaced isosmotically by Mg did not evoke any CA output (Fig. 4B). Subsequent application of Ca elicited a clear secretory response (10.12 ± 1.23 μg/20 min, N = 6). The same experimental design was used in the contralateral gland, except that a ouabain (10^{-4}M) pulse was first given, followed by a 20 min perfusion with Ca-free solution containing 144 mM Na and then with Ca-free high K solution. Application of Ca elicited a vigorous secretory response (22.71 ± 3.14 μg/20 min, N = 5) which was significantly higher than the secretory response observed in the control gland ($P < 0.05$).

It is interesting to note that if the Na-loading was done at a $[Na]_o$ of 25 mM and not of 144 mM Na as above, the additional release obtained in the ouabain-treated gland was not seen. In one paired experiment the normal and ouabain-treated glands released 8.58 and 6.08 μg/20 min of CA, respectively, in response to Ca reintroduction in the presence of 140 mM K.

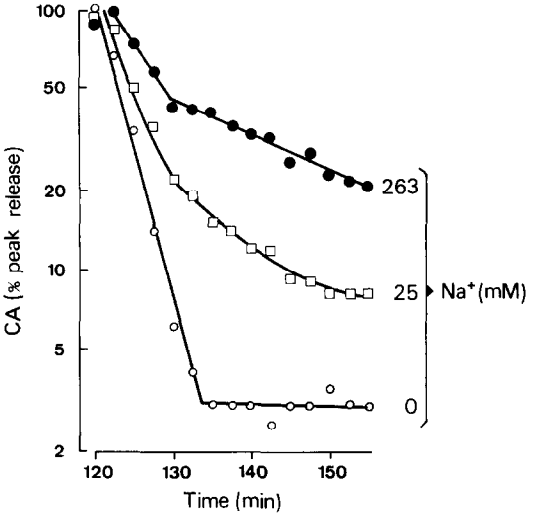

Fig. 3. Rate of decline of catecholamine (CA) secretory response to Ca introduction in ouabain-treated glands. After a ouabain pulse (10^{-4}M for 10 min) in 0 Ca, glands were perfused with 0 Ca-solution containing 144 mM Na for 60 min. At the end of this Na loading period, Ca (2.5 mM) was introduced in the presence of 0 (Na substituted by sucrose), 25 (Na substituted by sucrose) or 263 mM Na. Data of 263 Na and 0 Na are taken from two glands from the same animal. CA release in each 2 min sample is expressed as a percentage of the maximal CA output obtained after Ca introduction, which usually was the first 2 min sample; the ordinate is a logarithmic scale. The figure represents a typical result from four experiments.

DISCUSSION

We have shown in this paper that the secretory response from a ouabain-pretreated cat adrenal

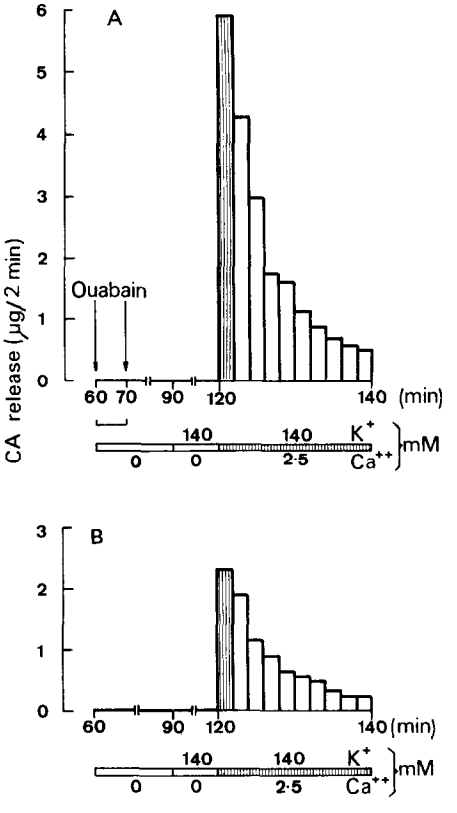

Fig. 4. Catecholamine (CA) secretory response to Ca introduction in adrenal glands treated with ouabain and depolarized with a high K solution. Glands were initially perfused with Ca-free solution. In A, a pulse of ouabain (10^{-4} M for 10 min) was applied; then both glands (A and B) were perfused with 140 mM K (as K_2SO_4) solution which contained 144 mM Na but no Ca. Ca was then introduced to evoke CA secretion. The figure represents a typical result from five (A) and six (B) experiments.

gland to Ca was related to the Na concentration in the perfusion medium during the loading period, and therefore to $[Na]_i$. Moreover, the decline in the peak secretory response was determined by the $[Na]_o$ at the time of Ca introduction but was independent of $[Na]_i$.

