Br. J. Pharmac. (1981), **74**, 673–680

POTENTIATION OF K^+-EVOKED CATECHOLAMINE RELEASE IN THE CAT ADRENAL GLAND TREATED WITH OUABAIN

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1 A vigorous catecholamine secretory response was evoked by small increments (2–10 mM) of the extracellular concentration of K^+ ($[K^+]_o$) in cat adrenal glands treated with ouabain (10^{-4} M), and perfused with Krebs-bicarbonate solution at room temperature.

2 The secretory response depends on $[K^+]_o$; increments of $[K^+]_o$ as small as 2 mM for 2 min evoked a clear secretory response; at 10–17.7 mM K^+, the maximal secretory response was observed. In normal glands, not treated with ouabain, no increase of the rate of catecholamine output was observed by raising $[K^+]_o$ up to 17.7 mM for 2 min.

3 The K^+ secretory response was time-dependent, requiring at least 1 min to be initiated; on continued exposure to 10 mM $[K^+]_o$, the enhanced response remained for at least 1 h.

4 In low $[Na^+]_o$, the K^+-secretory response was unchanged. However, in 0-Ca^{2+}, high-Mg^{2+} solutions, or in the presence of D600, an organic Ca^{2+} antagonist, it was abolished.

5 The K^+-induced secretory response was not altered in the presence of tetrodotoxin or tetraethylammonium.

6 It is concluded that ouabain potentiated the catecholamine secretory response to raised $[K^+]_o$ by increasing the amount of Ca^{2+} available to the secretory machinery through (a) mobilization of an enhanced pool of membrane-bound Ca^{2+}, (b) activation of membrane Ca^{2+} inward current; or (c) decrease of intracellular Ca^{2+} buffering systems. The activation by ouabain of a membrane Na^+-Ca^{2+} exchange system is not involved in this K^+-secretory response. It is suggested that the plasma membrane ATPase enzyme system, by changing the affinity of its Ca^{2+} binding sites, might control the availability of this cation to the secretory machinery and, therefore, modulate catecholamine secretion in the adrenal gland.

Introduction

The cardiac glycoside ouabain induces the release of catecholamines from the bovine adrenal gland (Banks, 1967), cat adrenal gland (García, Hernández, Horga & Sánchez-García, 1980; Esquerro, García, Hernández, Kirpeka & Prat, 1980; García, García-López, Montiel, Nicolás & Sanchez-García, 1981) and isolated bovine chromaffin cells (Aunis & García, 1981).

Since ouabain inhibits Na^+, K^+-dependent-Mg^{2+}-activated adenosinetriphosphatase (ATPase) of semipurified bovine adreno-medullary plasma membranes and these membranes contain high affinity binding sites for [^{3}H]-ouabain, it seems that the digitalis receptor mediating the ouabain-secretory effects in the adrenal medulla is the ATPase enzyme system (Aunis & García, 1981).

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Ouabain promotes the release of catecholamines and dopamine β-hydroxylase by an extracellular Ca^{2+}-dependent process (García *et al.*, 1980); it therefore seems probable that the action of the glycoside is achieved by a rise of the intracellular ionized Ca^{2+} concentration $[Ca^{2+}]_i$. The question arises as to whether this rise in $[Ca^{2+}]_i$ is secondary to redistribution of monovalent cation gradients within the chromaffin cell brought about by the inhibition of the Na^+ pump by ouabain, or rather to the inhibition of the membrane ATPase itself or some other effect. Previous experiments from our laboratory suggested that the secretory effects of ouabain could be interpreted in terms of activation, by intracellularly accumulated Na^+, of a membrane Na^+-Ca^{2+} exchange system similar to that described in the squid giant axon (Baker, Blaustein, Hodgkin & Steinhardt, 1969).

In this paper we describe the dramatic potentiation

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of the secretion of catecholamines, evoked by small increments of the extracellular K^+ concentration $[K^+]_o$, in the perfused cat adrenal gland, pretreated with ouabain. This sensitization of the gland to K^+ is still observed in low extracellular Na^+ solution ($[Na^+]_o$) and, therefore, cannot be attributed to indirect activation by ouabain of the Na^+-Ca^{2+} exchange system. Some other mechanism(s), probably related to ATPase inhibition itself, alterations of the membrane Ca^{2+}-binding properties or permeability, or some intracellular action of ouabain at the level of the Ca^{2+}-buffering system(s), could be related to this interesting potentiation of the K^+-secretory effects. A preliminary account of some of these findings has been published (Sánchez-García, Montiel, Frias, Nicolás, Pascual, Kirpekar & García, 1980).

