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Calcium channels in undifferentiated PC12 rat pheochromocytoma cells

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Undifferentiated rat pheochromocytoma PC12 cells were voltage clamped using the whole cell technique. After blockade of outward currents, calcium currents were elicited from –40 and –100 mV. A subpopulation of cells displayed only one current component activated at –10 mV and slowly decaying. In other cells this current coexisted with a component activated around –40 mV and decaying with a faster time constant. We conclude that undifferentiated PC12 cells can express two types of calcium channels, L (long-lasting) and N (neuronal)-type channels.

Calcium channel; Patch clamp; Neural growth factor; (PC12)

1. INTRODUCTION

PC12 is a cell line, initially isolated by Greene and Tischler [1] from a rat pheochromocytoma, which has been extensively employed in neurobiological studies. When cultured under conventional conditions these cells resemble chromaffin cells; when treated with nerve growth factor (NGF) PC12 cells stop growing, enlarge and acquire a neuron-like phenotype [1,2]. Because of these properties PC12 cells represent at the moment the most popular model of both neurosecretory and neuronal-type cells [3,4]. The control of the intracellular Ca^{2+} concentration, $[Ca^{2+}]_i$, is among the themes investigated in great detail [5]. In particular, previous studies established that PC12 cells express voltage gated Ca^{2+} channels, whose nature, however, appeared different before and after NGF-induced differentiation: naive cells were reported to express only channels of the L-type (high conductance, long lasting channels sensitive to dihydropyridine blockers) [6]; differentiated cells were thought to express also channels of the N-type (of lower conductance and more rapid inactivation) [7]. The latter channels could thus be envisaged as a differentiation marker in PC12 cells. Our present results demonstrate that this is not the case. In the population of naive PC12 cells investigated almost 50% exhibited in fact clear non-L, N-like Ca^{2+} currents.

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2. MATERIALS AND METHODS

PC12 monolayers were cultured in RPMI (Flow) 1640 medium supplemented with 5% fetal calf serum, 10% horse serum, penicillin 100 U/ml, streptomycin (0.1 mg/ml) and glutamine 2 mM. Cells were maintained at 37°C in a humidified atmosphere of 5% CO_2 and 95% O_2. Two to three days prior to patch clamp recording, monolayers were washed in PBS at 37°C and incubated in 0.02 g/ml trypsin (type III, Sigma). After washing with cold (4°C) RPMI 1640 the monolayers were gently detached and the cells sedimented by centrifugation at 800 rpm for 10 min. After mechanical dissociation, the cells were replaced in the culture medium.

Conventional whole-cell patch clamp techniques were used [8]. Voltage clamp experiments were carried out in 30 mm Petri dishes (Cel-Cult). The temperature was constantly held at 34°C. The patch clamp amplifier was designed by Professor Wanke, University of Milan. Fire-polished pipettes had a final resistance of 2–4 MΩ when filled with (mM): CsCl 122, $MgCl_2$ 2, $CaCl_2$ 0.5, EGTA-KOH 5, Hepes-KOH 10, ATP 5 and GTP 0.5 (pH 7.2). The extracellular solution consisted of (mM):

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NaCl 150, $MgCl_2$ 2, $CaCl_2$ 10, Hepes-NaOH 5, glucose 5 (pH 7.3). Recording pipettes were positioned by means of a remote controlled microstepper. After formation of a giga-seal (usually 5–6 GΩ) pipette capacitance was zeroed by capacitance compensation circuitry. After breaking into the cell, whole-cell membrane recordings were obtained. In some experiments capacitance was electronically reduced before eliciting calcium currents. Linear leakage was compensated electronically at −10 mV from the holding potential. Current signals were then filtered at 2 KHz. A 4-way superfusion pipette, positioned close (about 500 μm) to the cell, was used for exchanging solutions held at the temperature of the solutions in the Petri dish by means of a feedback circuitry. Data were displayed on a digital oscilloscope (Panasonic), digitized by an analog/digital converter (Sony) and stored on a videocassette for off-line analysis. Nitrendipine (Bayer) was dissolved in 100% ethanol and appropriate volume from the stock solution was added to the recording medium just before starting the experiment; ω-conotoxin (Peninsula) was dissolved in the extracellular solution.

3. RESULTS

As reported previously, the general morphology of naive PC12 cells is quite variable, with two groups predominating: round-shaped, phase-bright cells free of elongations and flat, polygonal cells, sometimes endowed with short projections. The general size was not significantly different in the two groups. Stable recordings were obtained in 103 cells. Average resting membrane potential was −41.5 ± 6.2 mV. Average cell input resistance was 378 ± 30 MΩ, average capacitance 22 ± 5 pF. Macroscopic Ca^{2+} currents were only rarely detected when recording from round cells, which in contrast exhibited outward currents. The study of Ca^{2+} currents was therefore conducted in the polyglonal cells. Fig.1 illustrates the *I/V* relationship results obtained in two subgroups of cells tested from two different holding potentials, −40 and −100 mV. In the group shown in fig.1A (46 out of 82 cells) no significant difference depending on the holding potential was observed, except for an increase of current reversal potential obtained from the holding potential of −100 mV. The current activated at −10 mV and peaked around +20 mV. Moreover, current decay analysis showed that the currents elicited from both holding potentials could be fitted with a single exponential (τ = 150–170 ms at +20 mV, see inset in fig.1A). In the remaining cells currents elicited from −40 mV were similar to those in the forementioned group whereas those elicited from −100 mV

