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DENSITY OF APAMIN-SENSITIVE Ca^{2+}-DEPENDENT K^+ CHANNELS IN BOVINE CHROMAFFIN CELLS: RELEVANCE TO SECRETION

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Abstract—Three objectives were defined when planning this study: (i) to identify binding sites for [^{125}I]-apamin in intact bovine adrenal medulla chromaffin cells and to estimate their density and selectivity; (ii) to determine whether apamin modified the release of catecholamines evoked by brief pulses of dimethylphenylpiperazinium (DMPP, 1 or 5 μM for 10 sec), histamine (10 μM for 10 sec) or high K^+ (20, 35 or 70 mM for 10 sec) applied to superfused cells; and (iii) to test whether apamin affected the profiles of the changes in cytosolic Ca^{2+} concentrations $[Ca^{2+}]_i$ obtained in suspensions of cells loaded with fura-2 and stimulated with DMPP or histamine. At equilibrium, increasing concentrations of [^{125}I]-apamin gave a saturation curve whose Scatchard transformation produced a K_d of 132 pM and a B_{max} of 0.72 fmol/10^6 cells. Quinine, tetraethylammonium, charybdotoxin or glibenclamide (blockers of various subtypes of K^+ channels) did not inhibit [^{125}I]apamin binding. Binding was blocked by apamin and by *d*-tubocurarine, two blockers of small-conductance Ca^{2+}-activated K^+ channels (SK channels). The number of binding sites for [^{125}I]apamin amounted to approx. 900 per single chromaffin cell, 0.72 sites per μm^2 surface area. Apamin (1 μM) enhanced the secretory response to histamine (10 μM), DMPP (1 or 5 μM) and high K^+ (20 or 35 mM) by 2–3-fold. The response to 70 mM K^+, however, was unaffected. Apamin also enhanced the peak $[Ca^{2+}]_i$ increase produced by DMPP or histamine by approx. 30%. Overall, these results strongly support the hypothesis that under physiological conditions, SK channels control some of the electrical activity of chromaffin cells and indirectly, the opening of voltage-dependent Ca^{2+} channels, the access of Ca^{2+} to the secretory machinery and the rate of catecholamine release to the circulation from the intact adrenal gland.

Key words: Ca^{2+}-dependent K^+ channels; catecholamine release; apamin binding sites; bovine chromaffin cells

In addition to large-conductance Ca^{2+}-dependent K^+ channels (BK‖ channels) [1], bovine chromaffin cells have also recently been demonstrated to contain small-conductance, Ca^{2+}-activated K^+ channels (SK channels) [2]. An outward K^+ current through SK channels is activated when cytosolic Ca^{2+} concentrations $[Ca^{2+}]_i$ are increased as a result of stimulation of bovine chromaffin cells with histamine; this current is blocked by apamin and causes hyperpolarization of such cells. Therefore, it was suggested that SK channels could contribute to the regulation of plasmalemmal electrical activity, the opening of voltage-dependent Ca^{2+} channels and Ca^{2+}-dependent exocytotic release of catecholamines [2].

To assess the relative importance of SK channels in the control of cell electrical activity in relation to other ionic currents, their density per unit area of chromaffin cell surface should be determined. This is now possible because [^{125}I]apamin, an octadecapeptide found in the venom of the honey bee [3], is commercially available. Being a hydrophilic peptide and having a very high specific activity, it could behave as an excellent radioligand in determining the density and characteristics of SK channels in primary cultures of living bovine adrenal chromaffin cells. This constitutes the first objective of this study.

The second objective relates to the hypothesis that SK channels might regulate $[Ca^{2+}]_i$ and secretory response in bovine chromaffin cells. This seems to be true in the perfused cat adrenal gland, where a healthy secretory response is observed upon stimulation of its medullary chromaffin cells with the muscarinic agonist methacholine. Such a response was potentiated by apamin and *d*-tubocurarine (another blocker of SK channels), suggesting that SK channels were regulating the muscarinic receptor-mediated secretory process [4]. However, bovine chromaffin cells lack a muscarinic-mediated secretory response [5]. This was why in a previous report we used histamine to characterize an outward K^+ current

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‖ Abbreviations: DMPP, dimethylphenylpiperazinium iodide; BK channels, large-conductance Ca^{2+}-dependent K^+ channels; SK channels, small-conductance Ca^{2+}-activated K^+ channels; DMEM, Dulbecco's modified Eagle's medium.

through SK channels [2]. Histamine has been shown to be a powerful secretagogue in bovine chromaffin cells, causing both release of Ca^{2+} from internal stores and Ca^{2+} entry from the extracellular medium [6–8]. On the other hand, information is also lacking on whether SK channels might be involved in the physiological nicotinic receptor-mediated secretory response. Therefore, we also performed experiments to analyse the effects of apamin on the changes in $[Ca^{2+}]_i$ and catecholamine release responses mediated by nicotinic or histamine receptor stimulation, as well as on secretion evoked by direct depolarization of superfused bovine chromaffin cells with high K^+ concentrations.