Methods

Both adrenal glands of the cat 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 about 1 ml/min.

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 7.4–7.5. Ca^{2+}-free or low-Ca^{2+} Krebs solution (0-Ca^{2+}) were made up by omission or reduction of $CaCl_2$; K^+-free solution (0-K^+) was prepared by omission of KCl and KH_2PO_4 and substitution of KH_2PO_4 by NaH_2PO_4; enriched K^+ solutions (10, 17.7 and 59 mM) were made by adding appropriate amounts of KCl; no osmotic adjustments were made in these solutions. Twenty-five mM Na^+-Krebs solution (25-Na^+) was prepared by substituting NaCl by iso-osmolar amounts of choline chloride.

Design of experiments and collection of perfusate samples

Both glands from the same cat were initially perfused for 1 h with normal Krebs solution at room temperature (22–24°C). Then, continuous collection of samples in acid-containing, chilled tubes was started at 2 min intervals from this moment (time zero) up to the end of the experiment. Introduction of ouabain and different modified solutions was started at time zero of the experiment. High-K^+ solutions were usually perfused for brief periods of time (2 min); in some experiments, they were perfused from 10 s to 60 min. Samples were acidified with perchloric acid to a final concentration of 0.05 N and assayed the same day for their total catecholamine content or frozen and assayed later.

Catecholamine assay

Total catecholamine content of the samples (noradrenaline plus adrenaline) was determined according to Shellenberger & Gordon (1971) without further purification on alumina. Total catecholamine values

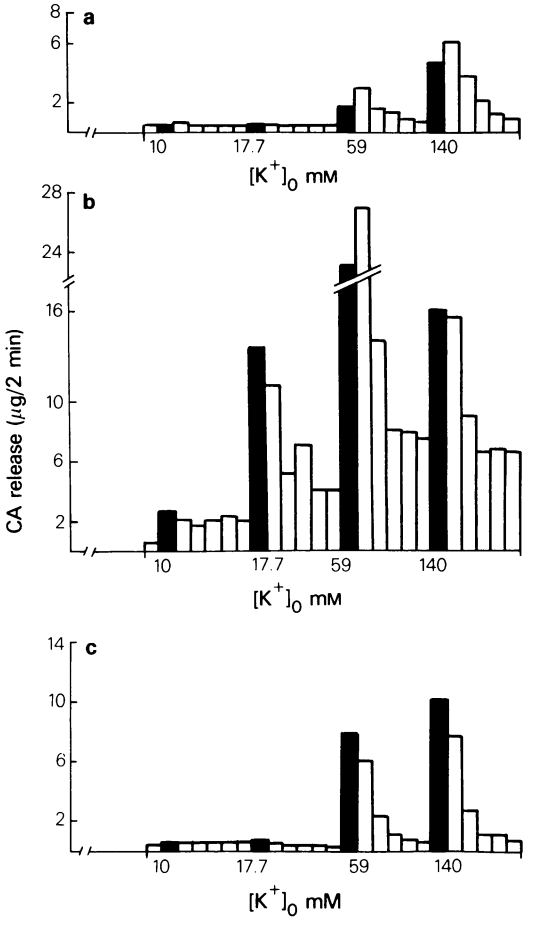

Figure 1 Effect of increasing extracellular K^+ concentrations ($[K^+]_o$) on the release of catecholamines (CA) from (a) control (b) ouabain-treated and (c) 0-K^+-treated glands. Glands were perfused initially with normal Krebs-bicarbonate solution and then, ouabain (10^{-4} M) or K^+-deprivation was started; after 20 min, high-K^+ concentrations were perfused for 2 min at 10 min intervals. Open columns represent CA outputs in normal Krebs, Krebs containing ouabain or 0-K^+ solutions; solid columns signify 2 min periods of raised (K^+). A typical experiment is shown.

are expressed as μg/2 min perfusion period. Net release of catecholamines was calculated by subtracting the basal, spontaneous release from the K^+-evoked release.

