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Prion protein fragment 106–126 potentiates catecholamine secretion from PC-12 cells

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Taylor, S. C., K. N. Green, I. F. Smith, and C. Peers. Prion protein fragment 106–126 potentiates catecholamine secretion from PC-12 cells. *Am J Physiol Cell Physiol* 281: C1850–C1857, 2001.—The toxic actions of scrapie prion protein (PrP^{sc}) are poorly understood. We investigated the ability of the toxic PrP^{sc} fragment 106–126 to interfere with evoked catecholamine secretion from PC-12 cells. Prion protein fragment 106–126 (PrP106–126) caused a time- and concentration-dependent augmentation of exocytosis due to the emergence of a Ca^{2+} influx pathway resistant to Cd^{2+} but sensitive to other inorganic cations. In control cells, secretion was dependent on Ca^{2+} influx through L- and N-type Ca^{2+} channels, but after exposure to PrP106–126, secretion was unaffected by N-type channel blockade. Instead, selective L-type channel blockade was as effective as Cd^{2+} in suppressing secretion. Patch-clamp recordings revealed no change in total Ca^{2+} current density in PrP106–126-treated cells or in the contribution to total current of L-type channels, but a small Cd^{2+}-resistant current was found only in PrP106–126-treated cells. Thus PrP106–126 augments secretion by inducing a Cd^{2+}-resistant Ca^{2+} influx pathway and alters coupling of native Ca^{2+} channels to exocytosis. These effects are likely contributory factors in the toxic cellular actions of PrP^{sc}.

regulated exocytosis; pheochromocytoma; amperometry; ion channels; toxic peptides

CELLULAR PRION PROTEINS (PrP^c) are naturally occurring, copper-binding glycoproteins found primarily on neuronal and glial plasma membranes as well as those of many other cell types (2, 9, 11). Their physiological function(s) is not well established, although available evidence [based largely on studies employing PrP^c-deficient mice (reviewed in Ref. 11)] suggests that they can modulate neuronal excitability, synaptic transmission, and intracellular Ca^{2+} homeostasis (6, 7, 12) as well as alter sleep patterns (33), learning, and memory processes (22).

Prion diseases are characterized by neurodegeneration, gliosis, and accumulation of plaquelike, extracellular deposits in the brain. These diseases include scrapie (in sheep), bovine spongiform encephalopathy (in cattle), and Creutzfeldt-Jakob disease (in humans). Collectively, these disorders are referred to as transmissible spongiform encephalopathies and arise from infection with the protease-resistant scrapie form of prion (PrP^{sc}). After infection, the presence of PrP^{sc} permits conversion of PrP^c to PrP^{sc}, leading to a progressive accumulation of PrP^{sc} that is believed to underlie neurodegenerative processes manifested in the above-mentioned diseases (11).

The cellular mechanisms by which PrP^{sc} causes neuronal dysfunction are currently unclear but are the subject of much interest. These studies, commonly employing the PrP^{sc} fragment 106–126 [which is functionally extremely similar to the full-length peptide (9)], suggest that PrP^{sc} has marked effects on Ca^{2+} homeostasis, altering transmembrane Ca^{2+} flux possibly by interacting with voltage-gated Ca^{2+} channels (23, 30, 31). In addition, prion protein fragment 106–126 (PrP106–126) has been demonstrated to form nonselective ionic channels in planar lipid bilayers (17, 18), which could permit Ca^{2+} entry into cells, although others have attempted and failed to reproduce such an effect (20). This notwithstanding, the possible channel-forming property of PrP106–126 is remarkably similar to one action of Alzheimer's amyloid β-peptides (AβPs) (1, 14). We have previously shown that AβPs can form Ca^{2+}-permeable channels in pheochromocytoma (PC-12) cells (a widely used neurosecretory model cell system; reviewed in Ref. 13) that contribute to the excessive catecholamine secretory response of these cells when they are depolarized with high-extracellular [K^+]-containing solutions (25). Such similarities in the functional effects of AβPs and PrP^{sc} have prompted us to examine the effects of PrP106–126 on the secretory responses of PC-12 cells. Our results indicate that this peptide enhances evoked catecholamine secretion by inducing a novel Ca^{2+} influx pathway that is distinct from known voltage-gated Ca^{2+} channels. We suggest that this influx may contribute to the neurodegenerative effects of PrP^{sc}.

MATERIALS AND METHODS

The PC-12 cells used in this study were obtained within the last year from American Type Culture Collection (Rockville, MD) and cultured as previously described (25, 26) in RPMI 1640 culture medium that contained L-glutamine and was supplemented with 20% fetal calf serum and 1% penicil-

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lin/streptomycin (GIBCO, Paisley, Strathclyde, United Kingdom). Cells were kept at 37°C in a humidified atmosphere of 5% CO_2-95% air, passaged every 7 days, and used for up to 20 passages. The prolonged period without medium change was believed to enhance evoked dopamine release (24). Cells used for experiments were transferred to smaller flasks in 10 ml of medium, and 1 μM dexamethasone (Sigma, Poole, UK; from a stock solution of 1 mM in ultrapure water) was applied for 72–96 h to further enhance dopamine secretion (32).

