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Differential coupling of voltage-gated Ca^{2+} channels to catecholamine secretion from separate PC12 cell batches

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Abstract

Amperometric recordings were employed to investigate the coupling of Ca^{2+} channels to catecholamine secretion in two batches of pheochromocytoma (PC12) cells. In 'new' (freshly obtained) cells (PC12n cells), secretion was dependent on Ca^{2+} influx through L-type and N-type Ca^{2+} channels. By contrast, in 'aged' cells (maintained in liquid nitrogen for 6–8 years; PC12a cells), secretion was mostly dependent on Ca^{2+} influx through N-type channels. Patch clamp recordings revealed that L-type channels accounted for only ca. 26% of total whole-cell current in PC12a cells (determined by blockade caused by 2 μM nifedipine). In contrast, nifedipine suppressed currents by ca. 59% in PC12n cells. Thus important differences in fundamental physiological properties can be observed in PC12 cell batches even when obtained from the same source and maintained under identical conditions.

Keywords: PC12 pheochromocytoma; Exocytosis; Catecholamine; Amperometry; Voltage-gated Ca^{2+} channels; Patch-clamp

Recent technological advances have considerably furthered our understanding of the mechanisms underlying exocytotic release of transmitters and hormones from neurones and endocrine cells [1,5,13]. One such technique, which has been established for some 10 years now, is that of single-cell amperometry [14]. This allows, through chemical oxidation of released transmitters, exocytosis to be monitored non-invasively from single cells in real time. Whilst its use is limited to studies of oxidizable material such as catecholamines and 5-HT, it has proved invaluable in investigating at high resolution numerous steps following fusion of vesicle with plasma membrane before a full exocytotic event occurs [2].

We have used amperometry to monitor quantal catecholamine secretion from the pheochromocytoma cell line, PC12. This line is derived from rat chromaffin tissue, and has been freely available for over 25 years [4]. It can be differentiated into neuronal-like tissue by exposure to nerve growth factor (when the cells extend numerous projections) and transmitter release is confined to nerve terminal-like structures [15]), and can also be differentiated by exposure to glucocorticoids to a more spherical, neuroendocrine phenotype, in which somatic transmitter release is observed [3,10]. Theoretically, PC12 cells, like numerous other cell lines, provide a reproducible and stable system for studying various aspects of cellular physiology. However, even a brief review of the literature reveals these and other cells to behave differently in different laboratories [6,7]. In this study, we have compared catecholamine secretion from two separate groups of PC12 cells, both obtained from the same source and maintained under identical conditions. The single difference between the two groups is that one was obtained some 8 years ago and has remained in storage under liquid nitrogen until 2 years ago ('aged' cells, hereafter termed PC12a). The second group was obtained within the past year ('new' cells, hereafter termed PC12n).

PC12 cells (all obtained from the American Type Culture Collection) were maintained as previously described [10] in RPMI 1640 culture medium containing L-glutamine, 20% fetal calf serum and 1% penicillin/streptomycin 37°C in a humidified atmosphere of 5% CO_2 /95% air. Cells were passaged every 7 days by re-suspension in fresh medium, diluted 1:2, and were used for experiments between passages 4 and 18. Cells were exposed 1 μM dexamethasone for 72–96 h before experiments, to enhance catecholamine secretion [12]. Each experimental day, cells were plated onto poly-D-lysine coated coverslips ($0.5–1.0 \times 10^5$ cells per coverslip) and allowed to adhere for ca. 1 h. Frag-

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ments of coverslip were then transferred to a recording chamber (volume ca. 80 μl) which was continually perfused under gravity (flow rate 1–2 ml/min) with a solution of composition (in mM): NaCl 135, KCl 5, $MgSO_4$ 1.2, $CaCl_2$ 2.5, HEPES 5, and glucose 10 (pH 7.4, osmolarity adjusted to ca. 300 mOsm with sucrose, 21–24°C). Ca^{2+}-free solutions contained 1 mM EGTA and no added Ca^{2+}. All drugs were applied in the perfusate except in the case of ω-conotoxin GVIA (ω-CgTx) the effect of which was investigated by pre-incubation of cells in extracellular solutions containing the toxin for at least 10 [11]. Experiments were conducted within 3 min of transfer of these cells to the perfused recording chamber. Experiments investigating the effects of nifedipine (applied from a stock solution of 20 mM in ethanol) were conducted at low light intensity. Secretion was evoked by elevating the $[K^+]_0$ to 50 mM (isotonic Na^+ substitution).

