

Protein Kinase C Regulates a Vesicular Class of Calcium Channels in the Bag Cell Neurons of *Aplysia*

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White, Benjamin H., Teresa A. Nick, Thomas J. Carew, and Leonard K. Kaczmarek. Protein kinase C regulates a vesicular class of calcium channels in the bag cell neurons of *Aplysia*. *J. Neurophysiol.* 80: 2514–2520, 1998. Protein kinase C (PKC) acutely increases calcium currents in *Aplysia* bag cell neurons by recruiting calcium channels different from those constitutively active in the plasma membrane. To study the mechanism of PKC regulation we previously identified two calcium channel α_1 -subunits expressed in bag cell neurons. One of these, BC- α_{1A} , is localized to vesicles concentrated primarily in somata and growth cones. We used antibodies to BC- α_{1A} to analyze its expression in the bag cell neurons of juvenile *Aplysia* at a developmental stage at which PKC-sensitive calcium currents have previously been shown to be low. We find that vesicular BC- α_{1A} staining is generally reduced in juvenile bag cell neurons but that its expression level can vary among juvenile animals. In 17 bag cell clusters examined, the percentage of neurons that displayed punctate α BC- α_{1A} staining ranged from 0 to 85%. Sampling of calcium currents from cells of the same clusters by whole cell patch-clamp techniques revealed that the PKC-sensitive calcium current density is significantly correlated with the degree of vesicular staining. In contrast, no correlation of basal calcium current levels with α BC- α_{1A} staining was found. These results strongly suggest that BC- α_{1A} , a member of the ABE-subfamily of calcium channels, carries the PKC-sensitive calcium current in bag cell neurons. They are consistent with a model in which PKC recruits channels from the vesicular pool to the plasma membrane.

INTRODUCTION

The essential role of calcium in neurosecretion and gene regulation and in the regulation of diverse enzymes makes calcium channels a prime target for the modulation of neuronal function. In recent years, G-proteins have emerged as prominent negative regulators of calcium channel function (Dolphin 1996; Hille 1994), and protein kinases have been frequently, although not exclusively, identified as positive regulators of calcium channels (Dolphin 1996; Tsien et al. 1988). Adenosine 3',5'-cyclic monophosphate-dependent protein kinase (Armstrong and Eckert 1987; Artalejo et al. 1990), guanosine 3,5'-cyclic monophosphate-dependent protein kinase (Paupardin-Tritsch et al. 1986), and protein kinase C (PKC) (Swartz 1993; Yang and Tsien 1993) have all been shown to potentiate calcium currents in various neurons or neuroendocrine cells. A particularly interesting example of calcium current enhancement by PKC is found

in the bag cell neurons of *Aplysia californica*, where PKC has been shown to enhance current not by modulating the activity of an already active calcium channel but by recruiting a previously inactive type of calcium channel (DeRiemer et al. 1985; Strong et al. 1987).

Patch-clamp analysis of bag cell neurons identified two types of calcium current, a basal current carried by a 12-pS channel constitutively present in the plasma membrane and a PKC-sensitive current carried by a 24-pS channel seen only after treatment of bag cell neurons with activators of PKC (Strong et al. 1987). A unique feature of calcium channel modulation in bag cell neurons is its sensitivity to whole cell recording conditions. Unlike kinase-mediated modulation of calcium channels observed in other cell types, recruitment is disrupted by the formation of whole cell patches before PKC activation (DeRiemer et al. 1985; Strong et al. 1987). The recruited channels themselves, however, are not sensitive to patch formation. Once they have been recruited, their activity can readily be recorded by either whole cell or cell-attached patch-clamp techniques. These observations suggest that the mechanism of recruitment is indirect. Strong et al. (1987) proposed that the PKC-sensitive channels may reside in a subcellular pool and be inserted into the membrane after PKC stimulation. This conclusion is consistent with calcium imaging studies, which show that PKC-sensitive calcium influx into bag cell neuron growth cones occurs at sites of apparent membrane addition (Knox et al. 1992). The identity of the channel regulated by PKC and its localization in bag cell neurons, however, remained undetermined.