Results

The effect of K^+ on release of catecholamines from control glands

Douglas & Rubin (1961) have previously shown that increase in $[K^+]_o$ causes the release of catecholamines from the perfused cat adrenal gland; omission of Ca^{2+} abolished the K^+ response. In our experiments, perfusion of normal glands with 10 or 17.7 mM K^+ solutions for brief periods of time (2 min) did not cause an apparent increase of the spontaneous rate of catecholamine output, which was 0.10 ± 0.02 μg/2 min ($n = 22$). Only by perfusing the gland with a 59 mM K^+ solution for 2 min was a clear, yet small secretory response obtained (Figure 1a).

The effect of K^+ on release of catecholamines from ouabain-treated glands

In an early experiment, attempts were made to reverse the ouabain secretory response by moderate increments (up to 15 mM) of $[K^+]_o$. To our surprise, instead of reversing the ouabain effect, raised $[K^+]_o$ evoked a dramatic, large secretory response. We then designed systematic experiments in order to define the characteristics of this response.

Figure 1b shows that increases of $[K^+]_o$ as small as 4 mM (normal $[K^+]_o$ is 5.9 mM) for 2 min caused a very substantial release of catecholamines in a ouabain-treated gland. Raising $[K^+]_o$ to 3 and 10 times the normal concentration (17.7 and 59 mM) caused still greater secretory responses. Usually, a normal cat adrenal gland contains 100 to 200 μg of catecholamines; perfusion of the gland with a 59 mM K^+ solution for only 2 min released as much as 59 μg of the amines (almost 50% of the total tissue catecholamine content), which is an indication of how dramatic is the potentiation by ouabain of the K^+-secretory response.

If the potentiating effect of ouabain were simply due to inhibition of ATPase activity and/or Na^+ pumping, then omission of K^+ from the perfusing medium should mimic the glycoside effect. Figure 1c shows that in glands perfused with 0-K^+ solution, a clear secretory response was observed only with 59 mM K^+; at 10 and 17.7 mM $[K^+]_o$, no secretory effects were detected. Probably, this is due to rapid reactivation of the enzyme after K^+ reintroduction.

Since K^+ reintroduction to K^+-deprived glands did not cause a secretory response, we decided to perfuse

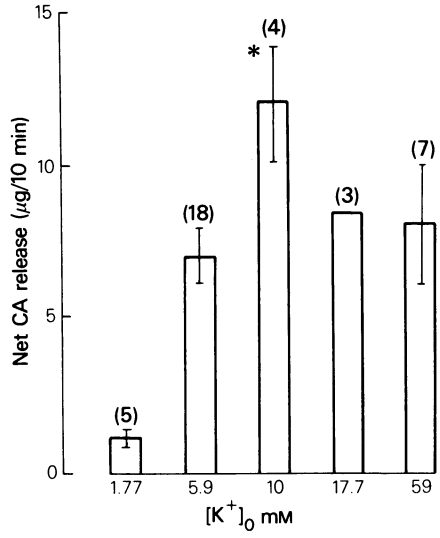

Figure 2 Net catecholamine (CA) release evoked by increasing the extracellular K^+ concentrations ($[K^+]_o$) in glands perfused with 0-K^+ solutions containing ouabain (10^{-4} M). Different $[K^+]_o$ were reintroduced for 2 min after 20 min of perfusing the glands with 0-K^+-ouabain solution. Net CA release was calculated by subtracting the amount of CA found in pre-stimulation samples from the 5 subsequently collected 2 min samples. In parentheses, number of experiments. Columns show mean values and vertical lines s.e.mean. * $P < 0.05$, compared with 5.9 mM K^+.

different glands with a 0-K^+ solution containing ouabain (10^{-4} M) in order to study the effects of K^+ reintroduction in conditions in which Na^+ pumping was inhibited by the glycoside. Figure 2 shows that reintroduction of K^+ in these circumstances caused a clear secretory response. The maximal secretory response was reached when 10 mM K^+ was reintroduced for 2 min, this response being about 120 times the spontaneous, basal catecholamine release. It is worth noting that 1.77 mM K^+ already evoked a significant increase of catecholamine release over basal levels of secretion. Also, it is interesting that catecholamine secretion was similar at 5.9 and 59 mM $[K^+]_o$.

Effect of the extracellular Na^+ concentration on the K^+ secretory response in ouabain-treated glands

Since ouabain increases the rate of spontaneous catecholamine release by activation of the Na^+-Ca^{2+} exchange system of the cell membrane (García *et al.*, 1980; Esquerro *et al.*, 1980), at this point it was critical to determine to what extent, if any, this glycoside-effect was also responsible for the potentiation of the K^+-secretory response.