Carbon fiber microelectrodes (proCFE, diameter 5 μm) were placed adjacent to individual cells and polarized to +800 mV to allow oxidation of released catecholamine. [2,14]. Currents were recorded using an Axopatch 200A amplifier (with extended voltage range), filtered at 1 kHz and digitized at 2 kHz before storage on computer. All acquisition was performed using a Digidata 1200 interface and Fetchex software from the pClamp 6.0.3 suite (Axon Instruments, Foster City, California, USA). Exocytosis is expressed as the frequency of quantal events, determined by counting the number of events over a 55 s period, 10 s after switching to test solutions, using Mini Analysis Program (Synaptosoft Inc., Leonia, NJ, USA). Quantal size was also obtained from these recordings, by integration of clearly resolvable events, as previously described [10,11].

Ca^{2+} channel currents were recorded using the whole-cell patch clamp technique. Patch pipettes (5–7 MΩ resistance) were filled with a solution of (in mM): CsCl 130, EGTA 1.1, $MgCl_2$ 2, $CaCl_2$ 0.1, NaCl 10, HEPES 10 and Na_2ATP 2 (pH 7.2). Cells were perfused with a solution of composition (in mM): NaCl 110, CsCl 5, $MgCl_2$ 0.6, $BaCl_2$ 20, HEPES 5, glucose 10 and tetraethylammonium-Cl 20 (pH 7.4). Osmolarity of the perfusate was adjusted to 300 mOsm by addition of sucrose. Currents were evoked using 200 ms voltage ramps applied from −100 mV to +100 mV at 0.2 Hz (inter-ramp holding potential −80 mV). All data are expressed as means ± SEM, and statistical comparisons were made using unpaired *t*-tests, with $P < 0.05$ being considered significant.

Fig. 1A,B shows that exposure of PC12n cells to 50 mM K^+ evokes marked secretion, which (like PC12a cells [9,11]) is wholly dependent on Ca^{2+} entry through voltage-gated Ca^{2+} channels, since it could be completely inhibited by either removal of extracellular Ca^{2+} (Fig. 1A, representative of six cells studied) or application of 200 μM Cd^{2+} (Fig. 1B, representative of six cells studied) a non-selective blocker of voltage-gated Ca^{2+} channels. Quantal size, $Q^{1/3}$, was not significantly different between the two cell groups, being 0.40 ± 0.10 $pC^{1/3}$ for PC12n (380 events

