Volume 196, number 1 FEBS 3333 February 1986

Inactivation of potassium-evoked adrenomedullary catecholamine release in the presence of calcium, strontium or BAY-K-8644

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Received 12 November 1985

The rate of catecholamine release from cat adrenal glands perfused with Krebs solution containing 59 mM K declined exponentially during the first few minutes of depolarization. The rate of decline was considerably slower when Ca was substituted by Sr. The late addition of Ca, Sr or the Ca-channel activator BAY-K-8644 evoked a revival of secretion when catecholamine release was inactivated by prior K depolarization; the revival of secretion was independent of the depolarization time. These data demonstrate that inactivation of catecholamine release is specifically dependent on Ca; the modulatory role of Ca on secretion seems to be exerted at a step distal to the transmembraneous Ca channel.

Catecholamine release *Ca^{2+} channel agonist* *BAY-K-8644* *Channel inactivation* *Depolarization* *Adrenal medulla*

1. INTRODUCTION

During prolonged stimulation of the cat adrenal gland with depolarizing high K concentrations, the amount of catecholamine released into the perfusate declines exponentially during the first few minutes [1]. The cause of this reduction in catecholamine release is not well understood, although some authors have invoked Ca-channel inactivation as being responsible for such a decline [2,3]. Nevertheless, experiments from some of these authors [3] as well as those carried out on permeabilized chromaffin cells [4] are compatible with the existence of intracellular mechanisms that might also account for the inactivation process. This paper describes the effects of the nature (Ca or Sr) and the concentration of the divalent Ca channel permeant cation used on the kinetics of catecholamine release from the perfused cat adrenal gland upon sustained depolarization with high K concentrations. By using several manipulations (addition of Ca or Sr in two steps after depolarization) and the novel dihydropyridine BAY-K-8644 that activates chromaffin cell Ca channels [5,6], an evaluation is also made of the role that Ca channels might play in this phenomenon. The experiments suggest that Ca, but not Sr, modulates the inactivation of the late adrenomedullary secretory response, and that this modulation seems to be exerted at a step distal to the Ca channel.

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2. MATERIALS AND METHODS

Cat adrenal glands were isolated and prepared for retrograde perfusion with Krebs-bicarbonate solution equilibrated with 95% O_2-5% CO_2 at room temperature [7]. Total catecholamine contents of each individual 2-min perfusate sample were determined by a fluorimetric assay [8]. Depolarizing stimuli consisted in the increase of the extracellular K concentration to 59 mM, decreasing iso-osmotically the Na concentration. When concentrations lower than 2.5 mM Ca or Sr

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

were used, the concentration of $MgCl_2$ was increased, keeping the total concentration of divalent cations constant at 3.7 mM; this avoided the surface charge screening effects on activation of channels [6,9]. Net catecholamine release evoked by different stimuli was calculated by subtracting the basal spontaneous release from the release obtained during the time of stimulation. In some experiments, the rate of catecholamine release was expressed as percentage of the maximal rate of release obtained in their stimulation period.

3. RESULTS AND DISCUSSION

All glands were initially perfused with Krebs solution for 40 min; then solutions containing Ca or Sr at different concentrations were perfused for an additional 20 min period. The spontaneous catecholamine outputs ranged between 0.03 and 0.05 μg/min at all Ca and Sr concentrations tested; BAY-K-8644 (1 μM) did not modify these secretory rates in the presence of Ca.

3.1. *Comparison of the profiles of potassium-evoked secretory responses in the presence of calcium or strontium*

Two parameters can be evaluated in the curve reflecting the time course of catecholamine release evoked by high K: peak release and the long-term profile of the secretory response. Upon stimulation with 59 mM K, catecholamine release increased in a manner proportional to the concentration of Ca or Sr. The peak responses obtained with either cation are listed in table 1. Although peak releases were similar in Ca or Sr, because the secretory response inactivated very little in the case of Sr, net

Table 1

Peak responses of catecholamine secretion (μg/min) in 2-min perfusate samples during 10 min of depolarizing stimulus (59 mM K) in the presence of calcium or strontium

Cation	Secretion	*n*
Ca, 0.25 mM	2.17 ± 0.26	8
0.75 mM	3.83 ± 0.79	3
2.5 mM	5.12 ± 0.59	8
7.5 mM	4.97 ± 0.36	3
Sr, 0.25 mM	1.03 ± 0.32	7
0.75 mM	3.32 ± 0.48	3
2.5 mM	4.71 ± 0.27	3
7.5 mM	2.61 ± 0.37	3

