Is the transient nature of the secretory response of chromaffin cells due to inactivation of calcium channels?

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Catecholamine release from chromaffin cells in response to carbamylcholine and high K^+ is transient. Monitoring intracellular free calcium ($[Ca^{2+}]_i$) using quin2 demonstrated a transient rise in $[Ca^{2+}]_i$ in response to carbamylcholine. The termination of secretion due to carbamylcholine is probably a consequence of the return of $[Ca^{2+}]_i$ to resting levels as the nicotinic receptors desensitise. Depolarisation with 55 mM K^+ led to a long-lasting rise in $[Ca^{2+}]_i$ which persisted after the secretory response had terminated. The maintained rise in $[Ca^{2+}]_i$ appeared to be due to continued opening of verapamil-sensitive Ca^{2+} channels. These results suggest that inactivation of voltage-dependent Ca^{2+} channels does not account for the transient nature of the secretory response in chromaffin cells.

Chromaffin cell *Calcium* *Calcium channel* *Secretion* *Quin2*

1. INTRODUCTION

Secretion from a wide range of cell types, in response to maintained stimulation, is transient. Cholinergic agonists and high K^+ elicit a transient release of catecholamines from adrenal medullary chromaffin cells [1–4] brought about by a rise in intracellular free calcium ($[Ca^{2+}]_i$) [5,6] as a result of Ca^{2+} influx through voltage-dependent Ca^{2+} channels [6–8]. In the case of cholinergic stimulation the transient nature of the secretory response can be attributed to desensitisation of the nicotinic acetylcholine receptors [9]. The transient release due to maintained depolarisation by high K^+ has been suggested to be due to activation followed by inactivation of Ca^{2+} channels [10]. Using the quin2 technique [11] we have found that depolarisation of bovine chromaffin cells leads to a long-lasting rise in $[Ca^{2+}]_i$ due to continued opening of verapamil-sensitive Ca^{2+} channels at a time when secretion has terminated. These results suggest that the transient nature of secretion by chromaffin cells is not simply a consequence of inactivation of voltage-dependent Ca^{2+} channels.

2. MATERIALS AND METHODS

Chromaffin cells were isolated from bovine adrenal medullae by enzymatic digestion [12] and preincubated for 1 h in a Krebs-Ringer buffer containing 3 mM $CaCl_2$ and 5 mg/ml bovine serum albumin. Catecholamine release was then examined as described previously [13] using a fluorimetric assay [14]. Total catecholamine released (adrenaline + noradrenaline) was expressed as a percentage of total cellular content. The results shown are means of duplicate determinations on cells from the same batch. Essentially the same results were obtained with three separate cell preparations.

Isolated chromaffin cells were loaded with quin2 by incubation in Krebs-Ringer buffer containing 3 mM $CaCl_2$, 5 mg/ml BSA and 10 μM quin2 acetoxymethyl ester for 30 min at room temperature. The cells were washed, resuspended in buffer without quin2 and incubated for a further 30–40 min. The cells were then washed, resuspended in buffer without BSA and fluorescence monitored at 20°C in a Perkin-Elmer

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LS5 fluorescence spectrophotometer (excitation 339 nm, emission 492 nm) fitted with a magnetic stirrer. Calibration of fluorescence following lysis with 50 μM digitonin and addition of 40 mM EGTA and correction for autofluorescence was as described [11]. Values of $[Ca^{2+}]_i$ found were similar to those of [6].

3. RESULTS AND DISCUSSION

Freshly isolated bovine chromaffin cells were stimulated by addition of 2×10^{-4} M carbamylcholine or 55 mM K^+, leading to a transient release of catecholamine (fig.1). Release due to carbamylcholine was both more extensive and longer lasting than that due to 55 mM K^+. Similar patterns of secretion have been reported previously [2–4]. If the cells were incubated with carbamylcholine or 55 mM K^+ in the absence of external Ca^{2+} for 15 min, catecholamine release was immediately initiated by readdition of 3 mM external Ca^{2+} (fig.1). In the case of 55 mM K^+ more catecholamine was released following readdition of external Ca^{2+} than in control experiments with external Ca^{2+} present throughout; the reasons for difference are unclear. The results from these experiments demonstrate that at a time when secretion would have been complete, Ca^{2+} channels remain open in the presence of carbamylcholine or 55 mM K^+ when external Ca^{2+} is absent.