On each experimental day, aliquots of PC-12 cells were plated onto poly-L-lysine-coated coverslips and allowed to adhere for ~1 h. Fragments of coverslips with attached cells were then transferred to a recording chamber (vol ~80 μl) that was continually perfused under gravity (flow rate 1–2 ml/min) with a solution of the following composition (in mM): 135 NaCl, 5 KCl, 1.2 $MgSO_4$, 2.5 $CaCl_2$, 5 HEPES, and 10 glucose, pH 7.4 (osmolality adjusted to ~300 mosmol/kgH_2O with sucrose, 21–24°C). For experiments using solutions of raised K^+ concentration, the Na^+ concentration was reduced accordingly to maintain isoosmolarity. Ca^{2+}-free solutions contained 1 mM EGTA and no added Ca^{2+}.

Drugs were applied in the perfusate except in the cases of ω-conotoxin GVIA (ω-CgTx) and ω-agatoxin GIVA (ω-Aga-IVA). The effects of these agents were investigated by preincubation of cells in extracellular solutions containing these agents for at least 10 min as previously described (25, 27). Experiments were conducted within 3 min of transfer of these cells to the perfused recording chamber. Experiments investigating the effects of nifedipine were conducted at low-light intensity, and nifedipine was added to the perfusate from a stock solution of 20 mM in ethanol, made fresh each day. PrP106–126 and its scrambled version (used for initial control experiments, see RESULTS) were purchased from BaChem, dissolved in ultrapure water at a concentration of 1 mM, and stored at −20°C in aliquots. They were added directly to cells in culture at the concentrations indicated in RESULTS for varying periods (1–48 h) immediately before experiments.

Carbon fiber microelectrodes (Dagan Instruments) with a diameter of 5 μm were positioned adjacent to individual cells and polarized to +800 mV to allow oxidation of released catecholamine. Resulting 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). Unless otherwise stated, each experiment consisted of current recordings of a brief control period during which cells were perfused with standard external medium (5 mM K^+ concentration). This was then exchanged for a test solution, and amperometric signals were recorded for a further period of 1–4 min. Catecholamine secretion was apparent as discrete spikelike events, each corresponding to the released contents of a single vesicle of catecholamine (5, 35). Quantification of release was achieved by counting spikes using Minian 16 software (Jaejin Software, Columbia, NY). This allowed visual inspection of each event so that artifacts (due, for example, to solution switches) could be rejected from analysis. Integration of individual amperometric events allowed measurement of quantal size (see Fig. 2), as previously described (25, 28).

To record Ca^{2+} channel currents, cells were perfused with a solution of the following composition (in mM): 110 NaCl, 5 CsCl, 0.6 $MgCl_2$, 20 $BaCl_2$, 5 HEPES, 10 glucose, and 20 tetraethylammonium-Cl, pH 7.4. Osmolality of the perfusate was adjusted to 300 mosmol/kgH_2O by addition of sucrose. Patch pipettes (5–7 MΩ resistance) were filled with a solution of (in mM) 130 CsCl, 1.1 EGTA, 2 $MgCl_2$, 0.1 $CaCl_2$, 10 NaCl, 10 HEPES, and 2 Na_2ATP, pH 7.2. After the whole cell configuration was established, cells were voltage clamped at −80 mV. To evoke whole cell Ca^{2+} channel currents, 200-ms voltage ramps were applied from −100 mV to +100 mV at a frequency of 0.2 Hz (10). As for amperometric recordings, evoked currents were filtered at 1 kHz, digitized at 2 kHz, and stored on computer for offline analysis.

All results are presented as individual examples or means ± SE, and statistical comparisons were made using an unpaired Student's *t*-test unless stated otherwise.

RESULTS

Secretory responses to high (50 mM) K^+-containing solutions of PC-12 cells are shown in Fig. 1, *A–C*. It is apparent from these examples and from the mean exocytotic frequency plot of Fig. 1*D* that 24-h exposure of cells to PrP106–126 caused a concentration-depen-