We recently identified two molecular species of calcium channel expressed in bag cell neurons and showed that one of them, BC- α_{1A} ,¹ is localized to vesicles in the somata and growth cones of adult bag cell neurons (White and Kaczmarek 1997). We now show that BC- α_{1A} is present in the cultured bag cell neurons of a subpopulation of juvenile *Aplysia* and that its presence in vesicles correlates with the presence of PKC-sensitive calcium current. Correlation of the vesicularly localized class of calcium channels with the channels regulated by PKC suggests that PKC may act to enhance calcium currents by recruiting channels to the plasma membrane from a subplasmalemmal pool.

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¹ BC- α_{1A} designates the bag cell neuron calcium channel BCCA-I originally described by White and Kaczmarek (1997). Similarly, BC- α_{1D} in this paper designates the previously described BCCA-II. The new designations indicate the class of mammalian α_1 -subunit to which each *Aplysia* channel appears to be most closely related.

METHODS

Animals

Juvenile *Aplysia californica* were distinguished by weight, which has previously been shown to be a good predictor of afterdischarge capacity (Nick et al. 1996). All juveniles used weighed between 4 and 11 g and were obtained from the Aplysia Resource Facility (RSMAS, University of Miami).

Immunocytochemistry

Whole mount staining was carried out as follows. Animals were anesthetized with an injection of isotonic $MgCl_2$ (to 50% of body weight), and the pleural, pedal, and abdominal ganglia were excised together with their connective nerves into 0.5% Disperse in artificial seawater [ASW; 460 mM NaCl, 10.4 mM KCl, 55 mM $MgCl_2$, 11 mM $CaCl_2$, 15 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), 1 g/l glucose, 100,000 units/l penicillin, 100 mg/l streptomycin, pH 7.8] for 30 min. Preparations were then fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) for 3 h at 4°C before 20 min treatment with 0.1% trypsin in PBS at 23°C. Samples were thoroughly washed and exchanged into 4% Triton X-100 in PBS for 1 h before blocking in blocking solution (4% goat serum and 0.5% Triton X-100 in PBS) for 12 h at 4°C. Each sample was incubated 2 days at 4°C with rabbit primary antibodies in blocking solution. Affinity-purified antibodies to the two bag cell neuron calcium channels, BC- α_{1A} and BC- α_{1D} , were used at 5 μ g/ml as described by White and Kaczmarek (1997). A rabbit immunoglobulin fraction to human glial fibrillary acidic protein from Sigma Chemicals (St. Louis) was also used at 5 μ g/ml as a control. Mammalian glial markers have previously been shown not to label glia in *Aplysia* (Lockhart et al. 1996). The rabbit antibody against egg-laying hormone (ELH) was the kind gift of Dr. Arlene Chiu and was used at a dilution of 1:1,000. Samples were washed 2 h in blocking solution and then incubated with fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit secondary antibody for 24 h before a final wash. Samples were mounted with Vectashield (Vector Laboratories, Burlingame, CA) on shimmied coverslips for analysis by confocal microscopy. Confocal microscopy was carried out with the BioRad MRC-600 laser system coupled to a Zeiss Axiovert 35 microscope outfitted with a fluorescein filter. Confocal imaging was carried out with a $\times 25$ oil-immersion objective, and five images were averaged to produce the final images.

For staining of cells in culture, the abdominal ganglia and pleural-abdominal connectives of anesthetized animals were excised into 1.3% Disperse in ASW for 18 h digestion at 23°C. Bag cell clusters were dissociated and plated in ASW on No. 1 coverslips coated with poly D-lysine (1 μ g/ml) as described by White and Kaczmarek (1997). One juvenile cluster was plated per coverslip. Bag cell neurons were fixed in 4% paraformaldehyde in ASW with 400 mM sucrose for 25 min and then immediately permeabilized for 5 min with 0.3% Triton X-100 in fixative before exchange into PBS and blocking with 5% goat serum in PBS. Bag cell neurons were stained overnight at 4°C with affinity-purified rabbit antibodies to the *Aplysia* calcium channel BC- α_{1A} . The primary antibody solution contained 5 μ g/ml α BC- α_{1A} in 5% goat serum-PBS. In control experiments, α BC- α_{1A} antibodies were preincubated for 3 h at room temperature with a 50-fold excess of either FP-I (the glutathione-*S*-transferase fusion protein to which α BC- α_{1A} was raised) or FP-II (the glutathione-*S*-transferase fusion protein to which α BC- α_{1D} was raised) before centrifugation and incubation. In other controls, the primary antibody was omitted, and neurons were incubated at 4°C in blocking solution overnight. Coverslips were washed thoroughly with PBS both before and after a 4-h incubation with a Texas red-conjugated goat anti-rabbit secondary

