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ORIGINAL ARTICLE

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Multiple calcium channel subtypes in isolated rat chromaffin cells

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Abstract By using the whole-cell configuration of the patch-clamp technique we have investigated the pharmacological properties of Ca^{2+} channels in short-term cultured rat chromaffin cells. In cells held at a membrane potential of −80 mV, using 10 mM Ba^{2+} as the charge carrier, only high-voltage-activated (HVA) Ca^{2+} channels were found. Ba^{2+} currents (I_{Ba}) showed variable sensitivity to dihydropyridine (DHP) Ca^{2+} channel agonists and antagonists. Furnidipine, a novel DHP antagonist, reversibly blocked the current amplitude by 22% and 48%, at 1 μM and 10 μM respectively, during short (15–50 ms) depolarizing pulses to 0 mV. The L-type Ca^{2+} channel agonist Bay K 8644 (1 μM) caused a variable potentiation of HVA currents that could be better appreciated at low rather than at high depolarizing steps. Increase of I_{Ba} was accompanied by a 20-mV shift in the activation curves for Ca^{2+} channels towards more hyperpolarizing potentials. Application of the conus toxin ω-conotoxin GVIA (GVIA; 1 μM) blocked 31% of I_{Ba}; blockade was irreversible upon removal of the toxin from the extracellular medium. ω-Agatoxin IVA (IVA; 100 nM) produced a 15% blockade of I_{Ba}. ω-Conotoxin MVIIC (MVIIC; 5 μM) produced a 36% blockade of I_{Ba}; such blockade seems to be related to both GVIA-sensitive (N-type) and GVIA-resistant Ca^{2+} channels. The sequential addition of supramaximal concentrations of furnidipine (10 μM), GVIA (1 μM), IVA (100 nM) and MVIIC (3 μM) produced partial inhibition of I_{Ba}, which were additive. Our data suggest that the whole cell I_{Ba} in rat chromaffin cells exhibits at least four components. About 50% of I_{Ba} is carried by L-type Ca^{2+} channels, 30% by N-type Ca^{2+}channels and 15% by P-type Ca^{2+} channels. These figures are close to those found in cat chromaffin cells. However, they differ considerably from those found in bovine chromaffin cells where P-like Ca^{2+}channels account for 45% of the current, N-type carry 35% and L-type Ca^{2+} channels are responsible for only 20–25% of the current. These drastic differences might have profound physiological implications for the relative contribution of each channel subtype to the regulation of catecholamine release in different animal species.

Key words Chromaffin cells · Ca^{2+} channels · Dihydropyridines · ω-Conotoxin GVIA · ω-Agatoxin IVA · ω-Conotoxin MVIIC

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Introduction

We have recently demonstrated that acetylcholine (ACh) and K^+-evoked catecholamine secretory responses from perfused rat adrenal glands are effectively modulated by 1,4-dihydropyridine (DHP) Ca^{2+} channel antagonists and agonists; however, a DHP-resistant component of the secretory process in response to splanchnic nerve stimulation was also observed [26]. Similarly, we have known for years that secretory responses in cat [15, 18] and bovine [13, 24] chromaffin cells are also highly sensitive to DHPs. However, in the bovine adrenal gland, the secretory response is partially preserved in the presence of DHPs [16]. These differences could be due to the coexistence of different populations of Ca^{2+} channels in bovine chromaffin cells, with respect to those of the cat and rat. In fact, the presence of DHP-sensitive as well as DHP-resistant Ca^{2+} channels using whole-cell [2, 5, 10, 17, 20] and single [6,11] Ca^{2+} channel current measurements has been demonstrated in bovine chromaffin cells. Another possible explanation comes

from experiments performed in cat chromaffin cells, where both DHP-sensitive and DHP-resistant Ca^{2+} channels have been recently characterized by using electrophysiological techniques [3], but only the L-type Ca^{2+}channel seems to be directly coupled to the secretory machinery [27]. A preferential coupling of L-type Ca^{2+} channels to the secretory mechanism has also been recently reported in bovine chromaffin cells [8].

In view of these results, the question arises as to whether other subtypes of Ca^{2+} channels, besides the L-type, are also expressed in rat chromaffin cells. If so, a second question emerges as to what the role of the DHP-resistant Ca^{2+} channels might be. Here we present a patch-clamp study showing that whole-cell Ba^{2+} currents (I_{Ba}) of cultured rat chromaffin cells can be dissected into subcomponents with different sensitivities for ω-toxins and drugs recognizing various subtypes of neuronal Ca^{2+} channels [32]. We also appreciate that the relative contribution of each channel subtype to the whole-cell I_{Ba} in rat chromaffin cells considerably differs from the bovine cells [2, 17]. Because the rat is the most widely used laboratory animal, and in contrast to the bovine preparation, can be manipulated easily, this study adds further interest to the knowledge of the Ca^{2+} channel subtypes present in its chromaffin cells and their relevance to the regulation of the catecholamine secretory process.

