Br. J. Pharmac. (1986), **88**, 757–765

Effects of Bay K 8644 on cat adrenal catecholamine secretory responses to A23187 or ouabain

Cristina R. Artalejo & Antonio G. Garcia

Universidad de Alicante, Facultad de Medicina, Departamento de Farmacología, Alicante, Spain

1 Calcium ionophore A23187 increases the rate of spontaneous catecholamine release from cat adrenal glands perfused at 37°C with oxygenated Krebs bicarbonate solution, in a time- and Ca-concentration-dependent manner. The secretory profile obtained with the ionophore was not modified in the presence of the Ca channel activator Bay K 8644.

2 Ouabain also enhanced the rate of spontaneous catecholamine outputs in a time- and concentration-dependent manner. The threshold ouabain concentration capable of producing a clear, yet delayed secretory response was 10^{-6}M. Increasing ouabain concentrations up to 10^{-4}M enhanced catecholamine release and shortened the time to peak release.

3 The dihydropyridine Ca channel activator Bay K 8644 (10^{-6}M) markedly potentiated the secretory effects of all ouabain concentrations used (10^{-7}–10^{-4}M). However, the most impressive potentiations were seen at 10^{-5}M ouabain; while at this concentration ouabain alone released 2.6 ± 0.07 μg catecholamines per 30 min, in the presence of Bay K 8644 the release was 73.4 ± 5.7 μg per 30 min. Conversely, at a fixed ouabain concentration (10^{-5}M), the potentiation was also dependent on the Bay K 8644 concentration (10^{-8}–10^{-5}M).

4 Although K deprivation inhibits Na pumping as does ouabain, Bay K 8644 did not modify the rate of catecholamine release evoked by K removal from the perfusion medium.

5 Potassium deletion, nimodipine or high Mg all reversed the fully developed secretory response evoked by ouabain plus Bay K 8644.

6 In glands depolarized by continuous perfusion with high K solutions, once the secretory response was inactivated, the introduction of ouabain caused an enhancement of the catecholamine secretory rate. This increase was dependent on the extracellular Na concentration and was not affected by Bay K 8644. In the presence of 6 mM Na the secretory effects of Bay K 8644 plus ouabain were abolished.

7 These results are compatible with the following conclusions: (i) Bay K 8644 potentiates only those catecholamine secretory responses that are known to be mediated through the activation of voltage-sensitive Ca channels; the drug does not seem to affect secretory responses by acting on the membrane Na/Ca exchange system or at some intracellular Ca-dependent component of the secretory machinery of Ca buffering systems. (ii) It is likely that ouabain enhances the rates of adrenal catecholamine release by a dual mechanism: chromaffin cell depolarization and activation of a membrane Na/Ca exchange system.

Introduction

In contrast to the inhibitory effects of nitrendipine (Ceña *et al.*, 1983), the dihydropyridine analogue Bay K 8644 potentiates markedly the release of catecholamines evoked by moderately high K concentrations (García *et al.*, 1984), nicotine (Sala *et al.*, 1985) or Ca reintroduction (Montiel *et al.*, 1984). Since the drug also increased ^{45}Ca uptake by bovine isolated adrenal chromaffin cells and displaced the specific binding of [^{3}H]-nitrendipine to bovine adrenomedullary membrane fragments (García *et al.*, 1984; 1985), it might well become the first available tool to activate selectively voltage-sensitive Ca channels favouring the access of Ca to the interior of the chromaffin cell. Therefore, it is of the utmost importance to define the degree of specificity of Bay K 8644 in acting on stimuli that cause catecholamine release by primary (high K) or secondary (nicotine, acetylcholine) activation of Ca channels, from other secretagogues (i.e., A23187, ouabain) that trigger the secretory process by mechanisms that seem to bypass

Ca channels. The Ca ionophore A23187 is known to release catecholamines by promoting the direct entry of Ca into chromaffin cells (García *et al.*, 1975) and ouabain enhances the amine output (Banks, 1967) probably through the indirect activation of a membrane Na/Ca exchange carrier system (García *et al.*, 1980; 1981a, b; Esquerro *et al.*, 1980).

The experiments described in this paper demonstrate that Bay K 8644 does not affect catecholamine release from the cat adrenal medulla in response to A23187 or K deprivation; in contrast, the dihydropyridine causes a considerable potentiation of the ouabain secretory response. These data add evidence in favour of the view that Bay K 8644 acts selectively on chromaffin cell voltage-sensitive Ca channels and suggest that ouabain enhances the rates of spontaneous catecholamine release by a dual mechanism: chromaffin cell depolarization and activation of the membrane Na/Ca exchange system.

Methods

Cats of either sex weighing 2.5–4 kg were anaesthetized with sodium pentobarbitone (50 mg kg^{-1}, i.p.). Both adrenal glands were isolated and prepared for retrograde perfusion with Krebs bicarbonate solution at 37°C (experiments with A23187) or 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, $CaCl_2$ 2.5, $MgSO_4.7H_2O$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2 and glucose 11. The solution was equilibrated with 95% O_2:5% CO_2, the final pH being 7.4. High [K] solution was made up by adding KCl to reach a final concentration of 59 mM (59K), with concomitant isoosmotic reduction of NaCl. In K-free solutions, both KCl and KH_2PO_4 were removed from the normal Krebs solution without osmotic adjustments. Low [Na] solutions were prepared by substituting all NaCl by equiosomotic concentrations of sucrose. High [Mg] solutions were prepared by adding $MgCl_2$ (20 mM) to normal Krebs solution without osmotic adjustments. Solutions containing several Ca concentrations were prepared by adding the appropriate amounts of $CaCl_2$; again, no osmotic adjustments were made.