MATERIALS AND METHODS

Isolation and culture of chromaffin cells. Mixed populations of bovine adrenal medulla chromaffin cells were prepared according to Moro *et al.* [9]. For secretion experiments, 5×10^6 cells were plated on 5 cm diameter Petri dishes (Falcon) and incubated in DMEM supplemented with 5% fetal calf serum, penicillin G (50 U/mL), streptomycin (50 μg/mL), gentamycin (30 μg/mL), and 10 μM cytosine arabinoside. For the [^{125}I]apamin binding experiments, cells were plated at a density of 1×10^6 cells/well in 24 multiwell Costar plates, and used 24–48 hr thereafter. For secretion experiments, cells were maintained 1–4 days before the experiment in a water-saturated incubator in an atmosphere of 5% CO_2/95% air at 37°. Medium was changed 24 hr later with fresh DMEM. Viability of the cells (usually greater than 90%) was estimated by Trypan blue exclusion.

Binding of [^{125}I]*apamin to intact cells.* [^{125}I]apamin equilibrium binding was performed in 24-well plates containing 1×10^6 cells/well. For equilibrium binding, cells were first washed twice for 10 min at 4° with 1-mL aliquots of binding buffer with the following composition (mM): 140 NaCl/5.4 KCl/2.5 $CaCl_2$/0.8 $MgSO_4$/10 glucose/25 HEPES, 0.25% BSA, 0.5% sodium azide, adjusted to pH 7.2 [10]. The cells were then incubated at 4° for 90 min with 1 mL Krebs–HEPES containing increasing concentrations of [^{125}I]apamin. In every experiment, each concentration of ligand was assayed in duplicate; non-specific binding was determined in the presence of 100 nM unlabelled apamin. Incubation was stopped by quickly washing the cells twice with fresh binding buffer solution. The cells were then collected in 0.5 mL of 10% trichloroacetic acid and their radioactivity counted in a γ-counter. Specific binding was estimated by subtracting non-specific from total binding and accounted for 85–90% of total binding. For displacement experiments, similar conditions were used. A fixed concentration of [^{125}I]apamin was used (50 pM) in the presence of increasing concentrations of the displacing agent. Binding in the presence at a given concentration of the displacing agent was expressed as a percentage of the total binding obtained in its absence (control). Analysis of experimental equilibrium data was performed with the LIGAND non-linear least squares computer program.

Measurements of catecholamine release. Five million cells (24–48 hr after plating) were transferred from the Petri dish to a microchamber (200 μL volume) and trapped with glass wool. In each individual experiment, cells were initially superfused with normal Krebs–HEPES solution for 15 min at room temperature (22–25°). Thereafter they were briefly (10 sec) and repetitively stimulated with several pulses of histamine, DMPP or high K^+ at the final concentrations indicated in Results and in the figure legends. Exchange of solutions was achieved by means of electronic valves placed near the superfusion microchamber.

Catecholamine release evoked by these three secretagogues was measured in the fluid emanating from the microchamber by two different methods. In the first, a perfusion rate of 4 mL/min was used and samples were continuously collected at 10-sec intervals in acidified tubes (0.05 M perchloric acid, final concentration); the total amount of catecholamines present in each sample was fluorimetrically measured [11]. The total catecholamine secretion evoked by each stimulus, expressed as ng/pulse, was calculated by adding the amines released during the pulse-sample to those collected in the subsequent 2 min samples. In the second method, the perfusate emanating from the chamber at a rate of 3 mL/min was passed through a Bio-Analytical System LC-4B electrochemical detector. A voltage of +0.65 V was applied to a glassy carbon working electrode versus an Ag/AgCl reference electrode. The oxidation current was continuously recorded onto a Houston Omniscribe polygraph. The detector was calibrated by perfusing known concentrations of adrenaline and noradrenaline; 100 nA were equivalent to 585 ng of adrenaline at a flow rate of 3 mL/min. The fluorimetric results were expressed as the net catecholamine release (after subtracting basal secretion) induced by the stimulus in the 2 min immediately after, in the absence or presence of apamin. The catecholamines released and detected electrochemically were expressed as the relation between the peak height evoked by each stimulus in the presence or absence of the toxin, considering that control values were normalized to 100%.

Measurements of $[Ca^{2+}]_i$. Chromaffin cells (24–48 hr after plating) were washed twice with Krebs–Tris buffer having the following composition (mM): 144 NaCl/5.9 KCl/1.2 $MgCl_2$/2.5 $CaCl_2$/11 glucose/10 Tris, pH 7.4. The cells (4×10^6/mL) were then incubated in Krebs–Tris buffer containing 2 μM fura-2/AM for 30 min at 37°. Next, they were washed twice with fresh Krebs–Tris and kept at room temperature. Fluorescence measurements were carried out in 2×10^6 cell aliquots in 2 mL buffer with continuous stirring at room temperature. Fluorescence was measured in a Perkin–Elmer LS-50 spectrofluorimeter, which allowed rapid alternation between 340 and 380 nm of excitation wavelengths. Emitted fluorescence was measured at 510 nm. $[Ca^{2+}]_i$ was estimated from the rate of the fluorescence values excited at 340 and 380 nm [12]. After estimation of basal $[Ca^{2+}]_i$ the cells were stimulated with DMPP (5 μM) or histamine (10 μM) for 10 min. When present, apamin (1 μM) was added 5 min before and during the stimulation period.