Materials and methods

Isolation and culture of rat adrenal medulla chromaffin cells

Rats of both sexes weighing 200–250 g were killed by cervical dislocation. The abdomen was opened and the adrenal glands exposed. The adrenal medullae were removed through extrusion by a surgical cut in the cortex. Adrenal medullae were isolated and decapsulated, trimmed and placed in Ca^{2+}- and Mg^{2+}-free Locke's buffer of the following composition (in mM): 154 NaCl, 3.6 KCl, 5.6 $NaHCO_3$, 5.6 glucose, and 10 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES) (pH 7.2) at room temperature. Tissues were collected under sterile conditions. Medullae digestion was achieved by incubating the pieces in 2 ml of a solution containing 0.15% collagenase, 0.3% bovine serum albumin, in Ca^{2+}- and Mg^{2+}-free Locke's buffer, for 30 min, at 37°C. The collagenase was washed out of the cells with large volumes of Ca^{2+}- and Mg^{2+}-free Locke's buffer. The cell suspension was then filtered through a 200 μm nylon mesh and centrifuged at 120 g. After washing twice, the cells were resuspended in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal calf serum containing 50 IU · ml^{-1} penicillin and 50 μg ml^{-1} streptomycin. Cells were plated on circular glass coverslips and incubated at 37°C in a water-saturated, and 5% CO_2 atmosphere; they were used within 1–3 days after plating.

Current measurements and analysis

I_{Ba} were recorded using the whole-cell configuration of the patch-clamp technique [19]. Coverslips containing the cells were placed on an experimental chamber mounted on the stage of a Nikon Diaphot inverted microscope. The chamber was continuously perfused with a control Tyrode solution containing (in mM): 137 NaCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 HEPES/NaOH, 0.005 tetrodotoxin (TTX), pH 7.4. For current recording, 10 mM Ba^{2+} (instead of 2 mM Ca^{2+}) was used as the charge carrier. Cells were dialysed with an intracellular solution containing (in mM): 10 NaCl, 100 CsCl, 20 tetraethylammonium (TEACl), 5 MgATP, 14 ethylenebis(oxonitrilo)tetraacetate (EGTA), 20 HEPES/CsOH, pH 7.2.

Whole-cell recordings were made with fire-polished electrodes (resistance 2–5 MΩ when filled with the standard Cs^+/TEA intracellular solution) mounted on the headstage of a DAGAN 8900 patch-clamp amplifier, allowing cancellation of the capacitative transient and compensation of series resistance. A Labmaster data acquisition and analysis board and a 386-based microcomputer with pCLAMP software (Axon Instruments, Foster City, Calif., USA) were used to acquire and analyse the data. Solutions were exchanged using electronically driven miniature solenoid valves coupled to a multi-barrelled concentration-clamp device, the common outlet of which was placed within 100 μm of the cell to be patched. The flow rate was low (<1 ml min^{-1}) and regulated by gravity to achieve complete replacement of the cell surroundings within less than 1 s. Experiments were performed at room temperature (22–24°C).

Solutions and chemicals

Stock solutions (10 mM) of Bay K 8644 and furnidipine were prepared in ethanol and diluted in the extracellular solution to the required final concentration (1–10 μM). At the highest concentrations used (0.1%), ethanol did not affect I_{Ba}. ω-Conotoxin GVIA (GVIA), ω-agatoxin IVA (IVA) and ω-conotoxin MVIIC (MVIIC) were prepared as 0.1 mM stock solutions in distilled water and kept in aliquots at −20°C until use.

Collagenase type A was purchased from Boehringer Mannheim, Madrid, Spain. DMEM, fetal calf serum, penicillin and streptomycin were purchased from Gibco, Madrid, Spain. BSA and TTX were purchased from Sigma. IVA was purchased from Peptide Institute (Osaka, Japan). GVIA and MVIIC were purchased from Bachem (Essex, UK). Furnidipine was supplied by Alter, Madrid, Spain. Other chemicals were obtained either from Sigma or Merck, Madrid, Spain.

Statistical analysis

Results are expressed as means ± SEM. The statistical differences between means of two experimental results were assessed by Student's *t*-test. A value of P equal to or smaller than 0.05 was taken as the limit of significance.