In low $[Na^+]_o$, the catecholamine secretory response to ouabain is almost abolished (Esquerro *et al.*, 1980). Therefore, if the same Na^+-Ca^{2+} exchange mechanism was also involved in the K^+ secretory response, one would expect that lowering $[Na^+]_o$ would abolish, or at least diminish such a response. However, when $[K^+]_o$ is briefly (2 min pulse) enhanced to 10 mM at 15 min intervals in a ouabain-treated gland perfused with 25 mM Na^+, a large secretory response is always observed (Figure 3b). When the same experiment is performed in normal $[Na^+]_o$, the background release is progressively increased with time, but the K^+ effect is identical (Figure 3a); here, both components of the effects of ouabain, activation of the Na^+-Ca^{2+} exchange (enhanced background release) and potentiation of K^+-evoked secretion are seen. Net catecholamine release evoked by 10 mM K^+ 30 min after ouabain (10^{-4} M), was 8.95 ± 1.15 μg/10 min ($n=21$) in 25 mM Na^+ solution and 9.16 ± 2.03 μg/10 min ($n=7$) in 144 mM Na^+ solution.

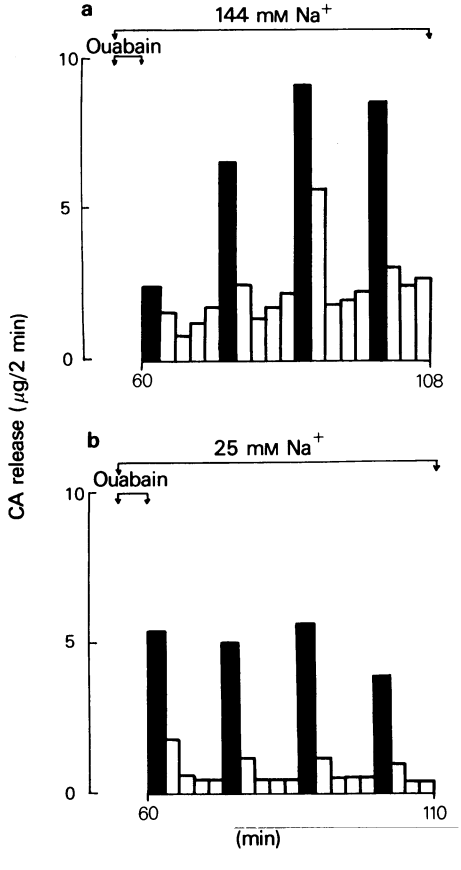

Figure 3 Effect of the extracellular Na^+ concentration on the K^+ secretory response in ouabain-treated glands. Two glands from the same cat were initially perfused with Krebs-bicarbonate solution; then, the perfusing fluid of gland (b) was changed to a 25 mM Na^+ (choline)-solution. Both glands were given a 10 min pulse of ouabain (10^{-4} M). Then, 10 mM $[K^+]_o$ pulses were applied at 20 min intervals. Results of a typical experiment are shown. Open columns 5.9 mM K^+; solid columns 10 mM K^+.

Time-dependence of the K^+-secretory response in ouabain-treated glands

If 2 min pulses of 10 mM K^+ solution are applied 0, 10, 20, 30, 45 and 60 min after introduction of ouabain (10^{-4} M) into the perfusion fluid, the K^+-evoked catecholamine secretory response increases, reaching a plateau at 20 min; the response was maintained fairly well up to 45 min, declining somewhat at 60 min (Figure 4). In all the following experiments, when several 10 mM K^+ pulses were used, the first pulse was given 20 min after the start of ouabain perfusion.

Perfusion of ouabain (10^{-4} M)-treated glands with a 10 mM K^+ solution for 10, 20, 30, 60 and 120 s, gave information on the time required for K^+ to