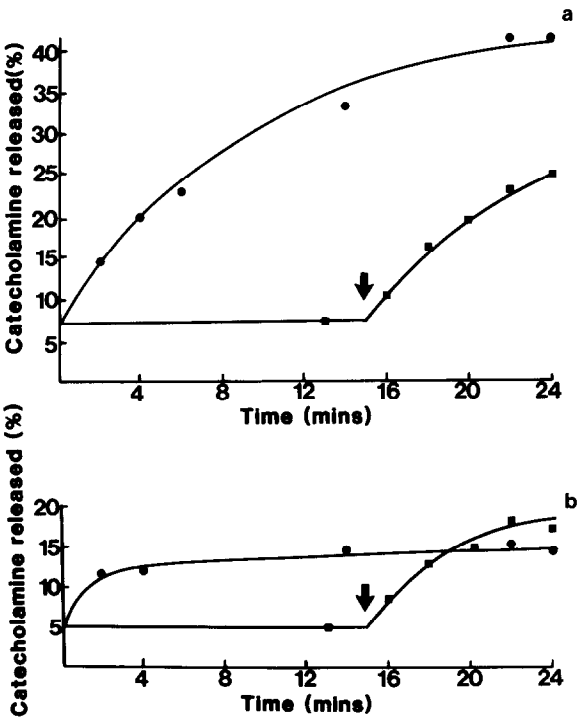

Fig.1. Catecholamine release from freshly isolated bovine chromaffin cells in response to 2×10^{-4} M carbamylcholine (a) or 55 mM K^+ (b). The level of catecholamine release was determined from cells with 3 mM external Ca^{2+} present throughout (●) or from cells incubated in the absence of external Ca^{2+} until after 15 min (arrow) when 3 mM external Ca^{2+} was added (■).

The results from Ca^{2+} readdition experiments have two possible interpretations. Either Ca^{2+} channel inactivation is Ca^{2+}-dependent or Ca^{2+} channels do not inactivate during the time examined. In order to distinguish between these two possibilities we examined $[Ca^{2+}]_i$ in chromaffin cells following stimulation and the effects of the Ca^{2+} channel blocker verapamil [15].

Addition of carbamylcholine caused an immediate rise in $[Ca^{2+}]_i$ (fig.2a) as shown previously. The rise in $[Ca^{2+}]_i$ decayed back to the original basal levels over the following 10–15 min. Prior addition of verapamil prevented the rise in $[Ca^{2+}]_i$ due to carbamylcholine (fig.2c) or if added after carbamylcholine reversed the rise in $[Ca^{2+}]_i$ (fig.2b). It should be noted that verapamil had little or no effect on basal $[Ca^{2+}]_i$. Results from experiments such as that shown in fig.2b indicate that verapamil-sensitive Ca^{2+} channels remain open during the decay of $[Ca^{2+}]_i$ to basal. The level of $[Ca^{2+}]_i$ at any time presumably reflects the net effects of Ca^{2+} influx through Ca^{2+} channels and Ca^{2+} efflux.

High (55 mM) K^+ caused an immediate rise in $[Ca^{2+}]_i$ in chromaffin cells (fig.3) to levels higher than those seen in response to 2×10^{-4} M carbamylcholine. Furthermore, the rise in $[Ca^{2+}]_i$ due to 55 mM K^+ was longer lasting. The rise in $[Ca^{2+}]_i$ due to high K^+ was prevented by prior addition of verapamil (fig.3b) and was reversed by verapamil up to 21 min (the longest time examined) after stimulation (fig.3a). These results suggest that the maintained rise in $[Ca^{2+}]_i$ elicited by depolarisation with high K^+ results from persistent opening of voltage-dependent Ca^{2+} channels.

Readdition of external Ca^{2+} to cells challenged with 55 mM K^+ or carbamylcholine in the absence of external Ca^{2+} resulted in rapid rises in $[Ca^{2+}]_i$