Materials. [^{125}I]apamin (specific activity 2.200 Ci/

mmol) was obtained from Amersham (Amersham, U.K.); D-tubocurarine was from Sigma (Madrid, Spain); apamin was from RBI (U.S.A.); charybdotoxin was from Alamone Labs (Jerusalem, Israel); collagenase was from Boehringer Mannheim (Mannheim, Germany); DMEM and fetal calf serum were from GIBCO; and Percoll was from Pharmacia. All other chemicals were of analytical grade from Sigma or Merck.

RESULTS

Binding of [^{125}I]apamin to intact bovine chromaffin cells.

Initial experiments were directed towards the definition of the quality of the signal for [^{125}I]apamin binding to intact bovine chromaffin cells. For instance, incubation of 10^6 cells in the presence of 100 pM [^{125}I]apamin in 1 mL binding medium for 90 min at 4° gave a value of 604 ± 22 cpm for total binding and 63 ± 8 cpm for non-specific binding (N = 7 wells containing cells from different cultures). Thus, specific binding amounted to 90% of total binding; the signal-to-noise ratio was very high.

Incubation of 10^6 cells with increasing concentrations of [^{125}I]apamin (5–500 pM) for 90 min gave binding curves that saturated at 400–500 pM (Fig. 1). Scatchard plotting of this saturation isotherm (inset) gave a K_d of 132 ± 33 pM and a B_{max} of 0.72 ± 0.13 fmol/10^6 cells (N = 5 experiments performed in duplicate with different batches of cells). The Hill coefficient was 1.03, suggesting the presence of a single and homogeneous population of binding sites.

Displacement experiments were also carried out with several compounds known to block various subtypes of K^+ channels. For example, *d*-tubocurarine is known to inhibit Ca^{2+}-dependent K^+ conductance in bullfrog sympathetic ganglion cells [13], an outward K^+ current induced by Ca^{2+} injections in mouse neuroblastoma × rat glioma hybrid NG108–15 cells [14] and Ca^{2+} mediated K^+ efflux from guinea-pig hepatocytes [15]. Because of its ability to inhibit after-hyperpolarization following an action potential, known to be due to activation of SK channels, it seems to be a quite specific blocker of such channels [13, 16]. D-Tubocurarine inhibited the binding of [^{125}I]apamin to intact chromaffin cells in a concentration-dependent manner. Inhibition of binding was 30% at 10 μM and 65% at 100 μM. These concentrations are in the range of those required for *d*-tubocurarine to potentiate the muscarinic secretory response in the perfused cat adrenal gland; 100 μM *d*-tubocurarine enhanced the catecholamine secretory response [4] by approx. 3-fold. Cold apamin displaced the binding of [^{125}I]-apamin with a K_i of 3 nM.

Quinine, a less selective agent which blocks BK as well as SK channels [15, 16], did not affect the binding of [^{125}I]apamin; some inhibition of binding was observed only at the highest concentrations used (100 μM). Tetraethylammonium, which blocks BK channels in bovine chromaffin cells [17], in NG108-15 cells [14] and in rat sympathetic neurones [18] did not modify the binding of [^{125}I]apamin to chromaffin cells at all; neither did the selective BK channel blocker charybdotoxin [16] affect such binding. Finally glibenclamide, a blocker of ATP-gated K^+ channels [19] did not inhibit the binding of [^{125}I]apamin.

Density of [^{125}I]apamin binding sites in intact bovine chromaffin cells

Table 1 summarizes calculations performed to determine the number of binding sites for [^{125}I]-apamin in intact chromaffin cells. From the Avogadro

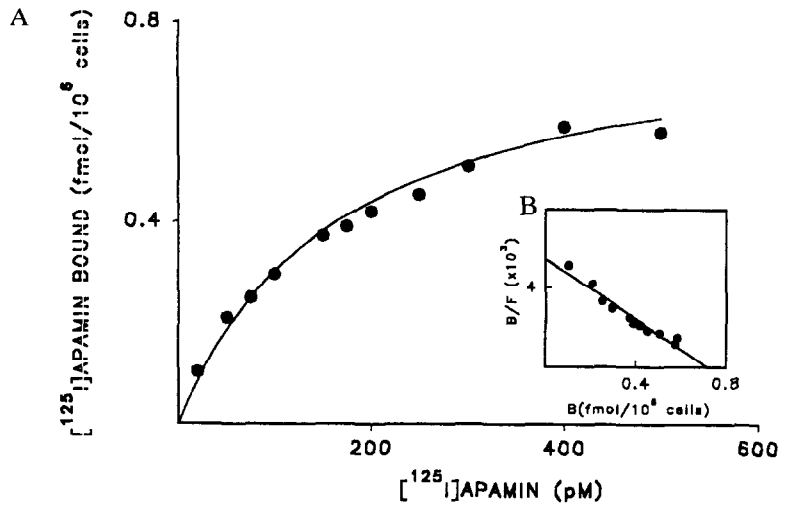

Fig. 1. Equilibrium binding of [^{125}I]apamin to intact isolated bovine adrenal chromaffin cells after 1–3 days in culture (see binding conditions in Materials and Methods). Inset: Scatchard plot of the saturation binding data shown in A (r = 0.98); B, [^{125}I]apamin bound; F, free [^{125}I]apamin concentration. Data are means from three experiments performed in duplicate with different cell cultures.