Experimental designs

After 1 h of initial perfusion with normal Krebs solution, collection of perfusate samples at 5 min intervals was started and continued during the entire experiment. The first two samples were collected to determine the spontaneous catecholamine output; then A23187 or ouabain, with or without Bay K 8644, were perfused in the presence of different media.

The protocol always ended with stimulation of the gland with high [K] in order to test its functional viability as far as its secretory response was concerned.

Catecholamine assay

Samples were collected in iced assay tubes containing enough perchloric acid to give a final concentration of 0.05 M. The total catecholamine content of perfusate samples (noradrenaline plus adrenaline) was determined fluorometrically according to Anton & Sayre (1962) without the intermediate alumina adsorption procedure. Catecholamine present in each collection tube was expressed as μg of noradrenaline equivalents per 1 or 5 min perfusion period. Adequate standards were prepared to avoid interferences with native fluorescence present in the drugs used, specially in the case of A23187. The sensitivity of the assay method, performed in a Perkin Elmer model 204 fluorescence spectrophotometer, was approx. 10 μg ml^{-1} of noradrenaline; its reproducibility was very high and the standards varied around 5% from one day to another.

Drugs and solutions used

Ouabain and A23187 were purchased from Sigma Chem. Co., St. Louis, MO. U.S.A.; Bay K 8644 and nimodipine were a gift from Bayer AG., Wuppertal, F.R.G. Ouabain was dissolved directly in Krebs solutions. A23187 and Bay K 8644 were dissolved in 96% ethanol at a 10^{-2}M concentration; appropriate dilutions were performed in Krebs solutions. The ethanol final concentration was smaller than 0.1% and had no effects on basal or evoked catecholamine release rates. All flasks containing these solutions were carefully protected from light and the experiments were performed under a sodium lamp.

Results

Effects of Bay K 8644 on catecholamine release evoked by A23187

In this experiment, both glands of the same cat were initially perfused for 30 min with A23187 30 μM dissolved in Krebs solution lacking Ca; then, perfusate samples were collected first in Ca-free solution. Later, Ca was introduced in several steps (0.25, 0.5, 1, 2.5 and 7.5 mM). A23187 was not perfused during the entire experiment because we had previously demonstrated that its effects on catecholamine release in the perfused cat adrenal gland are essentially irreversible (García *et*

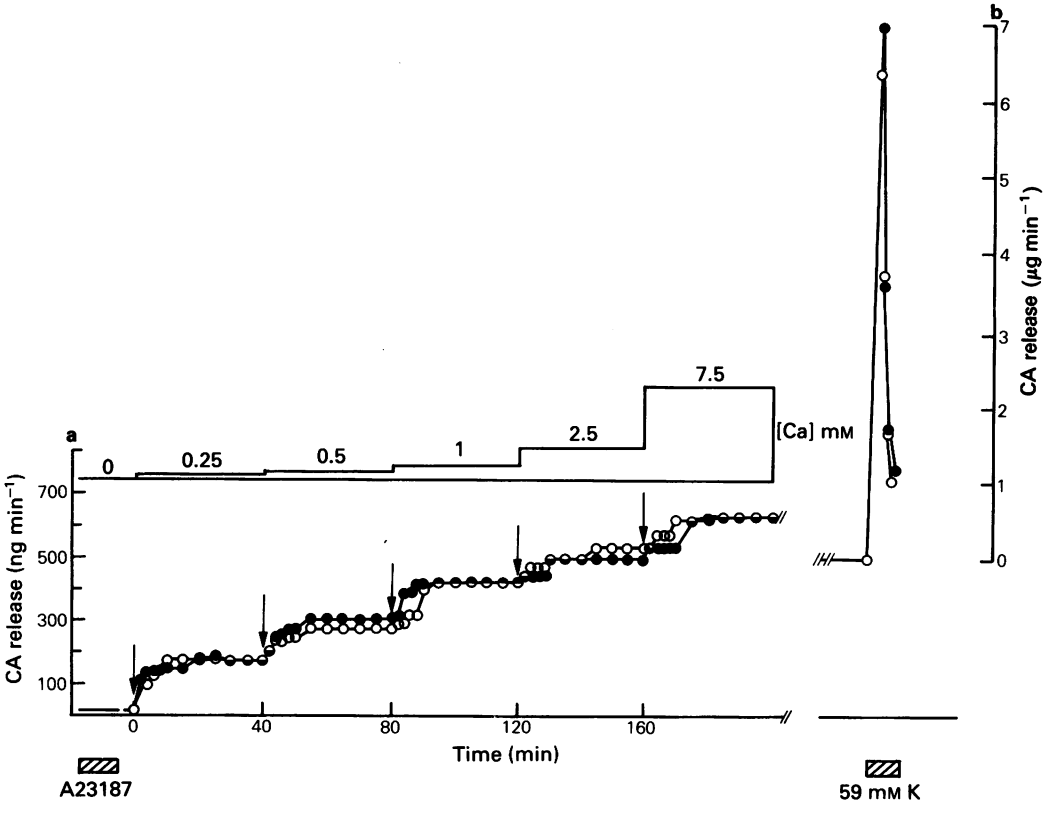

Figure 1 (a) Catecholamine (CA) release profiles from cat adrenal glands pretreated with the Ca ionophore, A23187. The ionophore (30 μM) was perfused and recirculated through both glands from the same animal for 30 min at 37°C in the absence of external Ca (left bottom horizontal bar). Then, increasing Ca concentrations were introduced into the perfusion solution in a step-wise manner at 20 min intervals. One gland was additionally perfused with Bay K 8644 (10^{-6}M, ●) during the entire experiment; control (○). After the 7.5 mM Ca step, normal Krebs-bicarbonate solution was perfused for 30 min and a 10 min pulse with high K (59 mM) was given to both glands (b). Data from a single paired experiment. A second similar experiment gave comparable results.