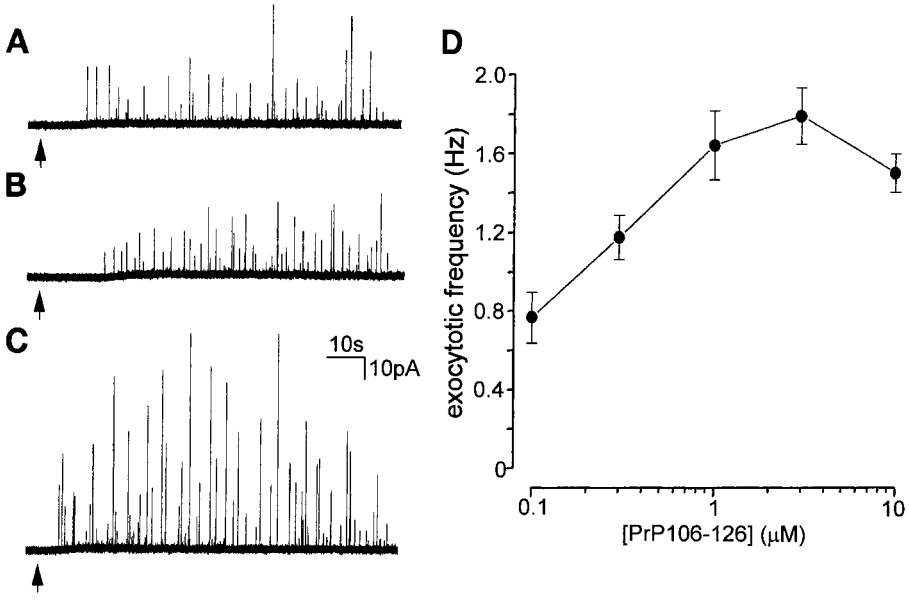

Fig. 1. Evoked, quantal catecholamine release is potentiated in prion protein fragment 106–126 (PrP106–126)-treated PC-12 cells. *A–C*: representative amperometric recordings obtained in response to elevated extracellular [K^+] from a control PC-12 cell (*A*), a cell treated for 24 h with 0.3 μM PrP106–126 (*B*), and one treated with 3 μM PrP106–126 for 24 h (*C*). Solution exchange to one containing 50 mM K^+ occurred at the point indicated by the arrow in each case. Scale bars apply to all 3 traces. *D*: concentration-response relationship for the effects of 24-h pretreatment of PC-12 cells with PrP106–126 on exocytotic frequency, determined over a 60-s period beginning 10 s after onset of 50 mM K^+ application. Each point represents the mean (with vertical SE bars) frequency determined from between 8 and 16 cells.

dent increase in the secretory response of cells, reaching a maximal increase at a PrP106–126 concentration of 1–3 μM, where the response to 50 mM K^+ was increased more than twofold. That this effect was specific to PrP106–126 was evidenced by the fact that a scrambled version of the peptide was without significant effect on K^+-evoked secretion (mean exocytotic frequency 0.76 ± 0.15 Hz, $n = 8$; not significantly different from control cells). In control solutions (containing 5 mM K^+), no secretory events were detected in control or PrP106–126-treated cells. Measurement of quantal size, obtained by integrating individual exocytotic events, revealed no statistical difference ($P > 0.2$) between control and PrP106–126-treated cells in terms of mean quantal size (Fig. 2), although quantal sizes had a somewhat broader distribution in PrP106–126-treated cells than was seen in controls. Mean ± SD values for controls were 0.37 ± 0.10 $pC^{1/3}$ and for PrP106–126-treated cells were 0.38 ± 0.14 $pC^{1/3}$.

We have previously demonstrated that K^+-evoked secretion of catecholamines from PC-12 cells is entirely dependent on Ca^{2+} influx through voltage-gated Ca^{2+} channels, since it can be abolished in Ca^{2+}-free solutions or by bath application of the nonselective Ca^{2+} channel inhibitor Cd^{2+} (26, 27). In PrP106–126-treated cells, K^+-evoked secretion was also fully and reversibly abolished by replacement of extracellular Ca^{2+} with 1 mM EGTA (Fig. 3*A*; representative of 13 cells examined). However, secretion was not abolished in the presence of 200 μM Cd^{2+} (Fig. 3*B*, representative of 13 cells examined). Instead, ~40% of the secretory response remained (see also Fig. 6). Release evoked in scrambled PrP106–126-treated cells was completely abolished in the presence of Cd^{2+} (data not shown). Thus complete blockade of voltage-gated Ca^{2+} channels failed to prevent fully the secretory response, strongly suggesting that exposure to PrP106–126 induced a distinct Ca^{2+} influx pathway that contributed to the secretory response. When PrP106–126 was applied for 24 h in the continual presence of Congo Red (500 μM), K^+-evoked secretion was not significantly altered (0.83 ± 0.19 Hz, $n = 10$), and Cd^{2+}-resistant secretion was reduced by ~80% (see Fig. 4) to 0.13 ± 0.05 Hz ($n = 10$).