Results

Characterization of I_{Ba} currents in rat chromaffin cells

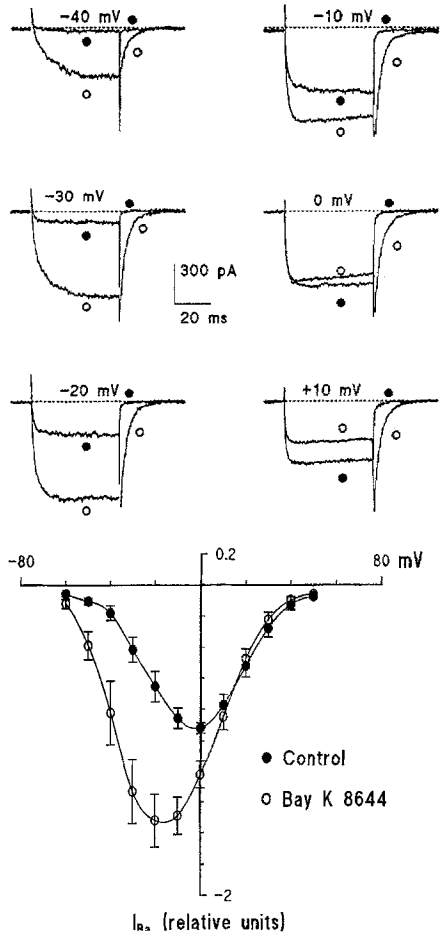
In freshly dissociated and short-term cultured rat chromaffin cells, I_{Ba} were elicited by short (50 ms) depolarizing pulses applied from a holding potential (V_H) of −80 mV in 10-mV steps up to ± 50 mV. Other ionic currents were suppressed by dialysing the cells with the Cs^+ − and TEA-based intracellular solutions, and by bathing them in a Na^+-based solution containing TTX (5 μM). I_{Ba} activated slowly and showed little or no time-dependent inactivation on this time scale. I_{Ba} activated around −40 mV, peaked around 0 mV and showed an apparent reversal potential at about +50 mV to +60 mV (Fig. 1). No evidence of low threshold

(LVA, T-type [12]) Ca^{2+} channels was observed. These results suggest that rat chromaffin cells are endowed with only high threshold (HVA) Ca^{2+} channels.

Sensitivity of I_{Ba} to Bay K 8644

Effects of the DHP agonist Bay K 8644 on HVA I_{Ba} were tested by applying a series of 50-ms depolarizing pulses of increasing amplitude to rat chromaffin cells held at a V_H of −80 mV. The depolarizing protocol was applied before and during the superfusion of the cells with a solution containing 1 μM Bay K 8644. As in cat chromaffin cells [3], Bay K 8644 produced a drastic potentiation of I_{Ba} in rat chromaffin cells and prolonged the time course of the tail currents recorded upon repolarization of the cells to negative potentials (−70 mV; traces to left in Fig. 1). The current increase was more evident at low membrane potentials where the agonist increased by about four fold to five fold the size of the current (Fig. 1). This contrasts with the more scarce effects seen in bovine chromaffin cells [5, 10, 14, 31], where the L-type component of the whole-cell I_{Ba} accounts for only 20–25% [2, 17]. There was some variability in the degree of increase of I_{Ba} between different cells, probably reflecting a different distribution of DHP-sensitive and DHP-insensitive Ca^{2+} channels in different cell subtypes. Figure 1 shows the averaged peak current voltage (I/V) relationship obtained in eight cells. Maximum increase was observed at −40 mV, where averaged I_{Ba} amplitude was 52 ± 14 pA (n = 8) in control conditions and increased further to 229 ± 58 pA (n = 8) in the presence of 1 μM Bay K 8644. At −30 mV, Bay K 8644 increased I_{Ba}, from a control value of 122 ± 28 pA to 379 ± 54 pA (n = 8).

Fig. 1 Drastic potentiation by Bay K 8644 of whole-cell Ba^{2+} currents (I_{Ba}) in voltage-clamped rat chromaffin cells. Averaged peak current voltage (I/V) relationship for I_{Ba}recorded from rat chromaffin cells either before (*control*) or after 3 min of superfusion of the cells with an extracellular solution containing Bay K 8644 (1 μM) and 10 mM Ba^{2+}. Cells were held at -80 mV and 50 ms pulses to different test potentials (V_T) were applied at 10 s intervals. Data were normalized with respect to the maximum inward current recorded in each individual cell and plotted as means ± SEM of 8 cells. *Inset* (*traces* to the *left*) show capacitance and linear leak-subtracted current traces obtained in the presence or the absence of Bay K 8644 at the test voltages indicated



Effects of furnidipine

To test the effects of DHP antagonists on I_{Ba}, furnidipine, a novel DHP derivative [1] which blocks L-type Ca^{2+} channels in bovine [2, 17] and cat [27] chromaffin cells was selected as a representative of this group of Ca^{2+} channel blockers. Furnidipine (1–10 μM) reversibly decreased the size of I_{Ba} during short (25 ms) depolarizing pulses to 0 mV (Fig. 2). Blockade by furnidipine had no significant effects on the activation time course of I_{Ba} and showed a dependence on the concentration used. Furnidipine blocked I_{Ba} by 21 ± 3% (n = 15), 32 ± 2% (n = 5) and 48 ± 2% (n = 24) at 1, 3 and 10 μM, respectively.