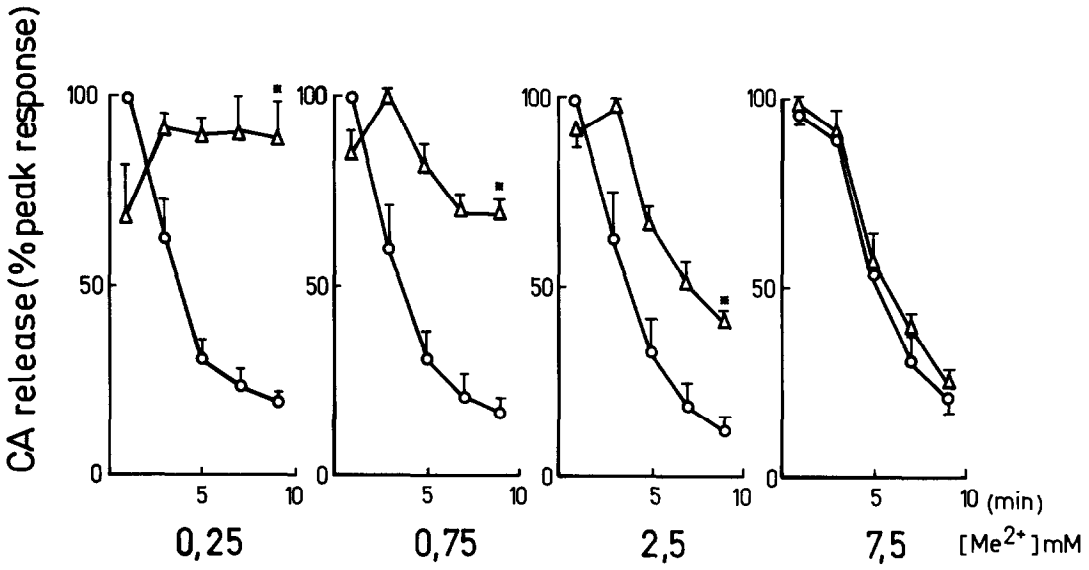

Fig.1. Effect of several concentrations of Ca (○—○) and Sr (△—△) on the inactivation of catecholamine (CA) release evoked by stimulation with high K solutions (59 mM) for 10 min. Data are expressed as % of maximal CA release in 2-min perfusate samples and represent the means ± SE of 3–7 paired experiments. * $P < 0.01$.

releases evoked in 10–20 min periods of depolarization were obviously greater when Sr was used as a cation that permeates Ca channels.

The secretory response to 59 mM K declined at the same rate along a 10 min depolarization period in a manner independent of $[Ca_o]$. However, when Sr was used instead of Ca as permeant cation, the secretory response did not inactivate at 0.25 or 0.75 mM and inactivated slowly at 2.5 mM. Only at 7.5 mM of each divalent cation did the response decline similarly with Ca or Sr (fig.1).

The different behaviour of Ca and Sr in modifying the profiles of the secretory response curves obtained with K stimulation may be explained on the basis of the different permeabilities of the Ca channel for both cations as well as differences in their abilities to inactivate such a channel; in fact, Ca current inactivation is delayed in the presence of Sr [10]. In chromaffin cells [11], as in other secretory cell types [12], Ca currents inactivate poorly. However, a type of slow inactivation (along several minutes) of Ca currents has been described [11]; it has been associated with a process of 'rundown' or 'washout' of Ca-channel activity by the loss of a hypothetical intracellular messenger that might keep the channel in an active state. Like Ba, Sr could prevent this rundown process delaying in this manner inactivation both of Ca channels and of the secretory response.

On the other hand, an intracellular event possibly associated with the exocytotic process, such as protein phosphorylation [13,14] promoted by Ca, might have different affinity for Sr [15,16] and could also explain the different rates of inactivation of catecholamine release in the presence of Ca or Sr. The experiments of fig.2 are compatible with this assumption. The late enhancement of $[Ca_o]$ or $[Sr_o]$ (from 0.25 to 2.5 mM) in glands depolarized with 59 mM K produced a large secretory peak of catecholamines that was similar at the 10th, 20th or 40th min of depolarization; this suggests that late after sustained depolarization, Ca channels remain open to allow the further entry of Ca and Sr.