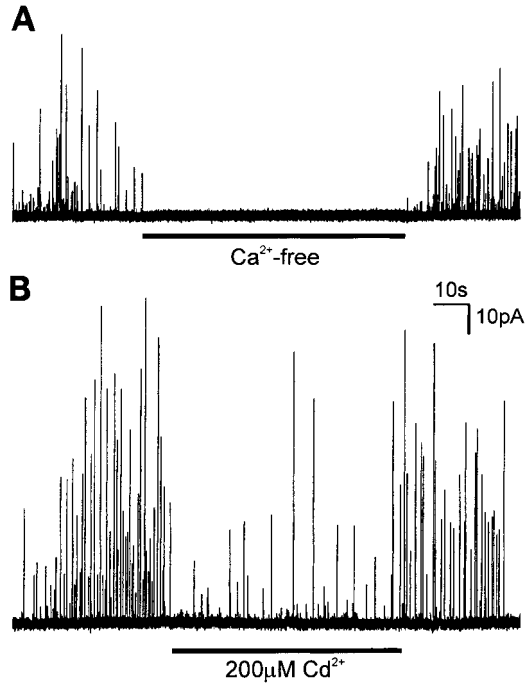

Fig. 3. Ca^{2+} dependence of K^+-evoked catecholamine release in PrP106–126-treated PC-12 cells. *A*: amperometric recording of ongoing secretion of catecholamines from a representative PC-12 cell in response to 50 mM K^+-containing solution. For the period indicated by the bar, the perfusate was exchanged for one containing no added Ca^{2+} and 1 mM EGTA in the continued presence of 50 mM K^+. *B*: as in *A*, except for the period indicated by the bar, when 200 μM Cd^{2+} was added to the perfusate in the continued presence of Ca^{2+} and 50 mM K^+. Note that Cd^{2+} reduced, but did not abolish, secretion. Scale bars apply to both traces.

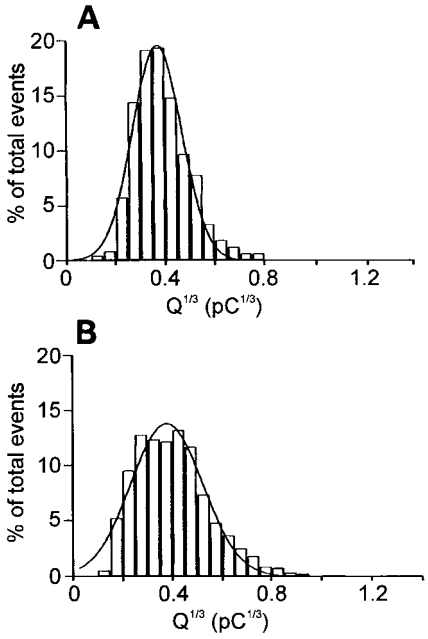

Fig. 2. PrP106–126 treatment does not alter quantal size. Plots of the distribution of $Q^{1/3}$ (determined by integration of exocytotic events evoked by 50 mM K^+) in 9 control cells (*A*; total number of events 485) and 9 PrP106–126-treated cells (*B*; total number of events 952).

The time course of emergence of the Cd^{2+}-resistant secretory response, and indeed the enhancement of the total secretory response, was studied by exposing cells to 1 μM PrP106–126 for between 1 and 48 h. Results are plotted in Fig. 4, which shows that the time course of enhancement of total secretory response (closed circles) was followed closely by the time course of emergence of the Cd^{2+}-resistant secretory pathway: in both cases, maximal enhancement was observed after a 6-h exposure period. This plot also reveals that the magnitude and time course of enhancement of total secretion can be accounted for by the emergence of the Cd^{2+}-resistant component of secretion.

Our findings thus far suggested that PrP106–126-induced potentiation of the secretory response of PC-12 cells was due to the formation of a Cd^{2+}-resistant Ca^{2+}

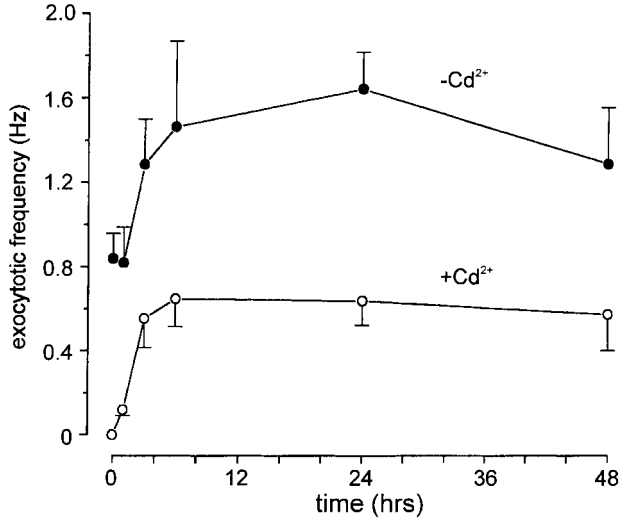

Fig. 4. Time course of enhancement of K^+-evoked catecholamine secretion in PC-12 cells. Shown is a plot of mean exocytotic frequency determined in cells exposed to PrP106–126 for the periods indicated (up to 48 h). Exocytosis was evoked by exposure of cells to 50 mM K^+-containing solution either in the absence (●) or presence (○) of 200 μM Cd^{2+}. Each point represents the mean (with vertical SE bars) frequency determined from between 7 and 16 cells.