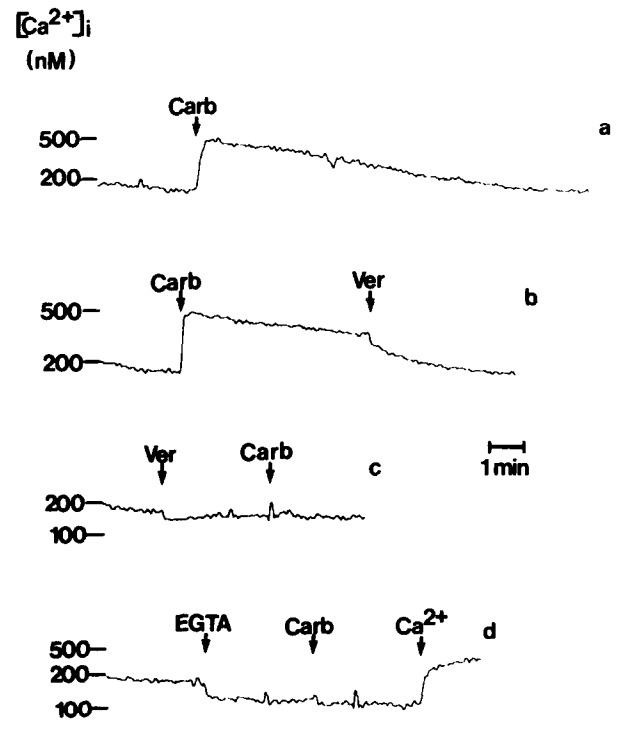

Fig.2. Changes in $[Ca^{2+}]_i$ in chromaffin cells in response to addition of 2×10^{-4} M (final concentration) carbamylcholine (Carb) and effects of verapamil (Ver). (a) Response to addition of carbamylcholine. (b) Response to addition of carbamylcholine followed by 500 μM verapamil. (c) Response to carbamylcholine after prior addition of 500 μM verapamil. (d) Response to carbamylcholine in medium made free of external Ca^{2+} by addition of 3 mM EGTA, followed by readdition of 3 mM $CaCl_2$.

(fig.2d,3c). This is consistent with the results showing catecholamine release following readdition of external Ca^{2+}. Readdition of external Ca^{2+} in the absence of carbamylcholine or high K^+ did not result in a rise in $[Ca^{2+}]_i$ beyond the original basal level in the presence of external Ca^{2+}.

The results presented here from quin2 experiments, and from an examination of catecholamine release following readdition of external Ca^{2+} to cells stimulated in its absence, suggest that Ca^{2+} channels remain open, in the presence or absence of external Ca^{2+}, at times when the secretory response has terminated. In the case of depolarisation with high K^+, in particular, the termination of secretion cannot be explained by inactivation of Ca^{2+} channels since these remain

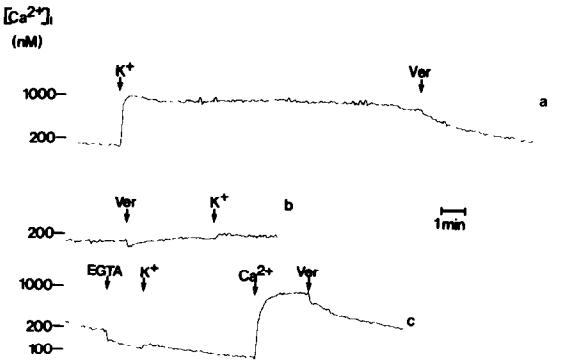

Fig.3. Changes in $[Ca^{2+}]_i$ in chromaffin cells in response to depolarisation by 55 mM K^+ and effects of verapamil (Ver). (a) Response to 55 mM K^+ followed by verapamil 14 min later. (b) Response to 55 mM K^+ after prior addition of verapamil. (c) Response to 55 mM K^+ in medium made free of external Ca^{2+} by addition of 3 mM EGTA, followed by readdition of 3 mM $CaCl_2$.

open for at least 20 min following K^+ addition with a maintained increase in $[Ca^{2+}]_i$. Previous studies [2,4], in which $^{45}Ca^{2+}$ uptake was monitored attributed the decrease in net uptake after the first few minutes of stimulation to inactivation of Ca^{2+} channels. These results could instead be explained as being due to the establishment of a new steady state in which there is no net uptake but the increased $[Ca^{2+}]_i$ is maintained at a level reflecting the balance of calcium influx and efflux.

The decrease in $[Ca^{2+}]_i$ due to verapamil is consistent with continued opening of Ca^{2+} channels. However, we cannot completely rule out a small degree of inactivation of Ca^{2+} channels. The results presented are not peculiar to verapamil since similar results were obtained with the alternative blocker nitrendipine. It should be noted that the present results are consistent with patch-clamp data [7] on Ca^{2+} channels in chromaffin cells which showed that there is probably no voltage-dependent inactivation of their Ca^{2+} channels.

The transient nature of the secretory response elicited by carbamylcholine may be a consequence of the return of $[Ca^{2+}]_i$ to basal levels following nicotinic acetylcholine receptor desensitisation [9] and closure of voltage-dependent Ca^{2+} channels. The termination of secretion despite maintained depolarisation by high K^+ is obviously independent of receptor desensitisation and does not ap-

pear, on the basis of the present results, to be a consequence of inactivation of verapamil-sensitive Ca^{2+} channels. An alternative explanation is required to account for this transient release of catecholamines. Such an explanation must also be able to account for the fact that 55 mM K^+ releases much less catecholamine than does 2×10^{-4} M carbamylcholine despite a rise in $[Ca^{2+}]_i$ to a similar or even higher level. One possible explanation is that for sustained secretion a signal other than the rise in $[Ca^{2+}]_i$ is required. We have previously presented evidence consistent with the presence of an additional signal generated in response to carbamylcholine [6,13]. Alternatively, the high $[Ca^{2+}]_i$ in cells depolarised by high K^+ may inactivate some aspect of the exocytotic mechanism.

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