al., 1975) and we had only a limited stock of the drug. Figure 1 shows the results of a paired experiment; after each change to a solution containing a higher Ca concentration, the secretory response increased to reach a new plateau, and remained constant with no apparent inactivation until the next increase in Ca. Ca addition in several stages in glands not pretreated with A23187 did not enhance the basal rates of catecholamine output.

It is interesting to note the different profile of the secretory curve obtained at the end of the experiment (Figure 1b) upon perfusion of the gland with 59 K for 10 min; the secretory rate increased rapidly to give a sharp peak and also declined very quickly to reach pre-depolarization output levels in about 6–10 min.

Addition of Bay K 8644 10^{-6}M, (a concentration known to potentiate markedly the secretory responses evoked by modest K depolarizations; García *et al.*, 1984) to the perfusion solution of the contralateral, A23187-treated gland gave secretory profiles very similar to those obtained in the absence of Bay K 8644. The dihydropyridine did not modify the extent of secretion nor the time course of the curves for each Ca concentration. In a second paired experiment, Ca steps were raised to 20 mM, with essentially similar results: an increase in the rates of catecholamine release to new plateaux with each Ca step and no modification of the secretory profiles in the presence of Bay K 8644.

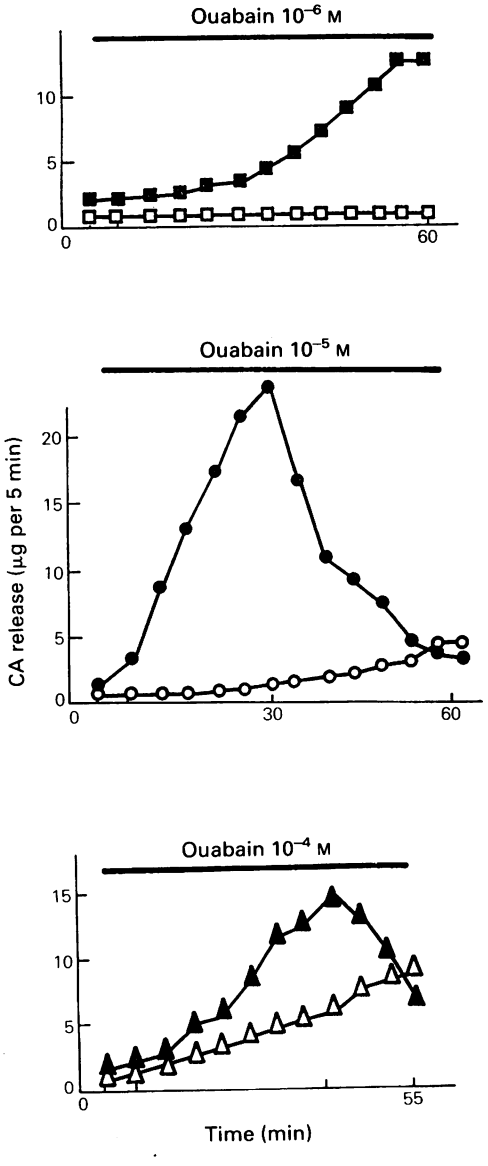

Figure 2 Effects of Bay K 8644 on catecholamine (CA) release evoked by several concentrations of ouabain. After the initial equilibration period, Bay K 8644 (10^{-6}M) was introduced into the perfusion fluid (filled symbols) and 10 min later ouabain (10^{-6}–10^{-4}M) was added; controls (open symbols). Data taken from single paired experiments; at least 3 experiments were done for each ouabain concentration. At 10^{-5}M ouabain, the peak release was 4.6 ± 0.7 μg per 5 min (mean ± s.e. of 7 glands); in the presence of Bay K 8644 the peak rose to 20.9 ± 3.2 μg per 5 min ($n = 8$). Differences between these two means were highly significant ($P < 0.01$).

Effects of Bay K 8644 on the rates of catecholamine release evoked by ouabain or K deprivation

After 1 h of initial perfusion with oxygenated Krebs-bicarbonate solution, spontaneous catecholamine release from cat adrenal glands was 247 ± 20 ng per 5 min ($n = 25$). Ouabain perfusion enhanced this catecholamine output in a time- and concentration-dependent manner (open symbols of Figure 2). The threshold concentration evoking a small secretory response was 10^{-6}M. At 10^{-5} and 10^{-4}M the secretory rates of spontaneous catecholamine output were clearly enhanced to reach peaks of 4.6 ± 0.7 (mean ± s.e., $n = 7$) and 6.4 ± 0.8 μg per 5 min, respectively. Times to peak releases were shortened as the ouabain concentration increased; so at 10^{-6}M,. 105 ± 7 min ($n = 3$) were required to reach a plateau, at 10^{-5}M, 76 ± 5.3 min ($n = 7$) were needed and at 10^{-4}M this time was reduced to 53 ± 4 min ($n = 8$).