influx pathway. To probe the properties of this pathway further, we investigated the ability of other cations to block Cd^{2+}-resistant secretion. We tested two divalent cations (Ni^{2+} and Zn^{2+}) and two trivalent cations (Gd^{3+} and La^{3+}), all at a concentration of 1 mM and in the additional presence of 200 μM Cd^{2+}, to compare results with those obtained when cells are treated with AβPs (25). These agents are known to block various Ca^{2+} influx pathways in other cell types (28). Results are exemplified and summarized in Fig. 5. All four cations suppressed secretion more than Cd^{2+} did alone, although for Ni^{2+}, this effect was found with the other cations. The potency order was $La^{3+} > Gd^{3+} > Zn^{2+} > Ni^{2+}$ (Fig. 5*E*), the same as that seen for AβP-treated cells (25).

Control PC-12 cells possess L-, N-, and P/Q-type voltage-gated Ca^{2+} channels (19, 27). Ca^{2+} influx through each of these channels can contribute to catecholamine release in a stimulus-specific manner (25–29). We compared the ability of selective inhibitors of these channels to modulate K^+-evoked secretion from control and PrP106–126-treated cells. Statistical analysis was performed using ANOVA with both Dunnett's and Bonferroni's multiple comparison post hoc tests (both of which produced the same significance values). As shown in Fig. 6, release evoked from control cells was mediated by Ca^{2+} influx through L- and N-type channels, since nifedipine (an L-type channel blocker) and ω-CgTx (an N-type blocker) each reduced the secretory response by ~50% ($P < 0.05$ and $P < 0.01$, respectively), whereas ω-AgaTx (a P/Q-type blocker) was without significant effect. Furthermore, exposure of cells to both nifedipine and ω-CgTx was almost as effective as Cd^{2+} in suppressing the secretory response. Responses in PrP106–126-treated cells were markedly different. In these cells, nifedipine was almost as effective as Cd^{2+} in suppressing the secretory response ($P < 0.01$), whereas ω-CgTx was without significant effect [as was ω-AgaTx; Fig. 6, although significant reduction ($P < 0.05$) was seen when both toxins were applied together]. Thus, in addition to inducing a Cd^{2+}-resistant Ca^{2+} influx pathway coupled to secretion, PrP106–126 appeared to prevent coupling of N-type channels to secretion, whereas coupling of L-type was promoted.

The altered pharmacology of the secretory response caused by PrP106–126 led us to investigate Ca^{2+} influx pathways more directly, using whole cell patch-clamp recordings. Figure 7, *A* and *B*, compares mean current densities in control (Fig. 7*A*) and PrP106–126-treated (Fig. 7*B*) cells. In both cases, current density

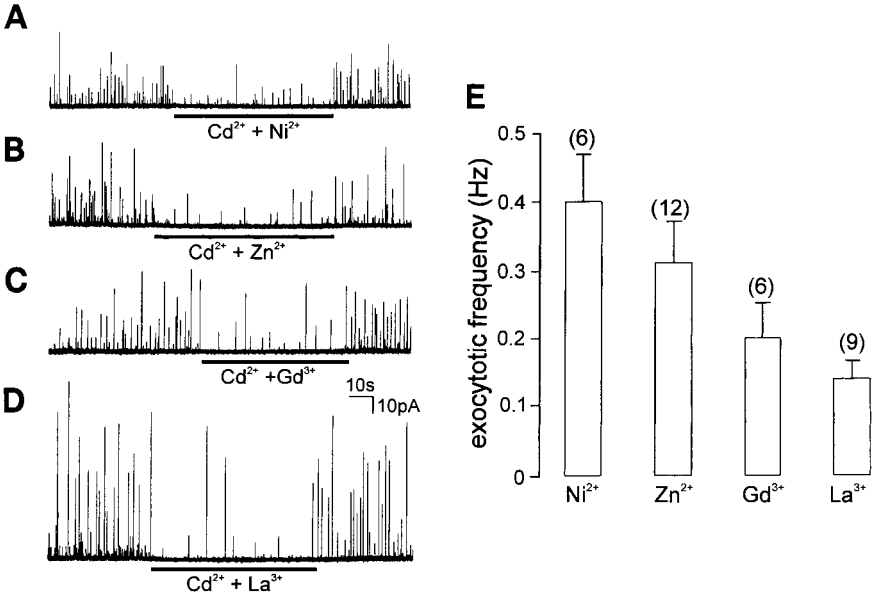

Fig. 5. Effects of inorganic cations on Cd^{2+}-resistant secretion from PrP106–126-treated cells. *A–D*: ongoing secretion evoked from cells by exposure to 50 mM K^+. For the periods indicated by the bars in each trace, cells were exposed to a perfusate containing 200 μM Cd^{2+} plus 1 mM of Ni^{2+} (*A*), Zn^{2+} (*B*), Gd^{3+} (*C*), or La^{3+} (*D*). Scale bars apply to all 4 traces. *E*: mean (± SE) exocytotic frequency evoked by 50 mM K^+ in PrP106–126-treated cells during exposure to 200 μM Cd^{2+} plus 1 of the 4 cations illustrated in *A–D*.