As for other DHPs, block of HVA currents in rat chromaffin cells by furnidipine seemed to show a marked dependence on V_H. Experiments were performed in GVIA-treated chromaffin cells, to which furnidipine was applied at two different values of V_H. First, furnidipine was applied to cells held at −80 mV. Under these conditions, furnidipine (10 μM) blocked I_{Ba} by 47 ± 4% (n = 12). Upon washing out the drug from the extracellular medium, recovery of I_{Ba} was fast and almost complete (Fig. 3). Then cells were held at −50 mV and 3 min later furnidipine (10 μM) was applied again. At a V_H −50 mV, blockade increased to 76 ± 4% (n = 12; P < 0.001 when compared to a VH of −80 mV). Another feature of this more depolarized V_H was the slower and incomplete recovery of I_{Ba} upon washing out furnidipine from the perfusion solution (Fig. 3), suggesting that in depolarizing conditions, the affinity of furnidipine for the DHP receptor and the L-type channel is greatly enhanced [9, 23]. Similar effects were obtained when comparing the effects of 1 μM furnidipine at a V_H of −80 mV (21 ± 3% blockade; n = 15 cells) versus a V_H of −60 mV (36 ± 5% blockade; n = 7 cells; p = 0.01).

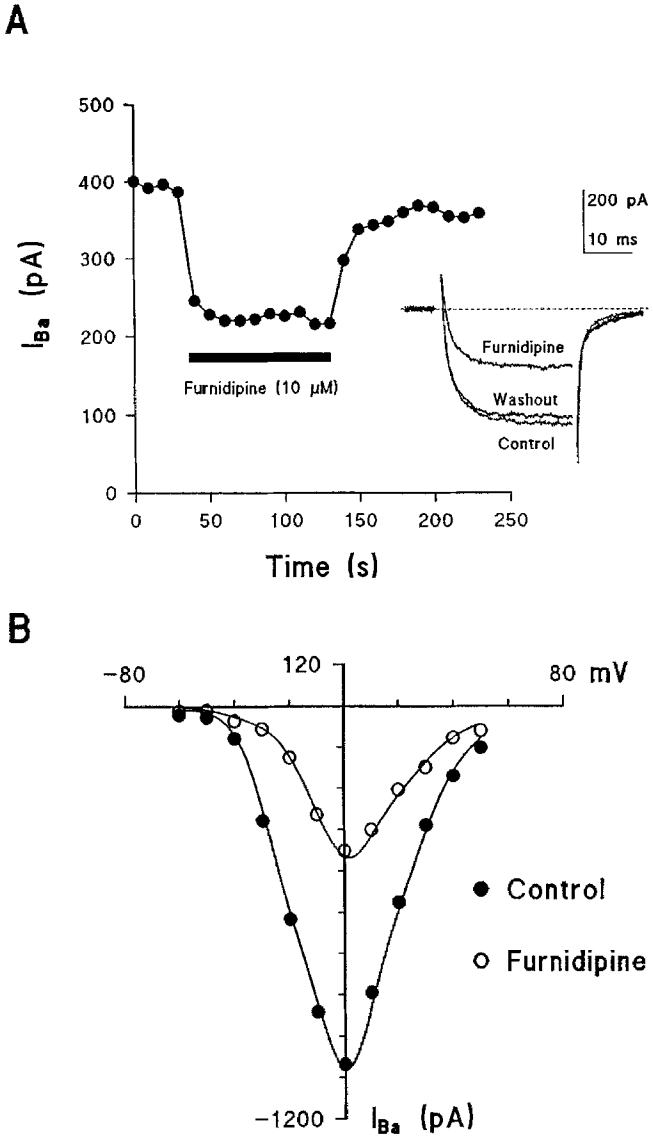


Fig. 2A, B Blocking effects of furnidipine on I_{Ba}. Panel **A** The time course of the peak current obtained during 25-ms test pulses to 0 mV applied to a rat chromaffin cell from a holding potential (V_H) of −80 mV. Test pulses were applied at 10 s intervals. *Inset* shows original current traces obtained under the different conditions. **B** The peak (I/V) relationship obtained in a different cell before and after the superfusion of the cell with a solution containing furnidipine (10 μM)

Sensitivity to GVIA of I_{Ba}

Sensitivity of I_{Ba} to the conus toxin GVIA was tested by acute superfusion of the toxin. When cells were superfused with a solution containing 1 μM GVIA, a variable percentage of blockade of I_{Ba} was observed. In 27 cells, the toxin blocked 31 ± 2% of the recorded current. The blocking effects were irreversible upon washing out the toxin from the extracellular medium (Fig. 4). Similar results have been obtained in bovine [2, 7, 17, 20] and cat [3] chromaffin cells.

Although the blocking effects of GVIA have been considered to be specific for N-type Ca^{2+} channels, such blocking effects could also affect L-type Ca^{2+} channels in neural tissue [4, 38]. In order to test such a possibility, effects of furnidipine were also tested after the irreversible blockade of GVIA was achieved. In these experiments, suppression of HVA currents by furnidipine (10 μM) also occurred after GVIA blockade (Fig. 4), suggesting that both types of blockers were acting at different Ca^{2+}channels.