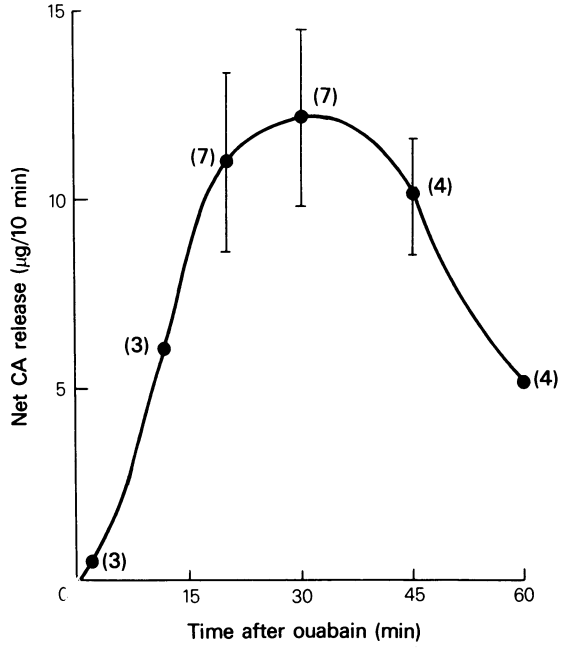

Figure 4 The effects of the duration of ouabain treatment on catecholamine (CA) secretion evoked by 10 mM $[K^+]_o$ (2 min pulses). The glands were perfused with 25 mM Na^+ (choline) solution, containing ouabain (10^{-4} M). Ten mM $[K^+]_o$ pulses were given after ouabain at the times shown on the abscissa scale. Means of the number of experiments shown in parentheses; vertical lines indicate s.e.mean.

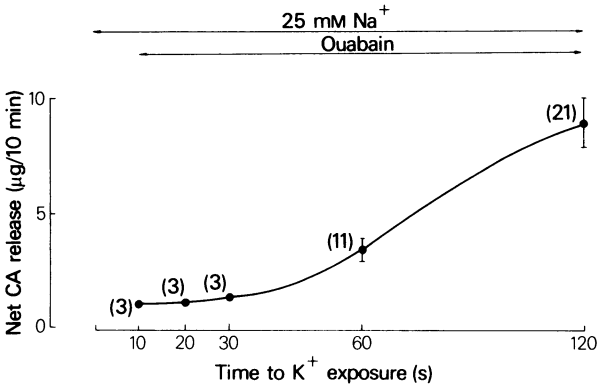

Figure 5 Time-dependence of the K^+-evoked catecholamine (CA) secretory response in ouabain-treated glands. Glands were perfused with a 25 mM Na^+ (choline) solution containing 10^{-4} M ouabain. At 20 min intervals, $[K^+]_o$ was raised from 5.9 to 10 mM for brief periods of time ranging from 10 to 120 s (abscissa scale). Means of the number of experiments shown in parentheses; vertical lines indicate s.e.mean.

reach its site of action and induce the secretory response. Figure 5 shows that the threshold response appeared at the 1 min perfusion time; 2 min perfusion drastically raised the catecholamine output; however, perfusion times lower than 1 min did not produce a remarkable secretory response.

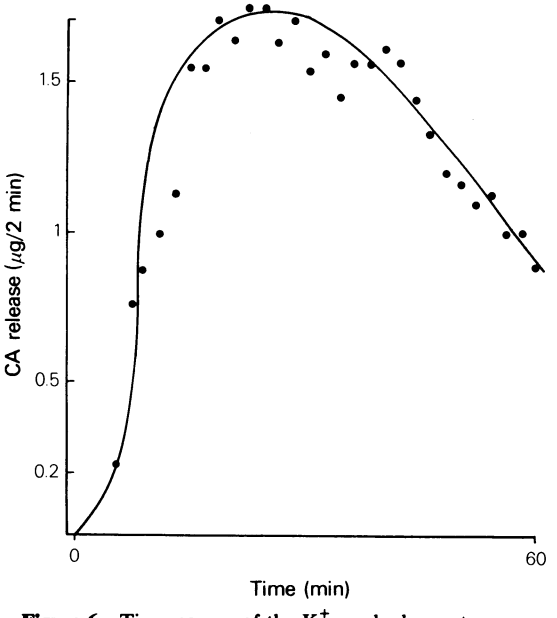

Figure 6 Time-course of the K^+-evoked secretory response. Experimental design as in Figure 5 but here, raised $[K^+]$ (10 mM) was left in contact with a ouabain-treated gland for 1 h. Results of a typical experiment (1 of 4) are plotted.

When 10 mM K^+ solution (in 25 mM Na^+) was left in contact with a ouabain-treated gland for as much as 1 h, the usual secretory response was evoked (Figure 6). The peak secretory response was reached about 20 min after K^+ introduction, maintained for an additional 20 min period and gradually fell during the last 20 min period of the experiment (Figure 6).