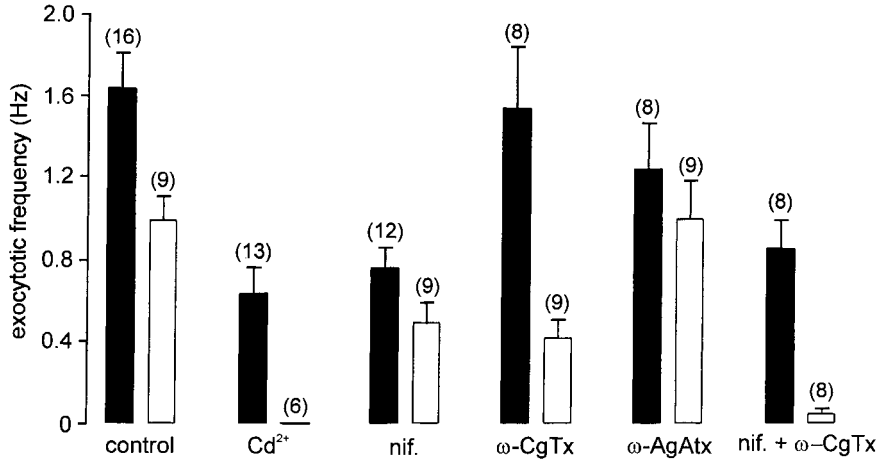

Fig. 6. Pharmacological profile of K^+-evoked exocytosis and its modulation by PrP106–126 treatment. Bar graph illustrating mean (±SE) exocytotic frequency evoked in control cells (open bars) and cells exposed to 1 μM PrP106–126 for 24 h (solid bars). Secretion was monitored in the absence or presence of the Ca^{2+} channel blockers indicated (200 μM Cd^{2+}; 2 μM nifedipine; pretreatment with 1 μM ω-CgTx; or 200 nM ω-AgaTx). Numbers in parentheses above each bar indicate number of cells studied. nif, Nifedipine.

vs. voltage relationships were bell-shaped, typical of Ca^{2+} channel currents, and were maximal in amplitude at +20 mV. Importantly, and perhaps unexpectedly, mean current density was not significantly different at any activating test potential in the two groups of cells. Furthermore, Cd^{2+} (200 μM) caused near complete inhibition of these currents (open symbols, Fig. 7, *A* and *B*). However, as shown in Fig. 7*C*, closer comparison of the Cd^{2+}-resistant Ca^{2+} channel currents revealed that these residual inward currents were much greater in amplitude in PrP106–126-treated cells than in controls, indicating the presence of a small but significant Cd^{2+}-resistant Ca^{2+} channel in this group of cells. Indeed, in control cells at higher test potentials, currents were outward, due to either incomplete block of outward K^+ currents or outward Cs^+ flow through Ca^{2+} channels. The inward, Cd^{2+}-resistant currents seen in PrP106–126-treated cells could be blocked by both Zn^{2+} and La^{3+} (both applied at a concentration of 1 mM in the presence of 200 μM Cd^{2+}), further suggesting that this current contributed to Cd^{2+}-resistant secretion following exposure to PrP106–126.

Given the different pharmacological profile of secretion following PrP106–126 treatment (Fig. 6), we next investigated the contribution of L-type channels to the total whole cell Ca^{2+} channel current in control and PrP106–126-treated cells by examining their responses to nifedipine. Results are plotted in Fig. 8, which reveals that in control cells, nifedipine (2 μM) reduced current amplitudes by 58.9 ± 7.3% (as determined at +20-mV test potential, $P < 0.002$, $n = 9$ cells). In PrP106–126-treated cells, the same concentration of nifedipine reduced currents measured at +20-mV test potential by 38.6 ± 5.4% ($P < 0.02$, $n = 9$). This value was not significantly different from the degree of blockade observed in control cells, indicating that the greater influence on secretion of L-type channels in PrP106–126-treated cells (Fig. 6) was not due to a selective enhancement of their expression or activity as determined at the level of whole cell patch-clamp recordings.