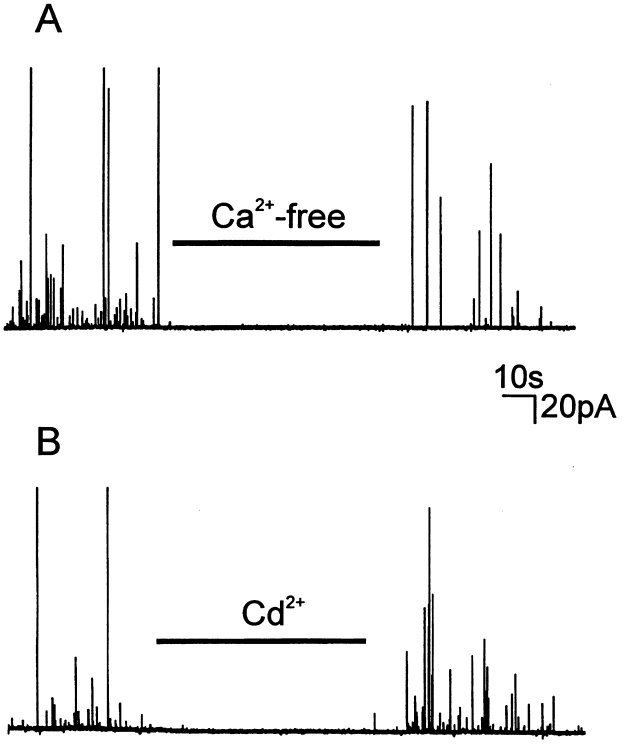

Fig. 1. Amperometric recordings of secretion of catecholamines from PC12n cells. In each case the perfusate was exchanged for one containing 50 mM K^+ immediately prior to the start of the trace. For the periods indicated by the bar, the perfusate was exchanged either for one in which the Ca^{2+} was replaced by 1 mM EGTA (A) or one containing 200 μM Cd^{2+} (B). Note that secretion was abolished in both cases. Scale bars apply to both traces.

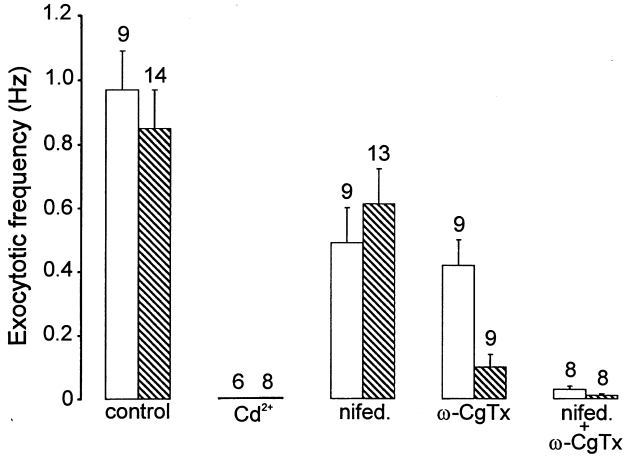

Fig. 2. Bar graph showing mean frequency of exocytosis evoked by 50 mM K^+ in PC12n cells (open bars) and PC12a cells (hatched bars), in the absence or presence of different blockers of voltage-gated Ca^{2+} channels. Cells were either exposed to Cd^{2+} (200 μM), nifedipine (2 μM; nifed.), pretreated with ω-conotoxin GVIA (1 μM; ω-CgTx) or pretreated with ω-CgTx and exposed to nifedipine, as indicated. Each bar represents mean ± SEM determined from the number of cells indicated.

from six cells) and 0.43 ± 0.15 $pC^{1/3}$ for PC12a cells (466 events, nine cells).

PC12 cells express multiple Ca^{2+} channel subtypes, [8,9], each of which is potentially capable of providing a Ca^{2+} entry route to trigger exocytosis. To examine the relative contributions of L- and N-type Ca^{2+} channels to secretory responses, we tested the ability of selective inhibitors of these channels to suppress K^+-evoked secretion. Results are summarized in the bar graph of Fig. 2. In PC12a cells, K^+ evoked secretion was reduced by ca. 90% by preincubation of cells with 1 μM ω-conotoxin GVIA ($P < 0.002$), a selective blocker of N-type Ca^{2+} channels. Secretion recorded in the presence of nifedipine (2 μM), a blocker of L-type channels, was not significantly different to that seen in the absence of blocker (Fig. 2). In contrast to these findings, secretion recorded from PC12n cells could be significantly suppressed by both ω-conotoxin GVIA and nifedipine (each drug causing ca. 50% inhibition, $P < 0.01$ for each condition), indicating that both N- and L-type channels are coupled to K^+-evoked secretion from these cells. In both cell batches, secretion in the presence of both selective inhibitors was almost completely abolished.