3.2. *Calcium channels remain active under prolonged depolarization*

In spite of a possible early inactivation of secretion and of a fraction of Ca channels [11] that would be undetected with the present experimental designs, it seems that a substantial fraction of such channels remain active for at least the 40 min period of depolarization with 59 mM K. Nevertheless, the secretory response inactivated in the presence of different Ca concentrations (fig.1) suggesting that an intracellular mechanism might be involved in such inactivation process.

In the presence of 2.5 mM Ca, a sharp peak of catecholamine release followed by a quick decline was observed upon sustained depolarization with 59 mM K (fig.2A); when 59 mM K was applied initially in the presence of 0.25 mM Ca, and 20 min later, once the secretory response inactivated, Ca was enhanced to 2.5 mM, the secretory rate rose to a large peak and then quickly declined again (fig.2B). Similar results were obtained using Sr as permeant cation (fig.2C,D).

The dihydropyridine BAY-K-8644 is known to increase the mean open time of cardiac Ca channels [17] and to prolong the activation of chromaffin cell Ca channels [5,6]. The late addition (20 min after depolarization with 59 mM K) of

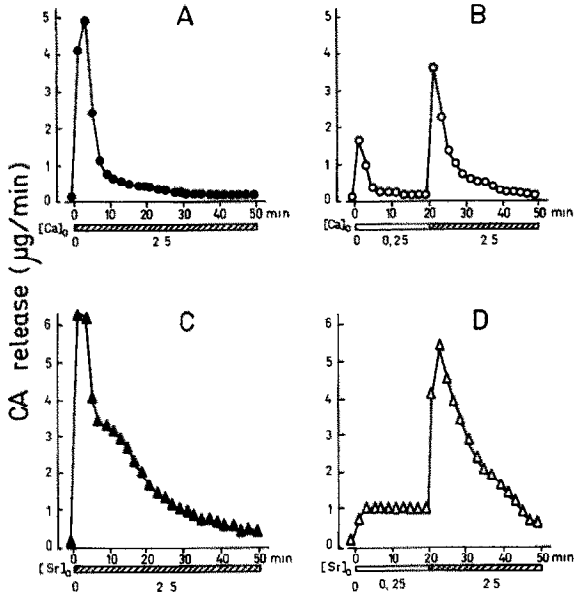

Fig.2. Catecholamine (CA) release evoked by a 50 min period of maintained depolarization (59 mM K). Effect of delayed increase (20 min) of extracellular Ca or Sr from 0.25 to 2.5 mM. The contralateral control gland was perfused in the presence of 2.5 mM Ca or Sr from the beginning of depolarization. Data are the results of a typical paired experiment out of 4.