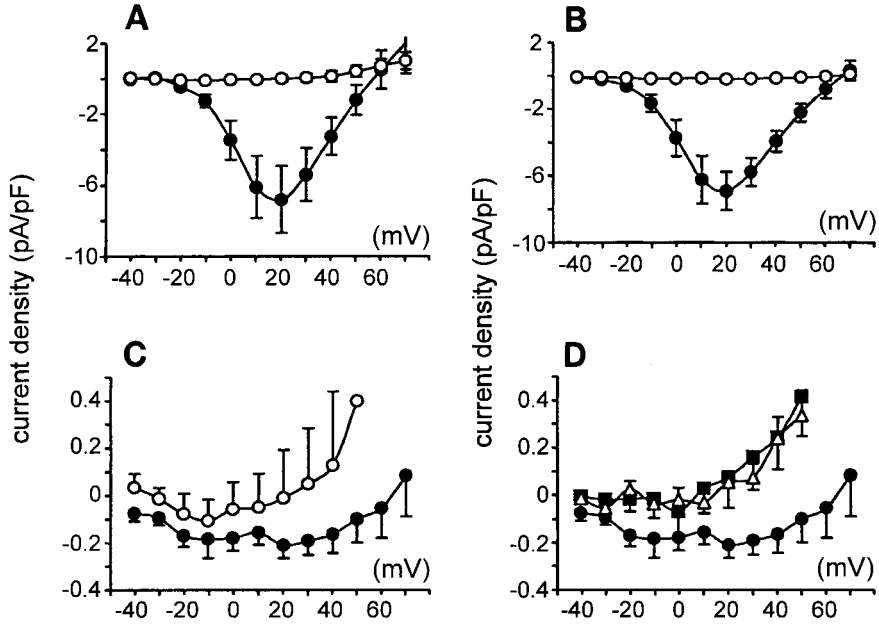

Fig. 7. Whole cell Ca^{2+} channel currents in control and PrP106–126-treated cells. Currents were evoked by ramp depolarizations (see MATERIALS AND METHODS) in control cells (*A*; $n = 8$) and cells pretreated with 1 μM PrP106–126 for 24 h (*B*; $n = 15$) in the absence (solid symbols) or presence (open symbols) of 200 μM Cd^{2+}. *C*: higher resolution plot of residual currents recorded in the presence of 200 μM Cd^{2+} in control (open symbols) and PrP106–126-treated cells (solid symbols). Data from open symbols of *A* and *B*, respectively. *D*: Cd^{2+}-resistant currents recorded in PrP106–126-treated cells in the absence of other blockers (●, taken from *C*) or in the additional presence of 1 mM Zn^{2+} (■, $n = 7$) or 1 mM La^{3+} (△, $n = 8$). Each point plotted in *A*–*D* represents the mean ± SE.

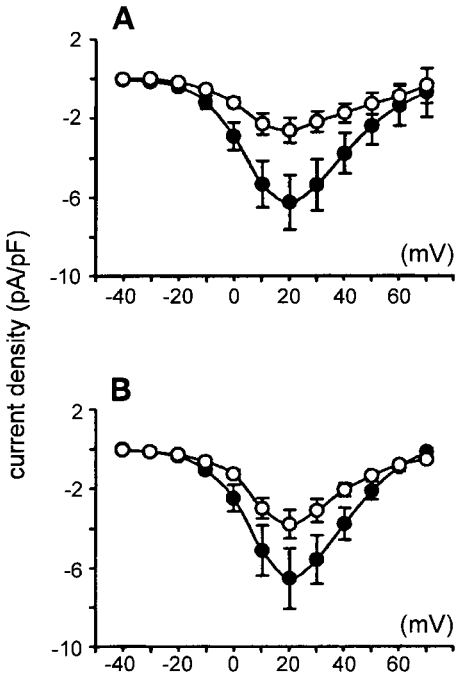

Fig. 8. Effects of nifedipine on Ca^{2+} channel currents in control and PrP106–126-treated cells. *A*: mean (±SE) current-voltage relationships from 8 control cells in the absence (●) and presence (○) of 2 μM nifedipine. *B*: mean (±SE) current-voltage relationships from 8 cells pretreated with 1 μM PrP106–126 for 24 h in the absence (●) and presence (○) of 2 μM nifedipine.

DISCUSSION

In this study, we examined the cellular effects of PrP106–126, the toxic fragment of PrP^{sc}, in the well-defined neuroendocrine cell line PC-12. Such effects of this peptide have not been extensively studied to date, despite the attention that transmissible spongiform encephalopathies have received in recent years due to contamination of the food chain and resultant implications for public health. The major observation of the present study is that PrP106–126 markedly augments evoked catecholamine release from PC-12 cells, as determined in real time using amperometry (Fig. 1). Amperometry limits our study to specific release of catecholamine from these cells (5), but the similar distribution of vesicular sizes in control and PrP106–126-treated cells (Fig. 2) strongly suggests that the same vesicular pool is mobilized during K^+ depolarization in both groups. Importantly, the enhancement of exocytosis can be accounted for by the emergence of a Cd^{2+}-resistant Ca^{2+} influx pathway coupled to secretion that is not present in control cells (Figs. 3 and 4) or in cells treated with a scrambled version of the peptide.