Effects of P-type Ca^{2+} channel blockers

The existence of P-type Ca^{2+} channels in rat chromaffin cells was studied by using the novel toxin isolated from the venom of the spider *Agelenopsis aperta* IVA, that has been recently reported to selectively block this subtype of Ca^{2+} channels in neurons [29, 30].

Superfusion of rat chromaffin cells with a solution containing a supramaximal concentration of IVA (100 nM) led to a significant blockade of I_{Ba} (15 ± 2%; n = 7). Such a blockade was almost irreversible upon removal of the toxin from the perfusion solution (Fig. 5). No additional blocking effects were observed when IVA concentrations were increased up to 300 nM (15 ± 2% blockade; n = 8 cells). Although IVA concentrations higher than 100 nM could block other subtypes of Ca^{2+} channels, it seemed not to be the case in rat chromaffin cells. As shown in Fig. 5, treatment of the cells with IVA did not prevent the blocking effects of other subtypes of Ca^{2+} channel blockers.

Effects of the novel conus toxin MVIIC

The effects of the novel conus magus toxin, MVIIC, were also tested in rat chromaffin cells. This toxin was initially described as an N- and P-type Ca^{2+} channel blocker in neurons [21]. Application of 5 μM MVIIC induced a fast blockade of I_{Ba} that amounted to 36 ± 4% (n = 11) of the HVA currents recorded. Blockade was partially reversible upon washing out the toxin from the extracellular medium, probably reflecting the reversible blockade of N-type Ca^{2+} channels induced by the toxin. MVIIC effects were also tested after treating the cells with 1 μM GVIA. Once the irreversible blockade induced by GVIA was reached, application of MVIIC induced an additional blockade of I_{Ba}, but under these conditions, only 19 ± 2% (n = 12; $P < 0.005$) of the remaining current was blocked, suggesting that MVIIC blockade was affecting both GVIA-sensitive as well as GVIA-resistant Ca^{2+} channels.

Recently, a novel subtype of Ca^{2+} channel has been described in neurons [34, 37], characterized by its insensitivity to blockade by DHP, IVA and GVIA, but its sensitivity to MVIIC. The presence of this new Q-type Ca^{2+} channel was also tested in rat chromaffin cells. Figure 5 shows a typical experiment in which a rat

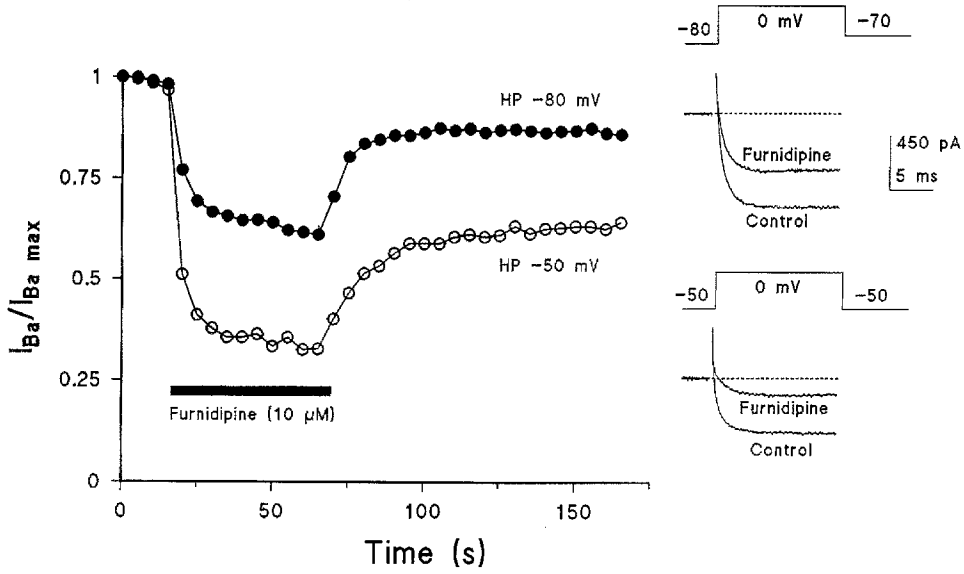


Fig. 3 Voltage dependence of the furnidipine blocking effects of I_{Ba} in rat chromaffin cells pretreated with 1 μM ω-contoxin GVIA (GVIA) to block the N-type component. Time course of the blocking effects of furnidipine (10 μM) at two different values of V_H potentials (*HP*). I_{Ba} was elicited by 15 ms depolarizing pulses applied at 5-s intervals either from a V_H of −80 mV (*solid circles*) or −50 mV (*open circles*). Superfusion with a solution containing furnidipine (10 μM) led to a stronger blockade when the V_H was fixed at −50 mV. Upon removal of furnidipine from the extracellular solution, recovery was faster and more complete with a V_H of −80 mV. Current was normalized with respect to the maximum current at the two V_H values tested. *Insets* show original current traces obtained at the two V_H values in the absence and presence of furnidipine