Table 1. Density of apamin binding sites in bovine adrenal chromaffin cells

B_{max} for [^{125}I]apamin (fmol/10^6 cells)	0.72
Cell diameter (μm)	20
Cell surface area (μm^2)	1,256
Number of [^{125}I]apamin binding sites per cell	452
Number of [^{125}I]apamin binding sites per μm^2 surface area	0.36
Mean capacitance of a bovine chromaffin cell (pF)	18.5
Surface of a chromaffin cell considering 1.08 $\mu F/cm^2$	1,713
Number of [^{125}I]apamin binding sites per μm^2 surface area	0.26

Two modes of calculations were performed. In the first, the chromaffin cell was considered as a sphere with a cell diameter of 20 μm. In the second, the capacitance of chromaffin cells was used to estimate their surface area.

number, the value of 0.72 fmol/10^6 cells comes to 452 sites per single cell. Considering an average diameter for bovine chromaffin cells of 20 μm [20] and taking chromaffin cells as spheres, the surface of a single cell comes to 1,256 μm^2. Therefore, the density of [^{125}I]apamin binding sites per μm^2 cell surface is 0.36.

Similar calculations were performed by measuring the capacitance of 155 bovine chromaffin cells using the path-clamp technique [2]. The mean capacitance of those cells was 18.5 ± 0.3 pF. Taking an average of 1.08 $\mu F/cm^2$, the surface of a single chromaffin cell comes to 1,713 ± 277 μm^2. Thus, the number of [^{125}I]apamin binding sites per μm^2 surface area is 0.26, a figure close to that obtained using a cell diameter of 20 μm.

Effects of apamin on catecholamine release from superfused bovine chromaffin cells

When bovine chromaffin cells were stimulated with histamine (10 μM, 10 sec), a healthy secretory response was evoked (Fig. 2A). Such a response was maximal during the 10-sec stimulation period (117.5 ng/10 sec) and returned to basal levels 2 min later. Two further histamine pulses induced new secretory reponses approx. 25% lower than the previous one. Thus, some desensitization to the histamine secretory response was evident. When apamin (1 μM) was present 3 min before, during and 2 min after the histamine pulse, a clear potentiation of secretion was obtained. Thus, apamin transformed the desensitization to histamine into an increasing potentiation of secretion (Fig. 2B). The net catecholamine release, after subtracting basal secretion, was 203 ng/2 min during the second pulse of histamine. In apamin-treated cells, net secretion was 401 ng/2 min. Application of DMPP (5 μM for 10 sec) after the three histamine pulses produced a sharp increase in net catecholamine secretion (351 ng/2 min, right side of Fig. 2A). In the second group of cells, the net secretory response was 648 ng/2 min when DMPP was applied in the presence of apamin (1 μM). Measurements of the net secretory responses induced by DMPP in seven different experiments gave a value of 462 ± 185 ng/2 min for the control cells, and 809 ± 226 ng/2 min for the apamin-treated cells ($P < 0.01$).

The effect of apamin was concentration-dependent, as shown in Fig. 3A. In these experiments, secretion was monitored on-line using an electrochemical detector. Pulses of histamine (10 μM, 10 sec) were applied at 5-min intervals. The presence of apamin (0.1 or 1 μM) 3 min before and during the pulse increased the histamine response to 145 ± 10% ($N = 4$; $P < 0.005$) and 191 ± 33% ($N = 4$; $P < 0.05$), respectively. A similar experiment was performed with DMPP pulses (5 μM, 10 sec). An increase to 137 ± 13% ($N = 4$; $P < 0.01$) and 182 ± 13% ($N = 4$; $P < 0.001$) over the control secretion was obtained in the presence of 0.1 and 1 μM apamin, respectively (Fig. 3B). When 1 μM DMPP was used instead of 5 μM, a secretory response 20–25 times lower was obtained. However, the enhancement of such DMPP-induced secretory responses by apamin was 220 ± 20% ($N = 3$; $P < 0.01$) in the presence of 0.1 μM apamin, and 336 ± 21% ($N = 3$; $P < 0.005$) in the presence of 1 μM apamin (Fig. 3C).

To analyse the effects of apamin on catecholamine secretion evoked by direct depolarization of bovine chromaffin cells, three different high K^+ solutions (osmotically adjusted through equimolar reduction of NaCl) were assayed (Figs 4A, B and C). An inverse correlation between the concentration of K^+ employed and the potentiation of secretion by apamin was observed. Using a mild depolarizing stimulus (20 mM K^+), apamin (1 μM) increased secretion to 192 ± 6% ($N = 4$; $P < 0.001$). Such potentiation was slightly less when 35 mM K^+ was employed (170 ± 17%; $N = 3$). Secretory response to 70 mM K^+ was hardly affected by apamin.