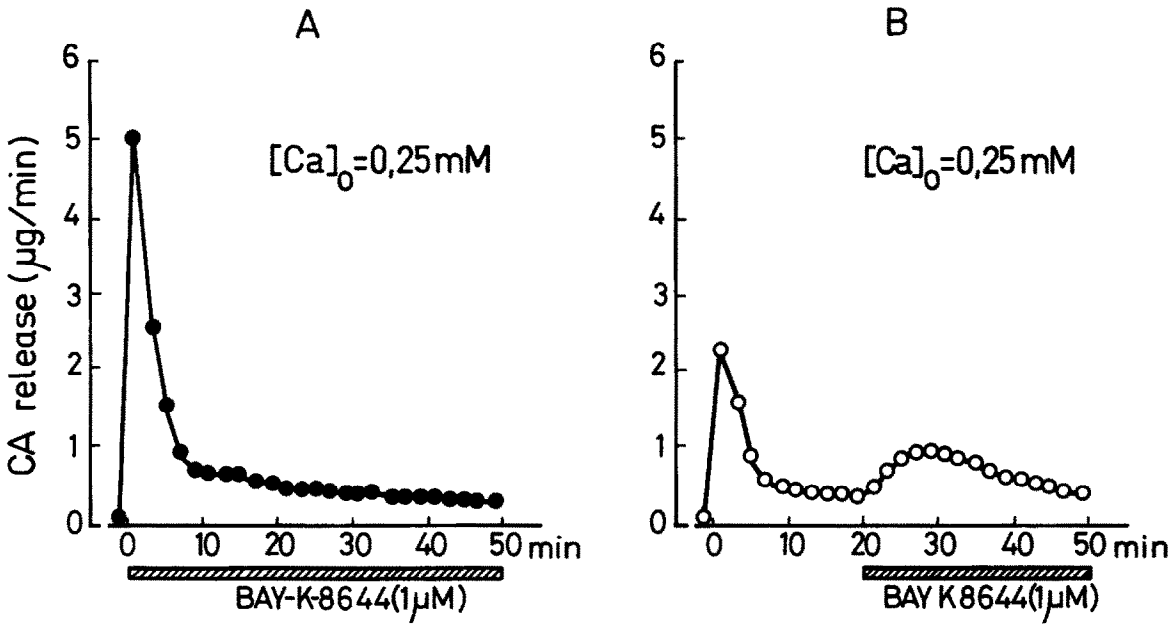

Fig.3. Catecholamine (CA) release evoked by a 50 min period of maintained depolarization (59 mM). Effect of delayed addition (20 min) of BAY-K-8644. The contralateral control gland was perfused with the drug from the beginning of depolarization. Data of both panels are the results of a typical paired experiment out of 3.

BAY-K-8644 (1 μM) enhanced the inactivated secretory response only at 0.25 mM Ca (fig.3B). This revival of catecholamine release is unlikely to be due to reactivation of previously inactivated channels because additional secretion was not seen when 2.5 mM Ca was used in the presence of BAY-K-8644 (not shown).

The overall secretion rates obtained in glands stimulated in one step (2.5 mM Ca or 0.25 mM Ca plus BAY-K-8644) or two steps (late addition of 2.5 mM Ca or 1 μM Bay-K-8644) were similar; this was true for all times elapsed between the first and second step (10, 20 or 40 min; fig.4). These experiments support the idea that Ca-channel activity remains constant throughout the depolarization period. If there were some loss of channel activity during the decline of the secretory responses, the total amounts of secreted catecholamines in glands stimulated in two steps should have been lower than the amounts secreted from paired glands stimulated in a single step, since less Ca would enter the cell during the second step to activate the

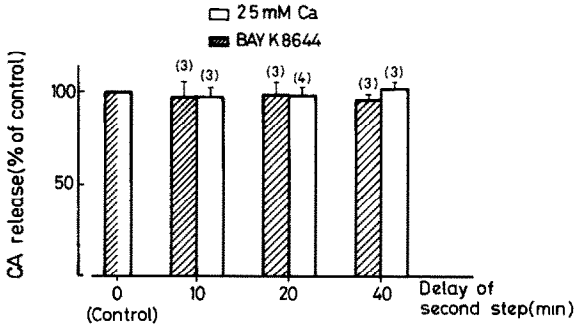

Fig.4. Total catecholamine (CA) release evoked by 59 mM K in two steps (experimental protocols as in figs 2 and 3). The first step was in 0.25 mM Ca, and the second was by adding BAY-K-8644 (1 μM) or by increasing extracellular calcium to 2.5 mM. The contralateral control glands were stimulated from the beginning under the conditions of the second step (2.5 mM Ca or 0.25 mM Ca plus 1 μM BAY-K-8644). Data are expressed as % of CA release obtained in control glands. Vertical lines are SE of the means of the number of experiments shown in parentheses.

secretory machinery. This interpretation is in line with that given by Knight and Baker [4] on the basis of experiments performed in leaky chromaffin cells where, in spite of bypassing the cell membrane, inactivation and revival of catecholamine release upon step addition of increasing concentrations of Ca was observed. Also, they agree with recent data of Burgoyne and Cheek [18] who observed sustained increases of $[Ca_i]$ after K depolarization (but see [19]).

In conclusion, these experiments show that the inactivation of the catecholamine secretory response during sustained depolarization of chromaffin cells with high K concentrations is substantially delayed when the permeant cation is Sr instead of Ca. In addition, the experiments suggest that the decline in the rate of secretion is critically dependent on Ca; this cation seems to exert a modulatory role on the secretory response at a step distal from the transmembraneous Ca channel.

ACKNOWLEDGEMENTS

This work was supported by grants from Fundación Ramón Areces, CAICYT (0626/81) and FISS, Spain. We thank Professor F. Hoffmeister for the kind gift of BAY-K-8644, Dr A.R. Artalejo and C. Montiel for useful comments, Mr Victoriano Mandado for technical assistance and Mrs Julia Romeo for typing the manuscript. The assistance of Mr Angel B.G. Moreno with illustrations is greatly appreciated.

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