Bay K 8644 lacked any secretory effects of its own even when 1 μM concentration was perfused through the cat adrenal gland for 1 h; nor did 10^{-6}M ouabain affect the rate of basal output of catecholamines during the first hour of perfusion (Figure 2). However, with the combination of both drugs each at a concentration of 10^{-6}M, a marked increase of catecholamine release (20.9 ± 3.2 μg per 5 min, $n = 8$) was reached at 21.9 ± 1 min after giving ouabain (in contrast to the tion was that seen with 10^{-5}M ouabain; here, the peak release (20.9 ± 3.2, μg per 5 min, $n = 8$) was reached at 21.9 ± 1 min after giving ouabain (in contrast to the 76 min required when using ouabain alone) and the amount of catecholamine release was quantitatively much greater. So, during the initial 30 min perfusion period, net catecholamine release in glands treated with ouabain alone was 2.6 ± 0.07 μg while in glands perfused with Bay K 8644 plus 10^{-5}M ouabain it was as much as 73.4 ± 5.7 μg ($n = 3$; $P < 0.01$). Finally, the potentiation of the secretory response to 10^{-4}M ouabain afforded by Bay K 8644, although clear, was less important (10.7 ± 2.3 μg per 5 min, $n = 4$). Because the interaction between the dihydropyridine and ouabain was best seen at 10^{-5}M ouabain, this concentration was used in the following experiments.

Bay K 8644 potentiated the ouabain-secretory effects in a concentration- and time-dependent manner. The rate of catecholamine release evoked by 10^{-5}M ouabain was enhanced in the presence of increasing concentrations of Bay K 8644. The curve depicting secretion with time was gradually displaced to the left when using 10 to 1.000 nM of the dihydropyridine. Peak releases were also higher and times to peak were considerably shortened as the concentration of Bay K 8644 was raised (Figure 3a). Concentrations as low as 10 nM were able to potentiate the secretory response and shifted to the left the curve obtained with ouabain alone.

In spite of its similar inhibitory action on the Na pump (Skou, 1957), catecholamine release evoked by a K-free solution was considerably smaller and slower than that evoked by ouabain (Figure 3b). Interestingly enough, the presence of Bay K 8644 (1 μM) did not modify at all the profile of the secretory curve obtained with K deprivation. Also, peak releases were similar in the absence (3.1 ± 0.2 μg per 5 min; $n = 5$) or the presence of Bay K 8644 (3.6 ± 0.8 μg per 5 min).

Effects of potassium removal on the secretory response to ouabain plus Bay K 8644

In the experiment shown in Figure 4a, b, two glands were perfused with ouabain (10^{-5}M) and in addition one of them with Bay K 8644 (10^{-6}M) from the beginning of the experiment. When on top of the secretory response evoked by the combination of ouabain plus Bay K 8644, K ions were suddenly removed, the secretory rate quickly declined to basal levels. Reintroduction of K to its normal level (5.9 mM) after a 20 min period of K deletion, allowed the full recovery of the secretory response. Note the time course of the secretory response in the gland treated only with ouabain (Figure 4a). Here, catecholamine release rates developed slowly, and K deprivation did not affect the rising tendency to reach a plateau of the secretory curve during the first hour of ouabain perfusion.

Figure 4c shows that increasing the Mg concentration of the perfusion medium from 1.2 to 20 mM produced a rapid fall to almost basal levels of the catecholamine release response evoked by ouabain plus Bay K 8644. Finally, when added on the peak of the secretory response, nimodipine (10^{-6}M) also evoked a quick decline of catecholamine release evoked by ouabain plus Bay K 8644.

Effects of extracellular sodium on the interactions between Bay K 8644 and ouabain

In this experiment, two glands from the same cat were perfused with a solution containing 59 mM K; in addition, one of the glands was treated with 10^{-6}M Bay K 8644. On introduction of high K, the rate of catecholamine release was quickly enhanced to reach a large peak (around 30 μg per 5 min) during the first 5 min collection period. The secretory rate declined to almost basal levels 10–20 min after continuous K stimulation. On ouabain addition, the secretory rate was enhanced again slowly but the usual large potentiation of secretion evoked by Bay K 8644 in control glands was not observed.

In the experiment shown in Figure 5 both glands were perfused with a solution enriched in K (59 mM) containing 10^{-6}M Bay K 8644; one of the glands was initially perfused with 25 mM Na. In these conditions,

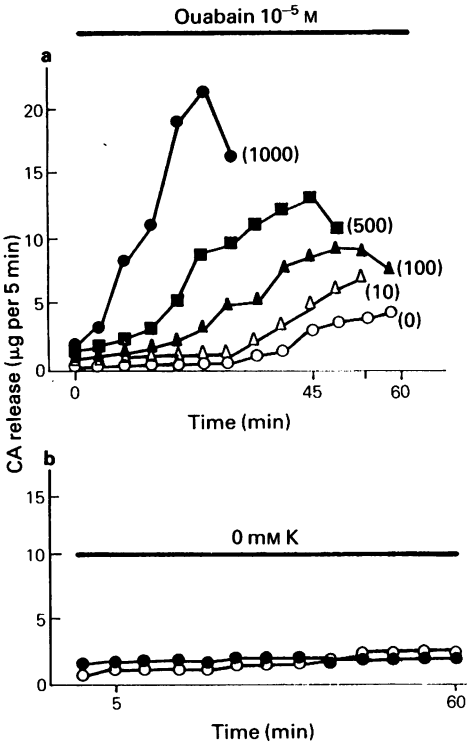

Figure 3 (a) Effects of increasing concentrations of Bay K 8644 on catecholamine (CA) release from glands perfused with 10^{-5}M ouabain. Each curve represents catecholamine outputs from separate glands perfused with ouabain alone or with ouabain plus increasing concentrations (nM, in parentheses) of Bay K 8644. Data are the means from 2–5 experiments. (b) Catecholamine output from adrenals perfused with solutions lacking K; data are the means of 3 paired experiments in which one gland (○) was treated with Bay K 8644 (10^{-6}M) from 10 min before and during the time of perfusion with the K-free solution; (●) control.