Effects of apamin on $[Ca^{2+}]_i$ changes induced by histamine or DMPP

In suspensions of chromaffin cells loaded with fura-2, basal $[Ca^{2+}]_i$ was approx. 100 nM. Spontaneous deviations from this value were not observed during a 15-min recording period. Addition of histamine (10 μM) induced a sharp increase in $[Ca^{2+}]_i$ which reached a peak at 600 nM in 10 sec (Fig. 5A). After the peak, the $[Ca^{2+}]_i$ slowly declined to a plateau at approx. 200 nM. Apamin itself (1 μM) did not modify basal $[Ca^{2+}]_i$. The response to histamine seemed to be modified by apamin in two ways: the $[Ca^{2+}]_i$ peak rose slightly above the control peak to approx. 800 nM while the declining phase was delayed, ending at a plateau of approx. 300 nM. Although peak $[Ca^{2+}]_i$ increased to 127 ± 8% of control in

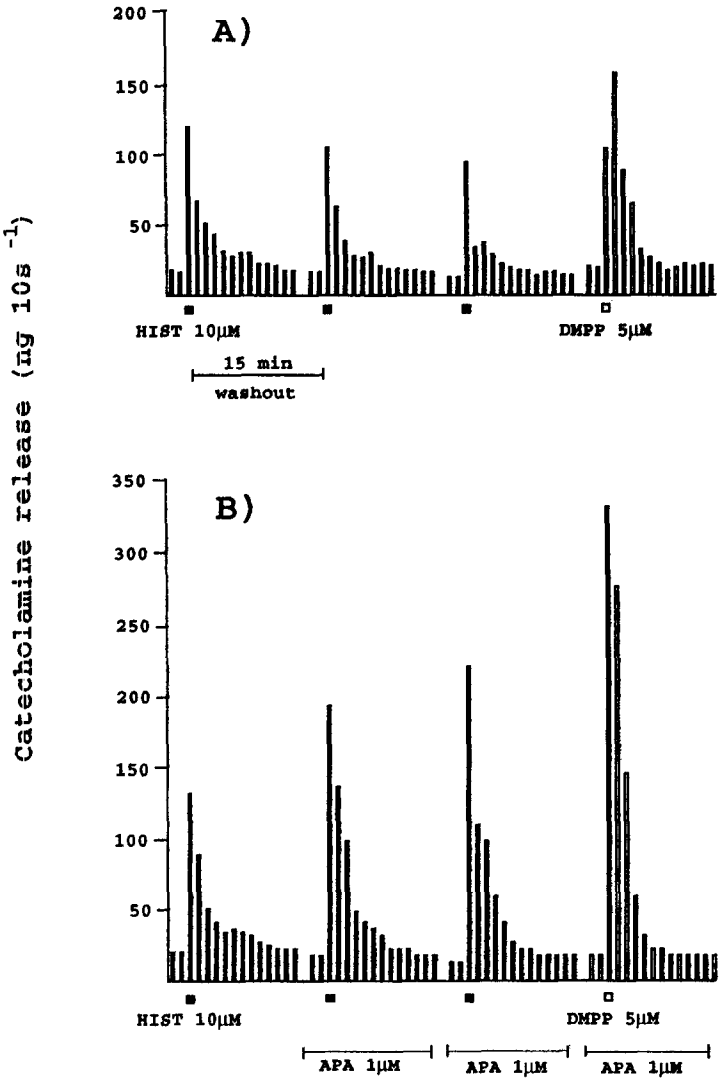

Fig. 2. Fluorimetric estimation of catecholamine release (ng/10 sec) evoked by histamine or DMPP. (A) Control secretion obtained during the 2 min period following the application of histamine pulses (10 μM, 10 sec), given 15 min apart, to 5×10^6 superfused bovine chromaffin cells. At the end of the experiment, a fourth pulse of DMPP (5 μM, 10 sec) was applied. (B) Using a similar protocol, except that apamin (1 μM) was present 3 min before, during and 2 min after the second and third pulses of histamine; apamin was also present during the DMPP pulse. Each column represents a 10-sec collection sample. Results are from one original experiment out of five.

three experiments, this difference was not statistically significant.

DMPP (5 μM) also induced a sharp increment in $[Ca^{2+}]_i$ to a peak of approx. 600 nM. In eight experiments, the net increase of $[Ca^{2+}]_i$ above basal values was 649 ± 109 nM in the absence, and 864 ± 129 nM in the presence of 1 μM apamin. After normalizing the control values to 100%, the increments induced by DMPP in the presence of apamin amounted to $135 \pm 7\%$ ($N = 8$; $P < 0.01$).

DISCUSSION

We have presented new information concerning the density of Ca^{2+}-dependent SK channels in single bovine adrenal medulla chromaffin cells, and their role in modulating the changes in $[Ca^{2+}]_i$ and the release of catecholamines triggered by the stimulation of such cells with various secretagogues. These data have been obtained through the use of [^{125}I]apamin as a selective tool to label SK channels in intact cells, and cold apamin to define its effects on the release of catecholamines and on changes in $[Ca^{2+}]_i$ in chromaffin cells.