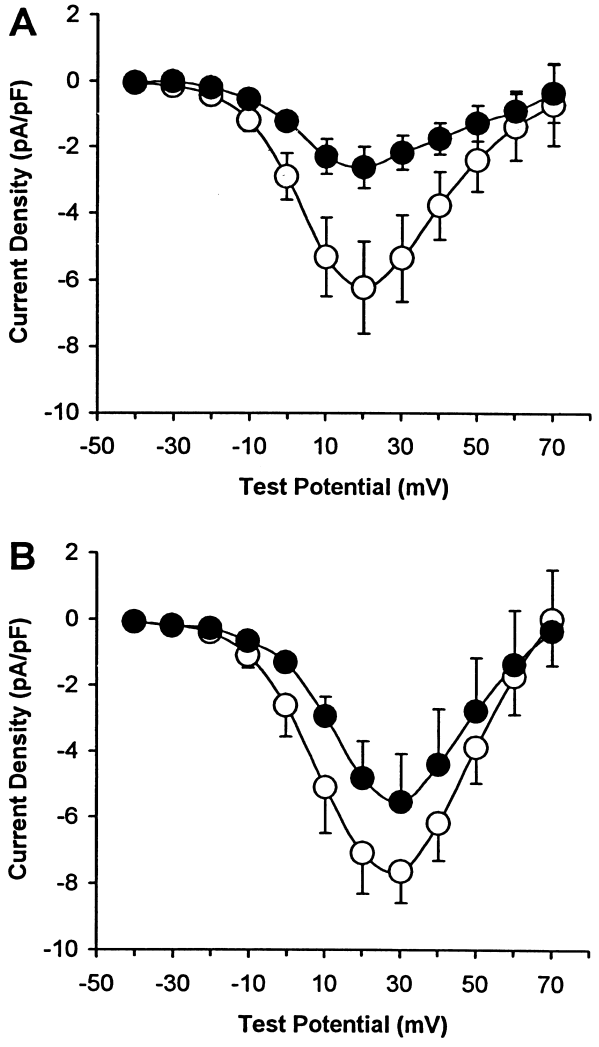

Fig. 3. Mean whole-cell Ca^{2+} channel current-voltage relationships (with vertical SEM bars) recorded in the absence (open symbols) and presence (filled symbols) of 2 μM nifedipine. (A) Recordings from PC12n cells ($n = 8$); (B) recordings from PC12a cells ($n = 7$). Note the significantly greater inhibition of currents by nifedipine in PC12n cells as compared with PC12a cells.

Whole-cell patch-clamp recordings revealed that, in both groups of cells, currents activated at approximately −30 mV and were maximal in amplitude at around +20 to +30 mV. Mean capacitance values were similar in the two cell groups (8.02 ± 1.65 pF for PC12a, 8.52 ± 0.99 pF for PC12n) and current densities in the two groups of cells were not significantly different (Fig. 3), indicating that expression of total Ca^{2+} channels were similar in the two groups of cells. Given the relative importance of L-type channels to secretion in PC12n cells (but not PC12a cells), we examined the sensitivity of whole cell Ca^{2+} currents in the two groups of cells to nifedipine. As shown in Fig. 3, 2 μM nifedipine suppressed whole cell Ca^{2+} currents in PC12n cells by 59.1 ± 8.3% ($n = 8$; Fig. 3A), a significantly greater suppression ($P < 0.008$) than was observed in PC12a cells, where whole cell Ca^{2+} currents were only suppressed by 23.6 ± 14.7% ($n = 7$; Fig. 3B).

Our results demonstrate that in two separate batches of PC12 cells, the contribution of L-type Ca^{2+} channels to the total whole-cell Ca^{2+} current can vary markedly, as can their contribution to Ca^{2+} influx leading to catecholamine secretion. Indeed, despite their presence in PC12a cells (revealed by patch-clamp recordings), they did not contribute to the secretory response significantly. This strongly suggests that PC12 cells (and possibly other cell lines) can vary from batch to batch in their fundamental physiological properties, even when obtained from the same source and maintained under identical conditions.

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