the usual fast secretory response to high K was seen in both glands, and also its quick decline to basal levels. Introduction of ouabain (10^{-5}M) into the perfusion fluid enhanced the rate of secretion provided that a normal concentration of Na ions was present; in 25 mM Na, the release rate was maintained at basal levels. Restoration of the normal Na concentration (144 mM) caused a rapid enhancement of the secretory rate.

A further experiment was performed perfusing both adrenal glands with a solution containing 25 or 6 mM Na ($NaHCO_3$ and NaCl substituted by sucrose, buffered with 15 mM HEPES and equilibrated with pure O_2). In these conditions, ouabain alone did not

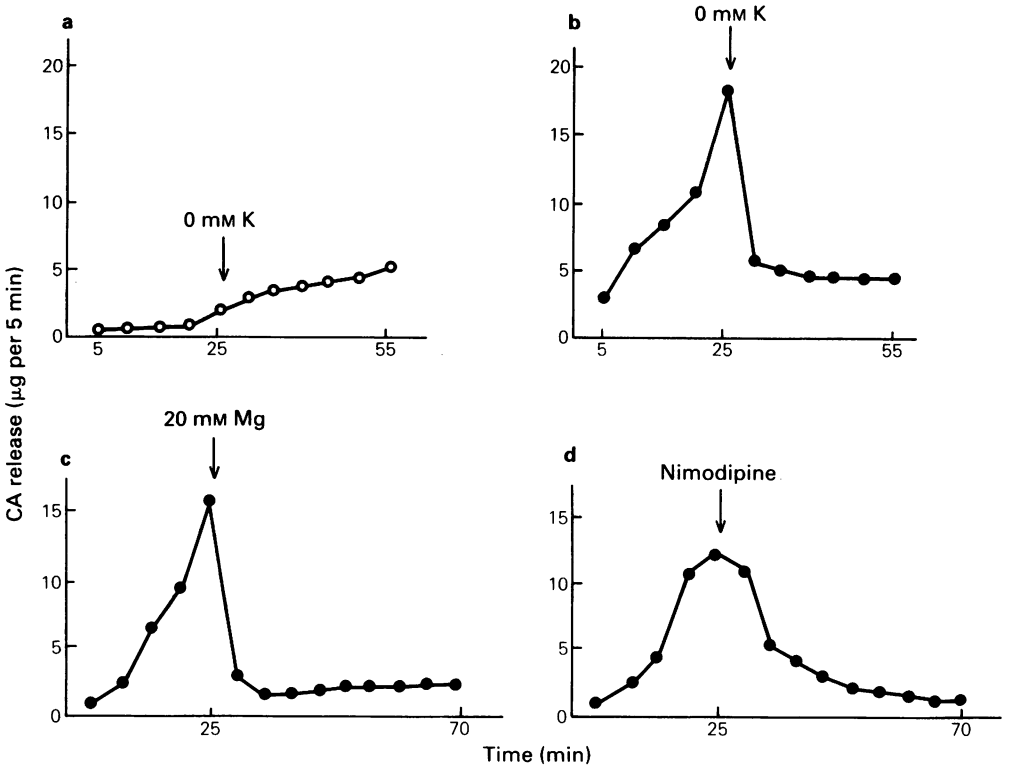

Figure 4 Time courses of catecholamine (CA) release from glands treated with ouabain alone (a) or with ouabain (10^{-5}M) plus Bay K 8644 (10^{-6}M) (b,c,d). In (a) and (b), K ions were removed from the perfusion media of both glands at the arrow. Data are the means of 3 experiments. At the arrow in (c) the Mg concentration of the Krebs solution was raised to 20 mM and in (d), nimodipine 10^{-6}M was added. Data are the means of 4 paired experiments.

increase the catecholamine secretory rates that remained at basal levels throughout the experiment, both in 25 or 6 mM Na (Figure 6). However, the combination of ouabain (10^{-5}M) plus Bay K 8644 (10^{-6}M) enhanced the secretory response in the presence of 25 mM Na but not in the gland perfused with 6 mM Na. Increasing the Na concentration to 144 mM produced a clear enhancement of the catecholamine release rate that was gradual in the gland treated with ouabain alone and faster in the gland perfused with the combination of ouabain plus Bay K 8644.

Discussion

The experiments presented here demonstrate that the dihydropyridine Bay K 8644 did not affect adrenomedullary catecholamine release responses evoked either by Ca ionophore A23187 or K deprivation, yet the secretory effects of the cardiac glycoside ouabain were markedly increased. Although measurements of secretion in perfused glands only provide an indirect indication of ionic transients that are probably responsible for the modifications of the rates of catecholamine output observed, the experiments suggest that Bay K 8644 does not affect the membrane Na/Ca exchange carrier system nor some intracellular Ca-dependent mechanism; rather, these experiments are consistent with the hypothesis that in potentiating catecholamine release, Bay K 8644 acts only on secretory stimuli that are known to depolarize chromaffin cells to cause the opening of voltage-dependent Ca channels.