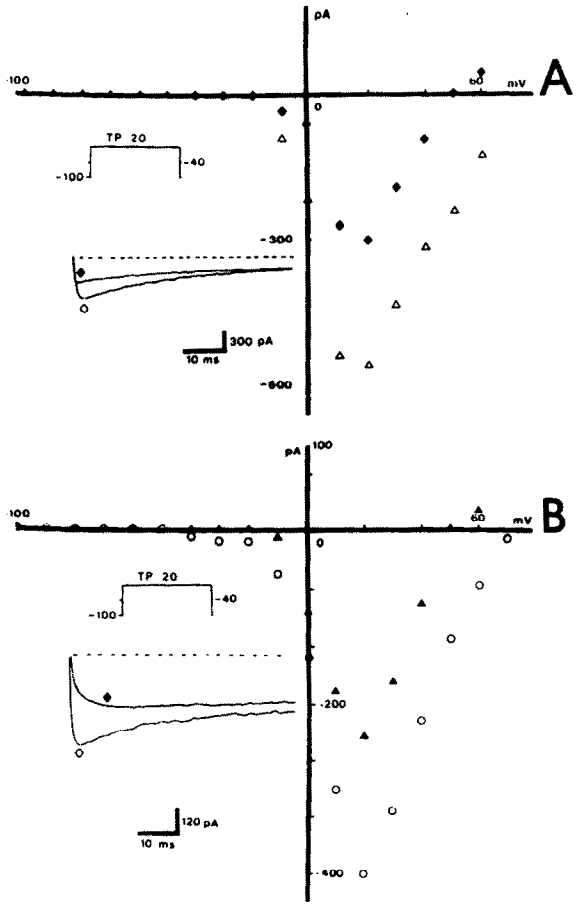

Fig.1. (A) Current/voltage plot of currents recorded in a cell following depolarizing steps from −100 mV (empty triangles) and −40 mV (filled diamonds). Note the inward current activation range and the slow current decay (inset). (B) Same experimental condition as in (A) but in a cell where current activation range and decay time constant depended on the pretest holding potential (−100 mV, empty circles; −40 mV, filled triangles).

were different. In particular, in the second group, currents from −100 were distinctly greater than those from −40, their activation occurred already at −40 mV, and their decay analysis revealed faster time constants (55 vs 250 ms for the cell shown in fig.1B, inset). On the average, in group two PC12 cells the faster inactivating channels were found to contribute for ~30% of the initial Ca^{2+} conductance.

In order to further characterize the Ca^{2+} currents, cells were studied after drug application. In group 2 cells the dihydropyridine Ca^{2+} antagonist,

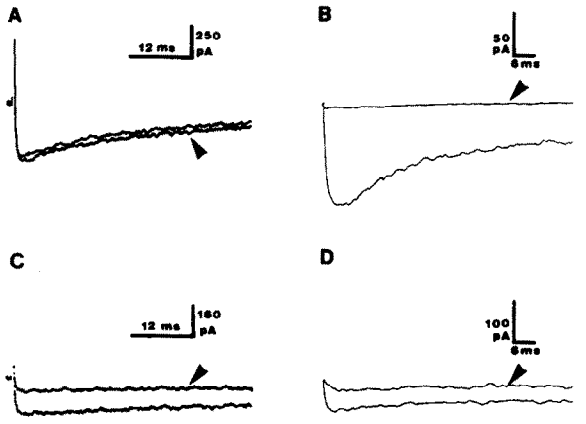

Fig.2. Effects of nitrendipine (10 μM, A and C) and ω-conotoxin (10 μM, B and D) on the calcium currents in group two PC12 cells elicited from either −100 mV (A,B) or −40 mV (C,D) holding potentials. The traces recorded 1 min after drug addition are marked by an arrowhead.

nitrendipine (10 μM, 6 cells) inhibited the current elicited from −40 mV by ~40% (fig.2C). This drug, however, was without consistent effect on the current elicited from −100 mV (fig.2a). In contrast, the snail toxin, ω-conotoxin (10 μM, 5 cells) proved to be a strong and irreversible inhibitor of the currents elicited from both −40 and −100 mV (fig.2B,C).

4. DISCUSSION

Our results demonstrate that, in contrast to previous reports, PC12 cells are markedly heterogeneous in their Ca^{2+} channel endowment. A considerable fraction of the cells failed to exhibit Ca^{2+} current activation under our experimental conditions, and may therefore be free of Ca^{2+} channels. The calcium current obtained in over half of the remaining cells appears compatible with the expression of channels of only the so-called L-type calcium channels. These channels are, in fact, activated from depolarized potentials (−40 mV in our experiments); they are slowly inactivating and sensitive to both dihydropyridine drugs and ω-conotoxin [8,9]. L-channels, however, cannot account entirely for the results in the remaining group. Here, in addition to L-channels, another type (activatable from negative holding potentials, rapidly inactivating, blocked by ω-conotoxin but not by dihydropyridines) was observed. All these features appear typical of the N-type channel so far described only in neurons and NGF-differentiated PC12 cells [7,10]. We conclude that exposure to NGF causes only the increase, not the appearance of N-type channels in PC12 cells. Naive cells, even before differentiation, are not simple secretory cells, but are therefore characterized by a typical channel normally expressed only in neurons. The N-channel is expected to play an important role in the control of various cell functions, including neurosecretion.

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