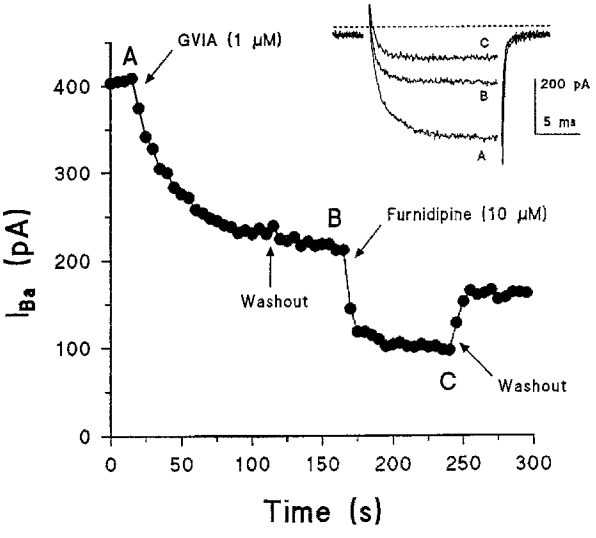


Fig. 4 GVIA irreversibly blocks I_{Ba}. Time course for the blocking effects of the N-type Ca^{2+}channel blocker GVIA (1 μM) on the peak I_{Ba} recorded during 15-ms depolarizing pulses to 0 mV, applied from a V_H of −80 mV at 5-s intervals. Effects of GVIA did not reverse when the toxin was removed from the perfusion solution (*washout*). Application of furnidipine (10 μM) after irreversible blockade with GVIA was achieved and induced an additional blockade of I_{Ba}. *Top panel* shows original current traces recorded at the points indicated in the figure

chromaffin cell was treated with IVA (100–300 nM) and GVIA (1 μM) and once the irreversible blockade of both toxins were reached, superfusion with a solution containing MVIIC (3 μM) led to an additional blockade of I_{Ba}. Average results of this subset of experiments show that the combination of IVA plus GVIA blocked I_{Ba} by 41 ± 2% (n = 22) and further addition of MVIIC increased the blockade to 59 ± 5% (n = 6; $P < 0.001$).

Discussion

The results of the present study demonstrate that, in addition to a predominant L-type Ca^{2+} channel, rat chromaffin cells are endowed with other subtypes of Ca^{2+} channels: GVIA-sensitive (N-type) and DHP- and GVIA-resistant (P- and Q-types) Ca^{2+} channels. Our conclusions are based mostly on pharmacological data that can be summarized as follows: (1) Bay K 8644 produced a marked potentiation of HVA currents; (2) the DHP antagonist furnidipine blocks the HVA currents only partially; (3) GVIA blocked a significant fraction of the current in an irreversible manner; (4) application of the novel toxins IVA and MVIIC also significantly decreased I_{Ba} when applied to the bath solution; and (5) combination of two or more blockers at supramaximal concentrations produced additive effects. It should be stressed that dissection of HVA Ca^{2+} channel subtypes in neurons can hardly be achieved through biophysical parameters and that the pharmacological strategy used here has a wide acceptance [32, 36]