Induction of a Cd^{2+}-resistant Ca^{2+} influx pathway by PrP106–126 is remarkably similar to the effects of treating PC-12 cells with AβPs that we have previously reported (25). Such similarities have been documented by others also. For example, in GT1–7 cells derived from hypothalamic tissue, AβPs and PrP106–126 raise intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) by stimulating Ca^{2+} influx (15). However, such influx was observed in the absence of depolarizing stimuli. Our results suggest that if PrP106–126 does raise basal $[Ca^{2+}]_i$, this is not sufficient to evoke catecholamine secretion per se. A depolarizing stimulus is still also required. The reason for this discrepancy is unclear at present, but it is noteworthy that Kawahara et al. (15) exposed cells to high peptide concentrations for brief periods of time, whereas in the present study, and in our previous investigation of the effects of AβPs (25), we employed much lower peptide concentrations, applied for much longer periods of time. This difference may lead to different patterns of peptide aggregation and insertion into the plasma membrane. Interestingly, these effects of PrP106–126 were almost completely inhibited by coincubation with Congo Red, which prevents aggregation of PrP and related peptides, indicating that aggregation is a requisite step in channel formation (see Ref. 17 and references therein).

Numerous similarities in the properties of ion channels formed from cytotoxic peptides have been highlighted (17). In accordance with this, our observation that PrP106–126 potentiates evoked secretion in a manner comparable with that of AβPs indicates further pathophysiological similarities between these peptides. We also report similar pharmacological properties. Figure 5 indicates that Cd^{2+}-resistant secretion is blocked by cations with a potency order $La^{3+} > Gd^{3+} > Zn^{2+} > Ni^{2+}$, identical to that for AβP-mediated, Cd^{2+}-resistant secretion induced by chronic hypoxia (25, 28). Perhaps surprisingly, given the marked influence on secretion of PrP106–126, when we investigated this Ca^{2+} influx pathway more directly with patch-clamp recordings, current amplitude was extremely small (<5% of total Ca^{2+} channel current amplitude, Fig. 7). Once again, however, this is comparable with the effects of AβPs (10). For such a small current to exert such a marked influence on secretion suggests that there may be specific localization of these channels to sites of exocytosis. For native Ca^{2+} channels, direct binding of channel proteins to proteins associated with the exocytotic apparatus has been demonstrated (3). It is conceivable, therefore, that PrP106–126 might interact in a comparable way such that the small amount of Ca^{2+} that enters the cell through these channels is targeted at release sites to optimize exocytotic efficiency. In this regard, it is of interest to note that PrP^{sc} has been localized in detergent-insoluble lipid "raft" domains within cells (16, 21). Such rafts in PC-12 cells are also enriched with soluble *N*-ethylmaleimide-sensitive factor attachment protein target receptor proteins essential for regulated exocytosis (4). Thus there is likely a direct association of prion proteins in the process of Ca^{2+}-dependent exocytosis.

Modulation of existing, native voltage-gated Ca^{2+} channels (particularly L-type channels) is a well-documented cellular effect of PrP106–126. However, the specific modulation of such channels appears variable. Thus, in astrocytes and microglia, PrP106–126 potentiates L-type channel activity (23, 30) and appears to enhance Ca^{2+} influx through voltage-gated Ca^{2+} channels in synaptosomes (34). In contrast, L-type channel

activity appears to be inhibited by PrP106–126 in neuroectodermal GH_3 cells and in isolated cerebellar granule neurons (8, 30, 31). The present study indicates that in PC-12 cells, PrP106–126 does not alter the activity of L-type Ca^{2+} channels, since their contribution to total whole cell Ca^{2+} current density was unaffected (Fig. 8). However, their influence on a specific cellular function was dramatically altered. Thus, after treatment with PrP106–126, nifedipine was virtually as effective as Cd^{2+} in suppressing the secretory response to 50 mM K^+, and, furthermore, ω-CgTx was without significant effect (Fig. 6). This observation suggests that PrP106–126 may act intracellularly to promote coupling of exocytosis to Ca^{2+} influx through L-type channels (without affecting their activity, as determined by patch-clamp recordings; Fig. 8) while inhibiting the contribution to secretion of Ca^{2+} influx through N-type channels. The mechanism(s) underlying such an effect remains to be determined but has important potential implications for transmitter release in other cells types, since Ca^{2+} channel expression within a given cell is targeted so that specific channel subtypes are present at transmitter release sites, such as nerve terminals or boutons (3).

In summary, the present study indicates that PrP106–126 markedly augments evoked catecholamine secretion from PC-12 cells. This occurs largely by inducing a Cd^{2+}-resistant Ca^{2+} influx pathway sensitive to blockade by divalent and trivalent cations. In addition, the coupling of native, voltage-gated Ca^{2+} channels is altered such that the influence of N-type channels on secretion is diminished and the influence of L-type is enhanced, without an increase in the contribution of these channels to total whole cell current. Our results provide novel data to indicate that PrP106–126 can exert multiple effects on secretory cells and have implications for understanding the pathophysiological actions of PrP^{sc} at the cellular level.

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