The mechanism of action of Bay K 8644

Previous studies from our laboratory concerning the

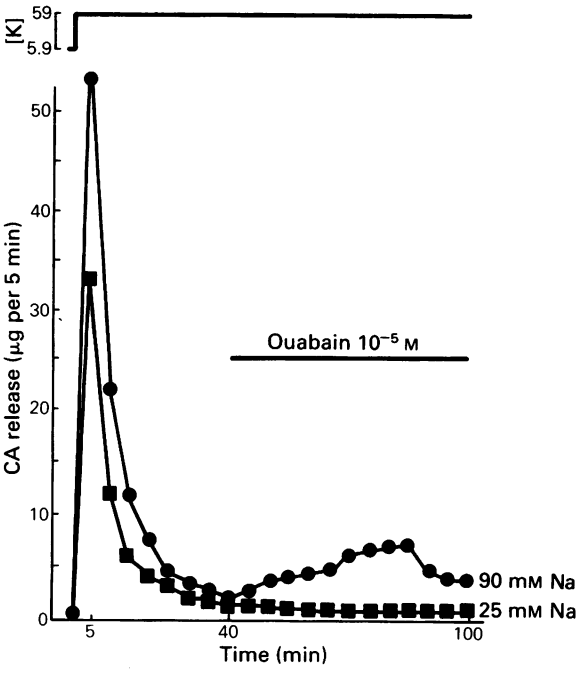

Figure 5 An experiment in which two glands were perfused with Bay K 8644 (10^{-6}M) throughout. The Na concentration was reduced to 25 mM in one gland (■) and to 90 mM in the other (●). K was increased as shown in the top horizontal line. The graph was drawn with data taken from a single paired experiment (one of three).

effects of Bay K 8644 on catecholamine release and ^{45}Ca uptake during depolarization, and on [^{3}H]-nitrendipine binding to adrenomedullary membranes suggest that its site of action might be located on or near the Ca channel (García *et al.*, 1984; Montiel *et al.*, 1984). On the other hand, the Ca ionophore A23187 enhances catecholamine release from cat adrenal glands by introducing Ca directly into the chromaffin cell, therefore bypassing the Ca channel (García *et al.*, 1975). The fact that the rates of catecholamine release obtained with several Ca concentration steps in A23187-treated glands were unmodified in the presence of Bay K 8644, speaks in favour of the selectivity of the drug for the Ca channel. In addition, this experiment indicates that the drug is not acting at some intracellular site to alter the capacity of Ca buffering systems or some Ca-dependent component of the secretory machinery. If Bay K 8644 were acting intracellularly in a step beyond the Ca channel, then secretory responses such as those induced by A23187 or activation of the Na/Ca exchange mechanism by K-free solution, ought also to be potentiated by the dihydropyridine.

Like ouabain, K deprivation inhibits the membrane Na^+,K^+-ATPase activity (Skou, 1957) causing the secondary activation by intracellularly accumulated Na, of a membrane Na/Ca exchange carrier that leads to an increased rate of catecholamine release (García *et al.*, 1981b). The experiment demonstrating that Bay K 8644 was unable to affect the profile of the secretory curve obtained with K-free solutions, strongly suggests that this drug is not acting on the Na/Ca exchange system of the chromaffin cell membrane to modify its activity.

The potentiation by Bay K 8644 of the ouabain-evoked catecholamine release seems to be exerted through a mechanism similar to the potentiation of the secretory response evoked by depolarization with high K since: (1) the potentiation was more pronounced at lower ouabain concentrations, and was only seen with modest increases in K concentration (García *et al.*, 1984); (2) the potentiation was quickly antagonized by K removal, a manipulation that hyperpolarizes the chromaffin cell and decreases the opening probability of Ca channels; (3) the response was also suppressed by high Mg concentrations or by the organic Ca channel antagonist nimodipine; and (4) the ouabain secretory effects were not manifested in glands pre-depolarized with high K concentrations. These experiments strongly suggest that as in brain neurones (Brosemer, 1985), ouabain might cause depolarization of the chromaffin cell; such depolarization could be due to Na accumulation by inhibition of the pump, as suggested by the experiment shown in Figure 6 performed in low Na concentrations. Therefore, Bay K 8644 might be potentiating the secretory effects of the glycoside probably through a mechanism similar to the potentiation seen with modest increments of the K concentration, namely by increasing the opening time of voltage-sensitive Ca channel (Hess *et al.*, 1984).

Dual mechanism of action of ouabain on adrenomedullary catecholamine release

Although the secretory effects of ouabain had been previously studied in our laboratory using the perfused cat adrenal gland (García *et al.*, 1980; 1981a,b; Esquerro *et al.*, 1980; Kirpekar *et al.*, 1982) or bovine isolated adrenomedullary chromaffin cells (Aunis & García, 1981), here we have re-examined this problem in more detail and demonstrated that ouabain enhanced the rate of secretion of catecholamines in a time- and concentration-dependent manner. In such previous papers, and in the original paper of Banks (1967), ouabain concentrations in the range 10^{-4}–10^{-3}M were used. These concentrations are several orders of magnitude higher than those required for inotropic effects of ouabain on the heart to be