One additional time-dependent property of the K^+ response is the slow reversibility of its effect. Once $[K^+]_o$ is restored to its normal level (5.9 mM), the catecholamine output returns to basal levels only after 10–12 min and in the presence of normal $[Na^+]_o$ it takes even longer (Figure 1b and 3a). Thus, the potentiation of the secretory response to K^+ by ouabain involves both parameters, the height and the duration of such response.

Influence of $[Ca^{2+}]_o$ and $[Mg^{2+}]_o$ on the K^+-secretory response in ouabain-treated glands

This experiment was designed to test how manipulation of the external concentrations of divalent cations affected the catecholamine secretory response evoked by K^+ in ouabain-treated glands, and was performed on two glands from the same cat perfused with 25 mM Na^+ Krebs solution. Three 2 min pulses of 10 mM K^+ (S_1, S_2 and S_3) were applied at 20 min intervals to ouabain-treated (10^{-4}M) glands perfused with 25 mM Na^+ solution. Between S_1 and S_2, Ca^{2+} was removed in one gland and in the contralateral, $[Mg^{2+}]_o$ was increased to 20 mM. In both cases the secretory response to K^+ (10 mM) was abolished (Figure 7 a,b). After restoration of the normal $[Ca^{2+}]_o$ and $[Mg^{2+}]_o$, the K^+ response was restored.

In two experiments it was observed that the Ca^{2+}-dependency of the K^+-secretory response was not strictly linear, and that at $[Ca^{2+}]_o$ of 0.25 and 0.75 mM, catecholamine outputs were similar to those obtained at 2.5 mM. However, the organic Ca^{2+} antagonist, methoxyverapamil (D600), at 10^{-5}M, almost abolished the K^+-secretory response. In two ouabain-treated glands, the net catecholamine release during S_1 was 13.5 µg/10 min. D600 was given 10 min before and during S_2; output in this case was only 2 µg/10 min; in S_3, even in the absence of D600, the secretory response was still greatly depressed (0.80 µg/10 min).

The effects of tetrodotoxin (TTX) and tetraethylammonium (TEA) on the K^+-secretory response in ouabain-treated glands

The K^+-secretory response in ouabain-treated glands could be indirectly mediated by activation and/or inactivation of voltage-dependent Na^+ or K^+ channels, respectively. Therefore, TTX and TEA were used in experiments similarly designed to those

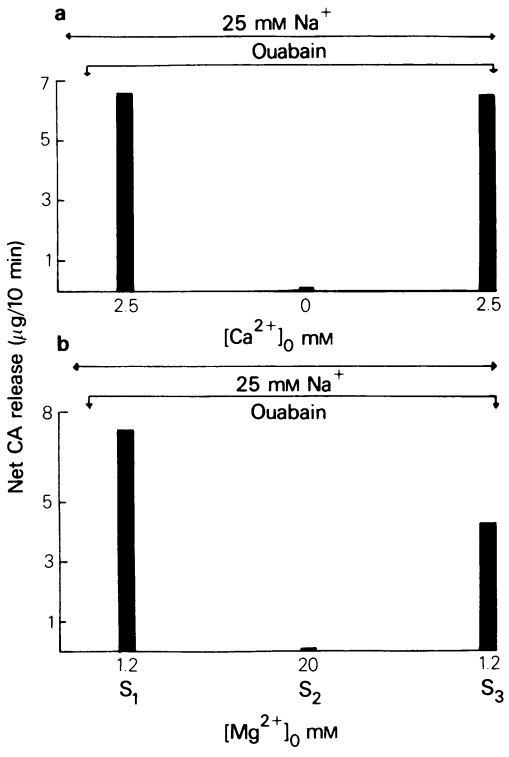

Figure 7 Influence of Ca^{2+} and Mg^{2+} on the K^+-evoked secretory response in ouabain-treated glands. The basic perfusion medium was 25 mM Na^+ (choline). Ouabain (10^{-4} M) was present during all the experiment. $[K^+]_o$ was raised to 103mM for 2 min periods at 20 min intervals (S_1, S_2 and S_3, solid columns). Between S_1 and S_2, Ca^{2+} was removed (a) or $[Mg^{2+}]_o$ increased to 20 mM (b). Ordinate scale, net catecholamine (CA) release; data taken from a single experiment (1 of 3).

described in the previous section. Each drug was introduced in the perfusion system between S_1 and S_2. S_2 (10 mM K^+ solution for 2 min), was applied in the presence of the drug.