The selection of apamin was based on reported data attesting to its ability to selectively block the SK channels of rat sympathetic neurones [21], cultured rat skeletal muscle fibres [22] and

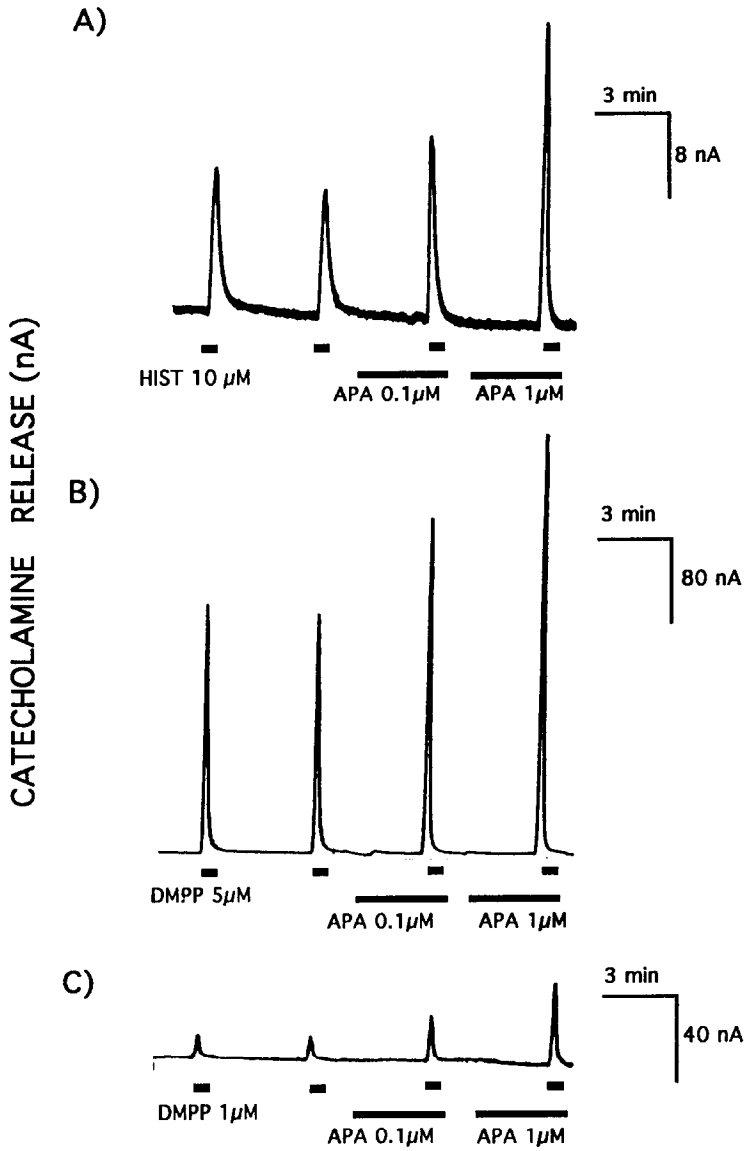

Fig. 3. On-line electrochemical monitoring of catecholamine release (nA) induced by histamine (10 μM, 10 sec) or DMPP (5 or 1 μM, 10 sec) pulses, in the absence or presence of apamin (0.1 or 1 μM). Pulses were applied every 5 min. Apamin was present 3 min before and during the pulse. Panels A, B and C show the original traces obtained in one experiment out of four, three and three, respectively.

hepatocytes [15, 23]. Thus, the presence of saturable binding sites for [^{125}I]apamin in bovine chromaffin cells support our recent electrophysiological studies demonstrating the presence of SK channels in such cells [2]. The specificity of apamin in blocking SK channels is strengthened given that apamin is unable to block BK channels in rat skeletal muscle [24], bull frog sympathetic neurones [25] and GH_3 rat anterior pituitary cells [26], and that charybdotoxin, a selective blocker of BK channels [16], did not affect the binding of [^{125}I]apamin to chromaffin cells. The lack of effect of TEA, glibenclamide and quinine on such binding further strengthens the view that [^{125}I]apamin selectively recognizes SK channels, but not other K^+ channels identified in bovine chromaffin cells [1, 17].

The K_D for apamin binding in bovine chromaffin cells agrees with values found in other cells [27], although the calculated number of SK channels (452 channels per cell) seems to be rather low in comparison with other ionic channels. For example, dihydropyridine-sensitive voltage-dependent L-type Ca^{2+} channels are present at a 4-fold higher density (approx. 2000 per cell) [28], and ω-conotoxin GVIA-sensitive Ca^{2+} channels at approx. 18,000 channels per cell [29, 30]. This difference is still more notorious if comparisons are made with the number of Na^+–K^+ pump sites, approx. 150,000 transport molecules