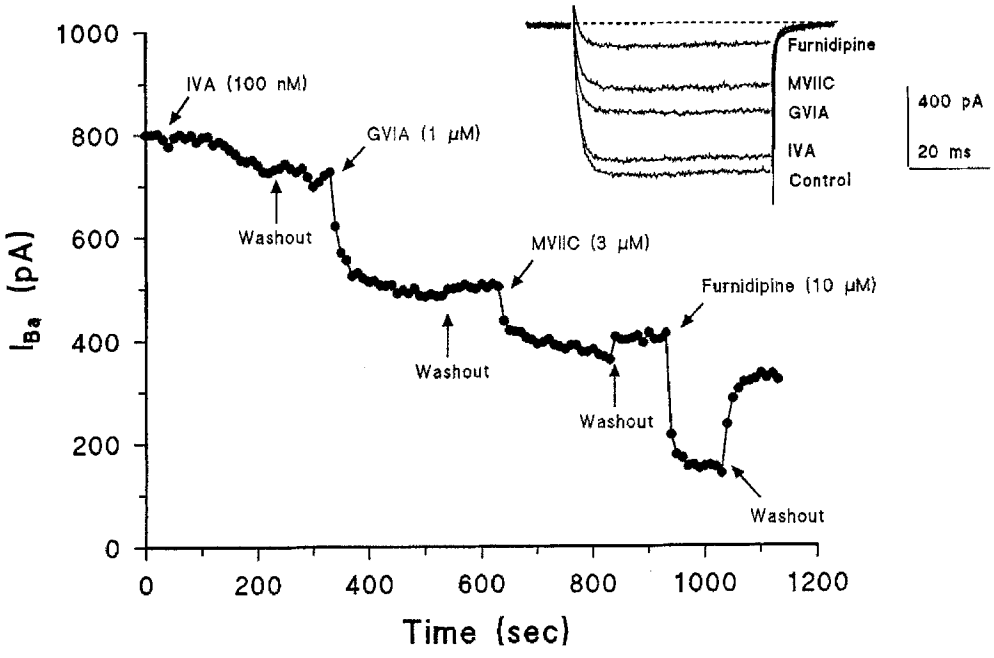


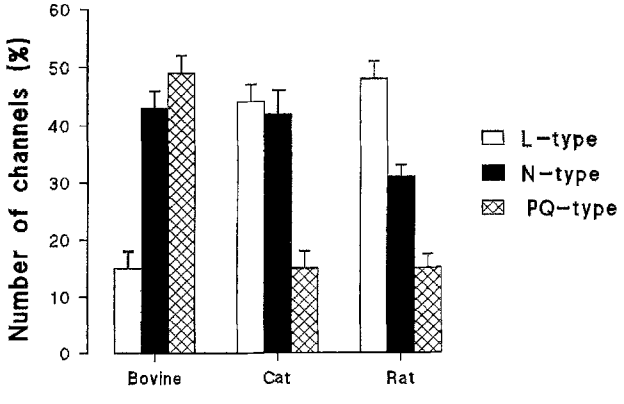
Fig. 5 Effects of the sequential addition of ω-agatoxin-IVA (IVA), GVIA (*GVIA*), ω-conotoxin MVIIC (*MVIIC*) and furnidipine on I_{Ba}. The cell was held at −80 mV and stimulated with 50-ms depolarizing pulses to 0 mV. Effects of IVA (100 nM) were not reversible upon washing out the toxin. Further application of GVIA (1 μM), MVIIC (3 μM) and furnidipine (10 μM) resulted in additional blockade of the current. *Inset* shows original current traces obtained at the different points indicated in the figure

The DHP-sensitive channel

The effects of DHPs observed in rat chromaffin cells are quite similar to those previously reported for cat and bovine chromaffin cells. Bay K 8644 has been shown to potentiate Ca^{2+} currents in bovine chromaffin cells [22] and to prolong tail currents in bovine [5, 10, 14, 31] and cat [3] chromaffin cells. Thus, it seems that L-type Ca^{2+} channels are ubiquitous in a variety of chromaffin cells and contribute significantly to the total HVA current. However, the degree of potentiation of HVA currents by Bay K 8644 markedly differed between these species, being only two fold in bovine chromaffin cells, where L-type Ca^{2+} channels contribute scarcely to the whole-cell current [2, 17], and four fold to five fold in cat [3] and rat (this study) chromaffin cells.

The effects of the DHP antagonist furnidipine in rat chromaffin cells are in good agreement with those of Albillos et al. [3] who observed a marked blockade of Ca^{2+} currents by nisoldipine in cat chromaffin cells. In contrast, blockade by furnidipine of I_{Ba} in bovine chromaffin cells was weaker [2, 17], likely reflecting a lower density of L-type Ca^{2+} channels than in rat or cat chromaffin cells. Our experiments with furnidipine show that rat chromaffin cells possess a significant fraction of L-type Ca^{2+} channels that can be effectively blocked if cells are maintained at more depolarized values of V_H.

Fig. 6 Distribution of Ca^{2+} channel subtypes in different species. Averaged data of blockade of I_{Ba} upon treatment of the cells with furnidipine (L-type), GVIA (N-type) and IVA (P-type). Data on bovine chromaffin cells were obtained from [2, 17]. Data on cat chromaffin cells were obtained from [3, 12]



The GVIA-sensitive channels

GVIA-sensitive channels have been consistently reported to be present in bovine [2, 7, 17, 20] and cat [3] chromaffin cells, and in rat pheochromocytoma PC12 cells [33, 35]. As in other species, blockade of HVA currents by GVIA varied from cell to cell, reflecting different distributions of the different subtypes of Ca^{2+} channels in these cells.

In the bovine, chromaffin cell, block of HVA currents by GVIA was largely irreversible but in cat chromaffin cells [3] and in PC12 cells [35], blockade was partially reversible. Our present data suggest that effects of GVIA on rat chromaffin cells are closer to those found in bovine chromaffin cells. The additive

effects of furnidipine on cells treated with GVIA (Fig. 4) suggest that both types of blockers are acting on different types of Ca^{2+} channels.