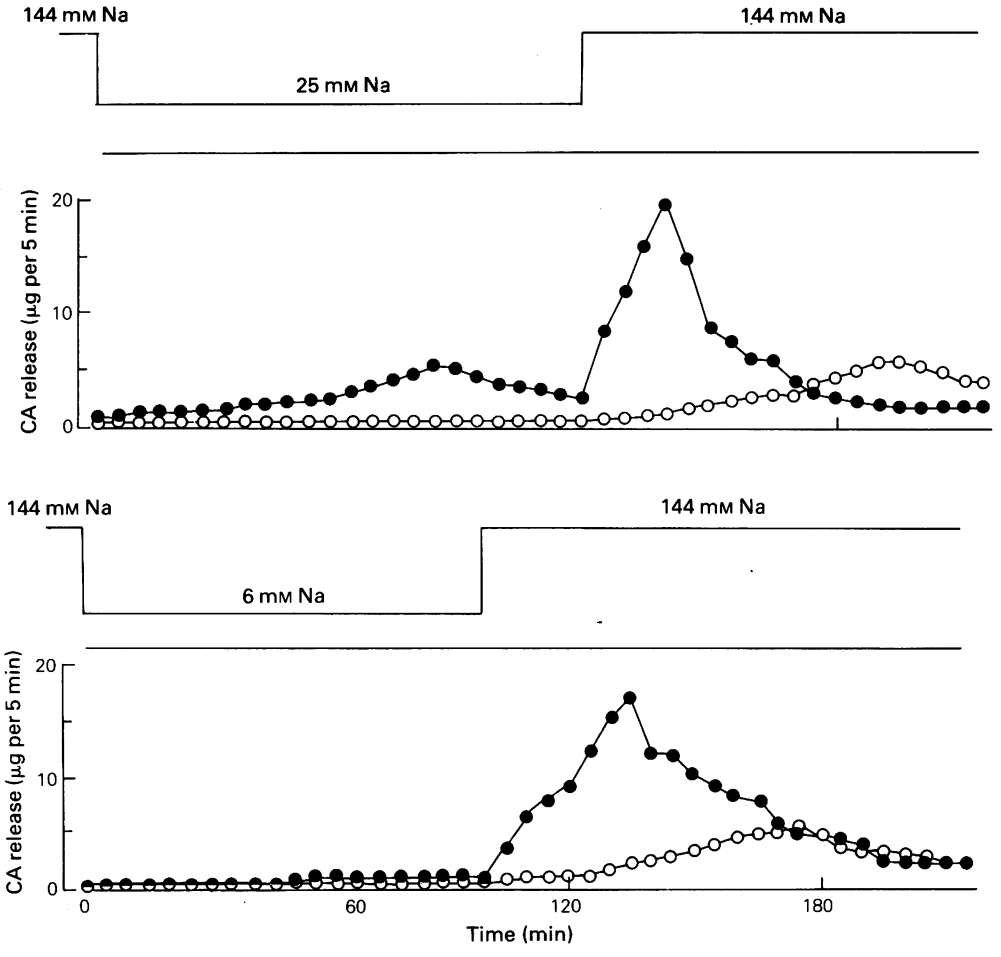

Figure 6 An experiment in which two glands were initially perfused with Krebs-solution containing 25 (a) or 6 (b) mM Na (top horizontal line) in the presence of ouabain (10^{-5}M); in addition, one of the glands (●) was also treated from the beginning with 10^{-6}M Bay K 8644. The Na concentration was raised to 144 mM as shown on the top horizontal line. The data are taken from a single paired experiment; in a second experiment with identical protocol, similar results were obtained.

manifested (Akera & Brody, 1978). The present experiments indicate that 10^{-6}M ouabain already enhanced the rate of catecholamine release provided that enough time (at least 1 h) was allowed for the drug to manifest its secretory effects.

An experiment directed to the separation of the depolarizing effects of ouabain from its ability to activate indirectly the Na/Ca exchange system was designed. It is known that on depolarization with high K concentrations, catecholamine release rates reach a quick peak to decline to basal levels in spite of the fact that chromaffin cells remain depolarized (Baker & Rink, 1975; Schiavone & Kirpekar, 1982). In such depolarized glands ouabain restored a Na-dependent secretory response (Figure 5), suggesting that in addition to cell depolarization, ouabain causes the activation of the Na/Ca exchange mechanism because: (1) in a depolarized gland, with an inactivated secretory response, ouabain still enhanced catecholamine release; (2) such enhancement was abolished by omission of Na and reactivated by Na reintroduction into the perfusion medium; (3) the

increased catecholamine release in such a depolarized gland was unaffected by Bay K 8644; and (4) Na reintroduction in a ouabain plus Bay K 8644-treated gland caused a sharp early increase of catecholamine release that strongly contrasts with the gradual increase observed in the gland treated with ouabain alone.

In summary, the combination of three mechanisms might explain the vigorous catecholamine secretory response obtained when ouabain and Bay K 8644 are jointly perfused through cat adrenal glands: small depolarizations caused by ouabain-inhibition of the Na pump will open voltage-dependent Ca channels; Bay K 8644 will delay their closing and inhibition of exchange mechanism that should slow down the extrusion of Ca from the cell and increase its uptake in exchange for Na. Overall, these combined mechanisms might lead to a large intracellular accumulation of Ca that is ultimately responsible for this vigorous secretory response.

Supported by grants from Fundación Ramón Areces, C.A.I.C.Y.T. no. 0626/81 (Ministerio de Educación y Ciencia) and F.I.S.S. (Ministerio de Sanidad), Spain.