The net catecholamine output was 8.5, 9.5 and 4 μg/10 min during S_1, S_2 and S_3, respectively, in the glands treated with TTX (10^{-6} M) 10 min before and during application of S_2 (means of 2 experiments). Net catecholamine outputs in 2 glands treated with TEA (3 mM) 10 min before and during S_2, were 7.39, 7.23 and 7.51 μg/10 min for S_1, S_2 and S_3, respectively.

Discussion

The results of the present study clearly show a novel catecholamine secretory response evoked by K^+ in the perfused cat adrenal gland. This response consists of a dramatic potentiation of the catecholamine output, evoked by exposure of ouabain-treated glands during brief periods of time to solutions containing small increments of K^+ over the normal concentration. The potentiation is so intense that an increment of $[K^+]_o$ as small as 1.77 mM for 2 min is sufficient to induce a clear secretory response. In normal glands, only a 59 mM K^+ solution, when applied for 2 min, evoked an appreciable, yet small enhancement of catecholamine output.

Properties of the K^+-secretory response in ouabain-treated glands

The catecholamine-secretory response evoked by raised $[K^+]_o$ has the following essential features: (1) it depends on $[K^+]_o$ but reaches its maximum at 10 mM K^+; (2) it is time-dependent; (3) it does not desensitize; (4) it remains unchanged in low $[Na^+]_o$; (5) it requires the presence of Ca^{2+}; (6) it is abolished by high $[Mg^{2+}]_o$ and (7) is not affected by TTX or TEA.

Catecholamine outputs in response to raised $[K^+]_o$ were, to a certain extent, proportional to the increase of $[K^+]_o$; usually, at 10 mM K^+ a maximal secretory response was observed. It is interesting to note that in many experiments, 59 mM or 140 mM K^+ solutions did not produce greater catecholamine outputs than 5.9, 10 or 17.7 mM K^+ solutions, probably because the mechanism responsible for this catecholamine secretion was already maximally activated at the lower $[K^+]_o$.

Raised $[K^+]_o$ required at least 1 min to evoked an appreciable secretory response. Ten to 30 s exposure of the gland to 10 mM K^+ did not induce a secretory response, probably because this time was not sufficient for K^+ to diffuse from the vascular bed to the site of its action. Another interesting point is the time-course of the K^+-response. When a ouabain-treated gland was continuously perfused with raised K^+, the output of catecholamines reached a peak and then, the rate of secretion remained high for a fairly long period of time with no apparent desensitization. This is in contrast to the rapid inactivation of the secretory response observed in normal preparations exposed to raised $[K^+]_o$ (Baker & Rink, 1975; García, Kirpekar & Sánchez-García, 1976).

Possible mechanism(s) involved in the K^+-secretory response in ouabain-treated glands

When most of the Na^+ gradient was abolished by perfusing the gland with a 25 mM Na^+ solution, it was unlikely that Na^+ could enter the cell; so, intracellular accumulation of this cation should be prevented and also the activation of the membrane Na^+-Ca^{2+} exchange mechanism (García *et al.*, 1980; Esquerro *et al.*, 1980). Under these conditions, the K^+-secretory response was unchanged. This finding

clearly indicates that the K^+-evoked catecholamine secretory response is not dependent on the activation of the Na^+-Ca^{2+} exchange mechanism and some other mechanism must be involved in this phenomenon.

It seems possible that the increase in the intracellular free Ca^{2+} concentration mediating the catecholamine secretory response to K^+ could be a result of mobilization of Ca^{2+} from a superficially bound membrane pool. In fact, recently Gervais, Lane, Anner, Lindenmayer & Schwartz (1977) proposed a mechanism of inotropic action of cardiac glycosides which provides a rational framework within which to discuss our experiments. According to this hypothesis, digitalis induces a specific conformational change in Na^+, K^+-ATPase such that the affinity of Na^+ binding sites for Na^+ is decreased relative to that for Ca^{2+}. This change in relative affinity would increase the amount of Ca^{2+} localized to a submembranal Ca^{2+} store, which could be discharged during membrane depolarization. Evidence that membrane depolarization releases membrane-bound Ca^{2+} has received some experimental support (Greenspan & Morad, 1975). In our system, a similar mechanism can explain the potentiation of the catecholamine output evoked by raised $[K^+]_o$ in the presence of ouabain. Small increments of 2 mM $[K^+]_o$, which would produce depolarizations of the chromaffin cell membrane of only 2 mV (Douglas, Kanno & Sampson, 1967; Ishikawa & Kanno, 1978), would not be capable by themselves of evoking such a large secretory response by opening voltage-dependent Ca^{2+} channels. However, when ouabain is present, the binding of the drug to the ATPase-enzyme system would allow the accumulation of a large membrane-bound Ca^{2+} pool. Raising $[K^+]_o$ could either directly or through a small membrane depolarization, initiate a small extracellular Ca^{2+} entry which in turn could induce release of such membrane-bound Ca^{2+} into the cytosol and trigger a massive secretory response.