The DHP- and GVIA-resistant channels

Experiments on the effects of the toxin IVA on HVA currents in rat chromaffin cells indicating that 15% of the total current can be irreversibly blocked by 100 nM IVA, suggest that a fraction of HVA current can be carried through P-type Ca^{2+} channels similar to those described in central and peripheral neurons [29, 30]. The existence of this subtype of Ca^{2+} channel has been recently reported in bovine [2, 17] and cat [3] chromaffin cells. The presence of P-type Ca^{2+} channels is also supported by the observed blockade of GVIA-resistant current by the conus magus toxin MVIIC, that has been recently reported to block P-type Ca^{2+} channels [20].

The additional blocking effects of MVIIC in cells pretreated with IVA and GVIA (Fig. 5) indicates the presence of a subset of Q-type Ca^{2+} channels in rat chromaffin cells, similar to those recently described in neurons [34, 37] and bovine chromaffin cells [28].

Comparison with other species

The results obtained in the present study indicate that in rat chromaffin cells L-type Ca^{2+} channels are predominant, but other subtypes of Ca^{2+} channels like N-type, P-type and Q-type Ca^{2+} channels (Fig. 6) are also present. These results contrast with those previously observed in bovine chromaffin cells [2, 17], where N-type and P-like Ca^{2+} channels are predominant (Fig. 6), while L-type Ca^{2+} channels represent only a minor subpopulation. On the other hand, results obtained in cat chromaffin cells [3], are closer to those obtained in rat chromaffin cells (present paper), with N- and L-type Ca^{2+} channels being predominant and with a minor subpopulation of P-like Ca^{2+} channels. It should be noted that the addition of the percentage blockade of different components of I_{Ba} gives figures greater than 100%; this could be explained on the basis of the variability of the different channel subtypes in cells cultured for different time periods, as well as to overlapping of the pharmacological profiles of toxins in blocking Ca^{2+} channel types. Also, the washout of I_{Ba} with time could alter the relative proportion of the initial current blocked by each toxin. It is interesting to also note that at the end of each experiment, after treating the cells with IVA, GVIA, MVIIC and furnidipine, a Cd^{2+}-sensitive component of I_{Ba} remained unblocked. Whether this is a novel Ca^{2+} channel which is typical in chromaffin cells, or whether it is due to the experimental protocol remains to be elucidated.

It is also worth noticing that the DHP component of the whole-cell I_{Ba} current in rat chromaffin cells can be recruited with single test depolarizing pulses, in contrast with the observation that L-type Ca^{2+} channels in bovine chromaffin cells seem to require strong depolarizing prepulses to be recruited [5] (but see references [2, 17] for bovine chromaffin cells, and [3] for cat chromaffin cells).

Functional implications for catecholamine secretion

Catecholamine release from perfused rat adrenal glands is potently inhibited by DHP antagonists when both nicotinic receptor activators or high K^+ concentrations are used as secretagogues; however, a DHP-resistant component of the secretory response to splanchnic nerve stimulation has also been described [26]. These results seem to indicate that although the regulation of secretion in rat chromaffin cells is likely to be mediated by L-type Ca^{2+} channels, a second pathway for Ca^{2+} entry into the cells seems to be relevant from this functional point of view.

The existence of multiple subtypes of Ca^{2+} channels in rat chromaffin cells raises the question to what their role might be in regulating Ca^{2+} entry into the cell, and whether Ca^{2+} entering through different channel pathways is equally efficient in triggering exocytosis. In bovine chromaffin cells, DHPs block the secretion of catecholamines in response to depolarizing stimuli only partially [16], but see Ceña et al. [13]. In contrast, secretory responses in cat adrenal glands exhibit high sensitivity to DHP agonists and antagonists [15, 18, 25]. In rat adrenal glands, a picture similar to that observed in the cat adrenal gland emerges. The secretory process in response to nicotinic receptor stimulation or to depolarizing stimuli is highly sensitive to DHP antagonists [26], but when a more physiological stimulus, such as the electrical stimulation of the splanchnic nerve is used, secretion of catecholamines is less sensitive to DHPs [26]. Under these circumstances both L- and non-L-type Ca^{2+} channels might co-operate in controlling exocytosis.

In a recent report [27], the proposal was made that despite the presence of L-, N-and, probably P-type Ca^{2+} channels, only the L-type Ca^{2+} channels are predominantly coupled to the secretory machinery in cat chromaffin cells. A similar suggestion has been made for bovine chromaffin cells [8], in which also the Q-type Ca^{2+} channels have been reported recently to be related directly to the secretory process [28]. Therefore, it is, plausible that the Ca^{2+} channel subtype dominating the control of catecholamine release during stressful conflicts varies from species to species. From the standpoint of comparative physiology, this variability could represent the existence of different forms of "fight and flight" responses to the same stressful conflicts in different animal species.

Further studies are needed to elucidate possible functional features of these multiple subtypes of Ca^{2+} channels found in rat chromaffin cells, for instance

how they are regulated by phosphorylation/dephosphorylation processes, selective modulation by neurotransmitters acting through regulatory G-proteins or various intracellular messengers.

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