We thank Mrs Julia Romeo for typing the manuscript and Mr Victoriano Mandado for expert technical assistance. We also wish to express our gratitude to Prof. F. Hoffmeister from Bayer A.G. for his kind gift of Bay K 8644 and nimodipine.

References

AKERA, T. & BRODY, T.M. (1978). The role of Na^+,K^+-ATPase in the inotropic action of digitalis. *Pharmac. Rev.*, **29**, 187–220.

ANTON, A.H. & SAYRE, D.F. (1962). A study of the factors affecting the aluminium oxide trihydroxyindole procedure for the analysis of catecholamines. *J. Pharmac. exp. Ther.*, **138**, 360–375.

AUNIS, D. & GARCIA, A.G. (1981). Correlation between catecholamine secretion from bovine isolated chromaffin cells and [^{3}H]-ouabain binding to plasma membranes. *Br. J. Pharmac.*, **72**, 31–40.

BAKER, P.F. & RINK, T.J. (1975). Catecholamine release from bovine adrenal medulla in response to maintained depolarization. *J. Physiol.*, **253**, 593–620.

BANKS, P. (1967). The effect of ouabain on the secretion of catecholamines and on the intracellular concentration of potassium. *J. Physiol.*, **193**, 631–637.

BROSEMER, R.W. (1985). Effects of inhibitors of Na^+,K^+ ATPase on the membrane potential and neurotransmitter efflux in rat brain slices. *Brain Res.*, **334**, 125–137.

CEÑA, V., NICOLAS, G.P., SANCHEZ-GARCIA, P., KIRPEKAR, S.M. & GARCIA, A.G. (1983). Pharmacological dissection of receptor-associated and voltage-sensitive ionic channels involved in catecholamine release. *Neuroscience*, **10**, 1455–1462.

ESQUERRO, E., GARCIA, A.G., HERNANDEZ, M., KIRPEKAR, S.M. & PRAT, J.C. (1980). Catecholamine secretory response to calcium reintroduction in the perfused cat adrenal gland treated with ouabain. *Biochem. Pharmac.*, **29**, 2669–2673.

GARCIA, A.G., GARCIA-LOPEZ, E., HORGA, J.F., KIRPEKAR, S.M., MONTIEL, C. & SANCHEZ-GARCIA, P. (1981a). Potentiation of K^+-evoked catecholamine release in the cat adrenal gland treated with ouabain. *Br. J. Pharmac.*, **74**, 673–680.

GARCIA, A.G., GARCIA-LOPEZ, E., MONTIEL, C., NICOLAS, G.P. & SANCHEZ-GARCIA, P. (1981b). Correlation between catecholamine release and sodium pump inhibition in the perfused cat adrenal gland. *Br. J. Pharmac.*, **74**, 665–672.

GARCIA, A.G., HERNANDEZ, M., HORGA, J.F. & SANCHEZ-GARCIA, P. (1980). On the release of catecholamines and dopamine-B-hydroxylase evoked by ouabain in the perfused cat adrenal gland. *Br. J. Pharmac.*, **68**, 571–583.

GARCIA, A.G., KIRPEKAR, S.M. & PRAT, J.C. (1975). A calcium ionophore stimulating the secretion of catecholamines from the cat adrenal. *J. Physiol.*, **244**, 253–262.

GARCIA, A.G., SALA, F., REIG, J.A., VINIEGRA, S., FRIAS, J., FONTERIZ, R.I. & GANDIA, L. (1984). Dihydropyridine Bay K 8644 activates chromaffin cell calcium channels. *Nature*, **309**, 67–71.

GARCIA, A.G., SALA, F. & REIG, J.A. (1985). Activation of chromaffin cell Ca^{2+} channels by novel dihydropyridine. *Nature*, **313**, 503–504.

HESS, P., LANSMAN, J.B. & TSIEN, R. (1984). Different modes of Ca channel gating behaviour favoured by dihydropyridine Ca agonists and antagonists. *Nature*, **311**, 538–544.

KIRPEKAR, S.M., GARCIA, A.G. & SCHIAVONE, M.T. (1982). Secretion of catecholamines from the adrenal gland by various agents. In *Advances in the Biosciences*, Vol. 36: Synthesis, Storage and Secretion of Adrenal Catecholamines, ed. Izumi, F., Oka, M. & Kumakura, K. pp. 55–62, Oxford: Pergamon Press.

MONTIEL, C., ARTALEJO, A.R. & GARCIA, A.G. (1984). Effects of the novel dihydropyridine Bay K 8644 on adrenomedullary catecholamine release evoked by calcium reintroduction. *Biochem. biophys. Res. Commun.*, **120**, 851–857.

SALA, F., FONTERIZ, R.I., BALLESTA, J.J., ALCARAZ, C., REIG, J.A. & GARCIA, A.G. (1985). Inactivation of the chromaffin cell calcium channel. Effects of Bay K 8644 and of calcium, strontium and cobalt gradients. (Abstr.) *J. Neurochem.*, **44** (suppl.), S63D.

SCHIAVONE, M.T. & KIRPEKAR, S.M. (1982). Inactivation of secretory responses to potassium and nicotine in the cat adrenal medulla. *J. Pharmac. exp. Ther.*, **223**, 743–749.

SKOU, J.C. (1957). The influence of some cations on the adenosine triphosphatase from peripheral nerves. *Biochim. biophys. Acta*, **23**, 394–401.

(Received September 27, 1985.
Revised February 1, 1986.
Accepted April 8, 1986.)