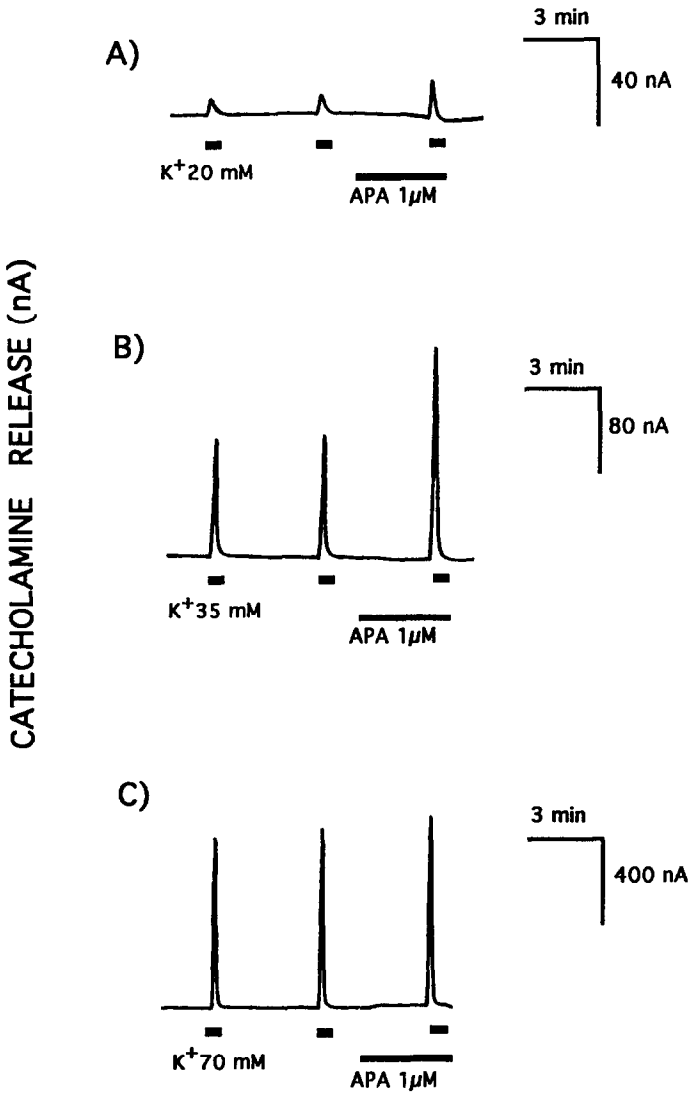

Fig. 4. Electrochemical detection of catecholamine release evoked by direct depolarization of the cells with high K^+ solutions. Ten-second pulses of high K^+ (20, 35 and 70 mM) were applied to three different batches of cells every 5 min in the absence or presence of 1 μM apamin. Panels A, B and C in the figure represent typical experiments out of four, three and three, respectively.

per single chromaffin cell [28]. But in spite of such low density, it is clear that the activation of SK channels, when $[Ca^{2+}]_i$ rises, causes a substantial hyperpolarization in bovine chromaffin cells stimulated with histamine [2].

Ca^{2+}-activated K^+ channels seem to serve as a link between changes in $[Ca^{2+}]_i$ and the electrical activity of excitable cells. It has been proposed that they mediate the hyperpolarization produced by noradrenaline in guinea-pig stomach smooth muscle [31]. This response is similar to the transient hyperpolarization occurring in GH_3/GH_4 pituitary cells on application of thyrotropin-releasing hormone [32] and would be equivalent to the increase in membrane potential that appears after the application of histamine to bovine chromaffin cells [2]. Apamin-sensitive SK channels have also been involved in the after-hyperpolarization that follows an action potential in many excitable cells [21, 24–26, 33]. In some of these cells, such as sympathetic neurones [21] and GH_3 cells [33], the blockade of SK channels with apamin increases the rate of action potential firing.

A similar mechanism could explain the effect of apamin in bovine chromaffin cells. An increased firing of action potentials in the presence of apamin will enhance the opening probability of voltage-dependent Ca^{2+} channels, thereby causing greater increments of Ca^{2+} entry, larger $[Ca^{2+}]_i$ signals and greater secretory responses. This might constitute the ultimate underlying mechanism for the potentiation by apamin of the release of catecholamines triggered by DMPP, histamine or high K^+.