Secretory response from a ouabain-pretreated gland was produced by Ca while the gland was being perfused with Ca-free solution. Previously, Douglas and Rubin [13] showed that introduction of Ca caused a massive release of CA from the cat adrenal gland perfused with Ca and Mg-free solution. The calcium response described in the present study is, however, different, because the response was clearly elicited even when high Mg was present during the initial perfusion of the gland with Ca-free solution.

The secretory response to Ca seems to be coupled to activation of the Na–Ca exchange mechanism, because the output of CA, which serves as an index of intracellular ionized Ca concentration, was directly related to $[Na]_o$ during the loading period, and therefore to $[Na]_i$. It should be noted, however, that in contrast to the effect of $[Na]_i$ the response to Ca introduction was markedly potentiated in the presence of low $[Na]_o$.

After inhibition of Na–K pump by the cardiac glycoside, the cell would gain increasing amounts of Na, resulting in the reduction of the Na gradient $[Na]_o/[Na]_i$) across the chromaffin cell membrane during the loading period. Reduction of the electrochemical gradient, e.g. by a rise in internal Na or a fall in external Na, would tend to reduce the Na-dependent Ca efflux from the cell and increase Ca uptake in exchange for Na loss. The magnitude of the Ca influx would then largely depend on the intracellular Na concentration, Ca influx being higher at higher $[Na]_i$. These presumed ionic changes would then be reflected in parallel changes in the rate of CA secretion. The data presented in this paper corroborate this suggestion and indicate that the Ca response is directly related to the intracellular $[Na]_i$ which in turn is determined by the $[Na]_o$ present during the loading period.

This effect of $[Na]_i$ on Ca response is in contrast to the effect of $[Na]_o$. In low $[Na]_o$, CA secretion was greatly potentiated whereas high $[Na]_o$ had the opposite effect. It seems that at low $[Na]_o$ Ca entry is promoted, but at higher concentrations, extracellular Na competes with Ca for its binding sites on the cell membrane with resultant inhibition of Ca influx and of CA secretion.

Another interesting aspect of the role of Na in the Ca response is its ability to modify the rate of decline of CA release. The decline from the peak CA release was much faster in low than in high $[Na]_o$. The interpretation of this finding is that after intracellular Na loading, Ca reintroduction stimulates the Na–Ca exchange with Na being lost from the cell at a rapid rate, thereby reducing the efficiency of the exchange mechanism. In low $[Na]_o$ the Na lost from the chromaffin cell is no longer replaced and the Na–Ca exchange mechanism becomes less efficient. On the other hand, Na–Ca exchange would continue to work effectively in high $[Na]_o$ because Na loss would be replenished by entry of extracellular Na by virtue of a favorable $[Na]_o/[Na]_i$ gradient. CA release which closely follows the movement of Ca ions will therefore fade much more slowly in high than in low Na.

The secretory response to Ca introduction could also be due to entry of Ca via a voltage-sensitive Ca conductance channel. Ouabain is likely to depolarize the chromaffin cell by interruption of the electrogenic pump, followed by changes in the distribution of Na and K ions leading to intracellular Na accumulation and K loss [1]. Arguments against the role of voltage dependent-Ca channels in the action of ouabain are: (a) CA release evoked by activation of voltage-dependent Ca channels is not sustained in chromaffin cells [12] and adrenergic neurones [14], whereas ouabain-evoked CA release is long lasting with little desensitization; (b) Ca response is not blocked by high Mg [9]; (c) the large additional CA secretory effect of Ca is obtained in an otherwise fully depolarized chromaffin cell by high K (see Fig. 4). These data, therefore, clearly suggest that the mechanism of action of ouabain is, if anything, barely related to its possible depolarizing effects.

It has been shown that the Na–Ca exchange mechanism operates in a variety of tissues [10, 15, 16]. It is interesting to note that this Na–Ca exchange system has recently been implicated in the development of hypertension. An alteration of the Na electrochemical gradient across the vascular smooth muscle cell plasma membrane may lead to a change in $[Ca]_i$ and, therefore, to an increase in basal vascular tension and hence of peripheral resistance [15]. In addition, metabolic alterations of the Na gradient may lead to an increased CA release from the adrenal gland and adrenergic neurones which could contribute to the development and/or maintenance of an elevated high blood pressure. If this is true, the development of drugs which specifically interfere with the Na–Ca exchange system at these cellular levels may prove clinically useful.

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