A second possibility could be a direct increase, by ouabain, of an inward Ca^{2+} current which could be activated by raised $[K^+]_o$. Even though Greenspan & Morad (1975) could not show any alteration by digitalis of the slow inward Ca^{2+} membrane current in frog ventricular myocardium, recent papers have demonstrated that cardiac glycosides can increase the slow inward Ca^{2+} current of cardiac Purkinje fibres from calf hearts (Weingart, Kass & Tsien, 1978) and smooth muscle of canine coronary artery (Belardinelli, Harder, Sperelakis, Rubio & Berne, 1979). On the other hand, digitalis has been shown to enchance ^{45}Ca influx in guinea-pig isolated auricle (Grossman & Furchgott, 1964). On this basis the possibility could be entertained that the small depolarization evoked by raised $[K^+]_o$ will induce the large secretory response in ouabain-treated glands by enhancing the number of Ca^{2+} channels or by allowing them to stay open longer.

Since high K^+ concentrations trigger Na^+-dependent action potentials in chromaffin cells (Brandt, Hagiwara, Kidokoro & Miyazaki, 1976), which in turn activate voltage-dependent Ca^{2+} channels, the potentiation by ouabain of the K^+ secretory response could be due to an increase in the number of action potentials evoked by raised K^+. However, this is unlikely since the secretory response was not affected either in low Na^+ solution or in the presence of TTX.

Finally, an effect of ouabain on intracellular organelles cannot be excluded. Ouabain can block sequestration of Ca^{2+} in intracellular organelles; also, Ca^{2+} entering the cell during K^+ stimulation may trigger a greater release of intracellular Ca^{2+} stores. Mitochondria are a very efficient intracellular Ca^{2+} sequestering system which might modulate the cytosolic concentration of ionized Ca^{2+} and, indirectly, transmitter and hormone release (Fiskum & Lehninger, 1980). We have recently found that mitochondrial membranes of bovine adrenal medulla contain high-affinity binding sites for [^{3}H]-ouabain (Sánchez-García *et al.*, 1980); experiments are now being performed in order to clarify whether these binding sites are associated to some functional role, i.e., modification of mitochondrial Ca^{2+} transport.

In conclusion, it seems that ouabain acts in two ways to evoke catecholamine release in the adrenal gland: (a) a slowly developed mechanism which is mediated by inhibition of Na^+ pumping, rise of $[Na^+]_i$ and activation of the membrane Na^+-Ca^{2+} exchange mechanism; and (b) increase of the Ca^{2+} available to the secretory machinery through mobilization of membrane-bound Ca^{2+}, activation of membrane Ca^{2+} inward current and/or greater availability of intracellular Ca^{2+} through an action on cellular organelles. Which of these mechanisms is mainly involved in the potentiation by the glycoside of the K^+-secretory response, remains to be elucidated. In any case, it seems attractive to suggest that the plasma membrane ATPase enzyme system, by changing the affinity of its Ca^{2+} binding sites, might control the availability of this cation to the secretory machinery and, therefore, modulate catecholamine secretion in the adrenal gland. Small increments in the $[K^+]_o$ such as those responsible for the large catecholamine secretory response seen in our experiments are likely to occur at synapses and neuro-effector junctions as a result of neuronal activity (Orkand, 1980). These changes, might modulate neurotransmitter release at these levels in conjunction with the ATPase enzyme system.

Supported in part by grants from Comisión Asesora de Investigación and INSALUD, Madrid, Spain; and also grants HL22170 from NIH and 212-2181-A from the New York Heart Association. We thank Ms M.C. Molinos for typing this manuscript.

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(*Received March 31, 1981.*
Revised June 18, 1981.)