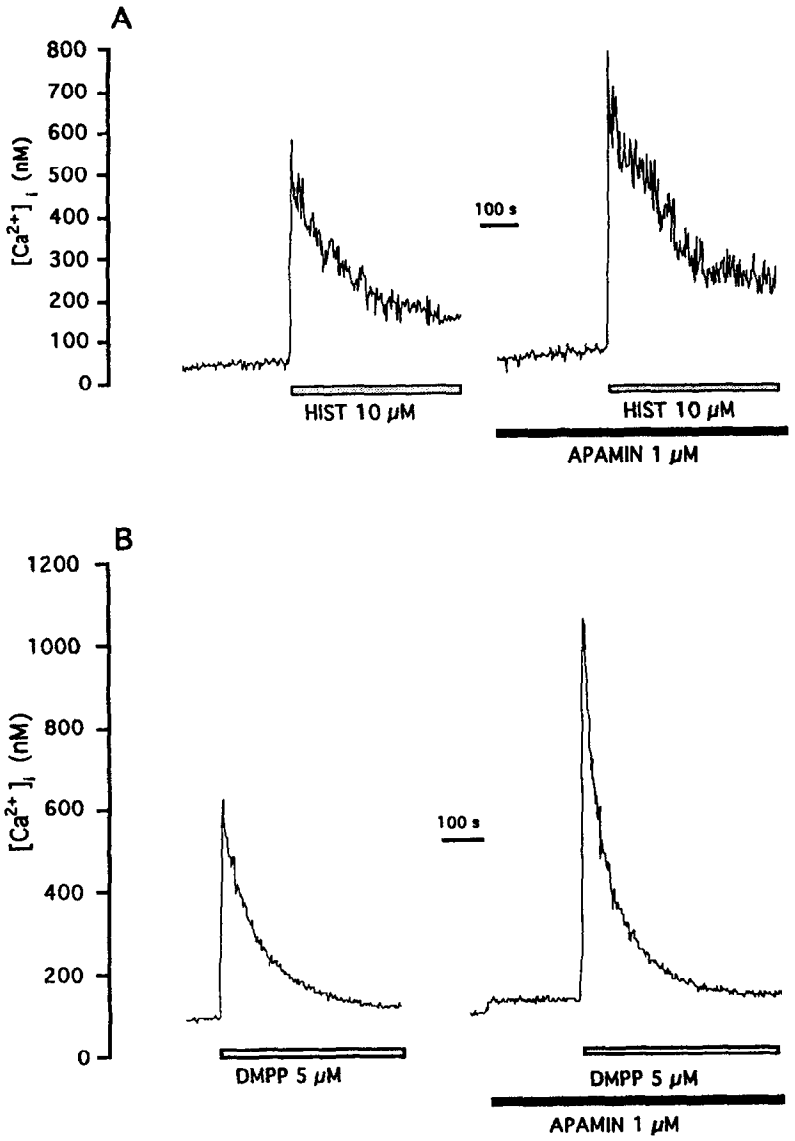

Fig. 5. Changes in cytosolic Ca^{2+} concentrations $[Ca^{2+}]_i$ in suspensions of bovine chromaffin cells loaded with fura-2 (see Materials and Methods for details). Histamine, DMPP and apamin were added to the cuvette containing the cells at the concentrations and time-periods shown by the horizontal bars at the bottom of each trace. Traces represent single experiments. Averaged results from various experiments are given in the text.

However, the potentiation by apamin of secretion seems to be more efficient than the potentiation of the $[Ca^{2+}]_i$ increase. For example, the nicotinic receptor-mediated secretory response was enhanced 2–3-fold while $[Ca^{2+}]_i$ was increased by only 30%. Still more striking was the comparison of the changes in these two parameters in the case of histamine; the $[Ca^{2+}]_i$ signal was not significantly changed yet the secretory response was enhanced at least 2-fold. This apparent dissociation between these parameters might have two possible explanations. One relates to the fact that changes in $[Ca^{2+}]_i$ and secretion can be fitted with a co-operative function while the total amount of secretion is a steep function of $[Ca^{2+}]_i$ [34]. Thus, at a certain range of $[Ca^{2+}]_i$, a small increment in cytosolic Ca^{2+} might lead to a drastic potentiation of secretion. It is also possible that apamin produces more drastic changes in $[Ca^{2+}]_i$ but at local sites in the cell, near exocytotic subplasmalemmal hot spots [35] where Ca^{2+} channels controlling secretion might be preferentially located [36, 37]. These local changes cannot be detected with the fura-2 technique which measures averaged whole-cell $[Ca^{2+}]_i$ changes.

Finally, it is worth considering the fact that apamin potentiated the responses to the three secretagogues

used, suggesting that SK channels were equally activated in cells stimulated with DMPP, histamine or moderate high K^+ concentrations. Secretion of catecholamines is triggered by Ca^{2+} entry through Ca^{2+} channels in the case of nicotinic receptor stimulation or high K^+ [38]. In addition to this Ca^{2+} entry pathway, histamine also causes the mobilization of Ca^{2+} from internal stores [6–8]. The recruitment of SK channels by such disparate stimuli may be explained by (1) the high affinity of SK channels for Ca^{2+} which may be fully activated even after small increments in $[Ca^{2+}]_i$ from whatever source [2, 16]; and (2) the fact that these secretagogues, including histamine, produce fast and considerable transients of cytosolic Ca^{2+}.

In conclusion, we have demonstrated here that a single bovine adrenal chromaffin cell contains approx. 450 binding sites for [^{125}I]apamin, which are likely to correspond to SK channels. In addition, we have shown that apamin considerably potentiated the secretory responses evoked by nicotinic and histamine receptor stimulation, as well as those evoked by moderate elevations in K^+. The changes in $[Ca^{2+}]_i$ in apamin-treated cells seem to be milder than those seen with secretion. Overall, these results strongly support the hypothesis that under physiological conditions, SK channels control the electrical activity of chromaffin cells, the access of Ca^{2+} to the secretory machinery and the rate of catecholamine release to the circulation.

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