antibody. Coverslips were finally mounted on glass slides shimmied on both sides with No. 1 coverslips for fluorescence microscopy. Bag cell neurons were identified morphologically.

In experiments where staining followed recording of calcium currents, bag cell neurons were fixed and permeabilized ≤ 4 h after recording. In most cases, incubation with α BC- α_{1A} was started the same day, and a rat α ELH antibody (kindly provided by Dr. Richard Scheller) was typically included at 1:100 dilution to help positively identify bag cell neurons. Control incubations without α BC- α_{1A} were also typically performed on cultured neurons from paired bag cell clusters to verify the specificity of punctate staining. Secondary antibody staining was performed as described previously except that an FITC-conjugated goat anti-rat secondary antibody was included in those cases where rat α ELH was used.

The percentage of neurons showing punctate staining on each coverslip was measured as follows. Bag cell neurons on a coverslip were scored as punctate if they contained a minimum of five of the "puncta" characteristic of staining with the α BC- α_{1A} antibody (White and Kaczmarek 1997). Typically, each cell encountered was scored as the field of plated cells on a coverslip was systematically traversed. In most cases, all bag cell neurons on the slide were scored, but in cases where the trend was unambiguous (e.g., very little punctate staining in any of the neurons) a minimum of 20 bag cell neurons per coverslip was scored. A "percentage punctate" value was assigned to each coverslip by dividing the number of cells scored as punctate by the total number of cells scored ($\times 100$).

Electrophysiology on juvenile bag cell neurons

Bag cell neurons were dissociated as described previously and cultured at 15°C for 1 day before current measurements. Calcium currents were measured by the whole cell patch-clamp technique (Hamill et al. 1981) by using electrodes with resistances < 1.5 M Ω . A 486DX computer with a Digidata 1200 A/D converter (Axon Instruments, Foster City, CA) was used to deliver current pulses in current-clamp mode and voltage steps in voltage-clamp mode with pClamp 6 software (Axon). Whole cell electrical signals were amplified with an EPC-7b patch/whole cell clamp (List-electronic, Germany). A 50-G Ω resistor was used for measurement of currents. Calcium currents were filtered at 3 kHz with an eight-pole Bessel filter and sampled at 600 Hz. Voltage step duration was 300 ms, except when measuring capacitance, in which case it was 120 ms. Series resistance and capacitance transients were compensated before ionic current measurement, and currents were leak-subtracted with a P/8 protocol.

The pipette solution used was designed to block outward currents. It contained (in mM) 10 reduced glutathione, 5 $MgCl_2$, 20 ethylene glycol-bis(β -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid, 4.14 $CaCl_2$, 470 CsOH, 100 TEA-OH, 570 K^+ -aspartate, 5 ATP, 0.1 GTP, 1 mg/ml glucose, 10 HEPES, pH 7.3. The external solution was a modified ASW with choline chloride substituted for NaCl to remove sodium current. Pipettes were Sylgard coated to reduce capacitive noise. I_{Ca} was revealed by holding at -40 mV and stepping to $+90$ mV in 13 10-mV steps with a 5-s interpulse interval. PKC-sensitive calcium current was estimated as described subsequently from the whole cell currents elicited by the same protocol after addition of 20 nM phorbol 12-myristate 13-acetate (TPA).

Values for raw currents were obtained with the Clampfit subprogram of pClamp 6. Current values near the peak (20–40 ms after pulse initiation) were averaged from four traces for each voltage step to lower the signal to noise ratio. Currents were normalized for cell size by dividing by the cell capacitance to give current densities. Mean current densities are presented with error bars indicating the SE of the mean.

The basal and PKC-sensitive calcium currents for the population

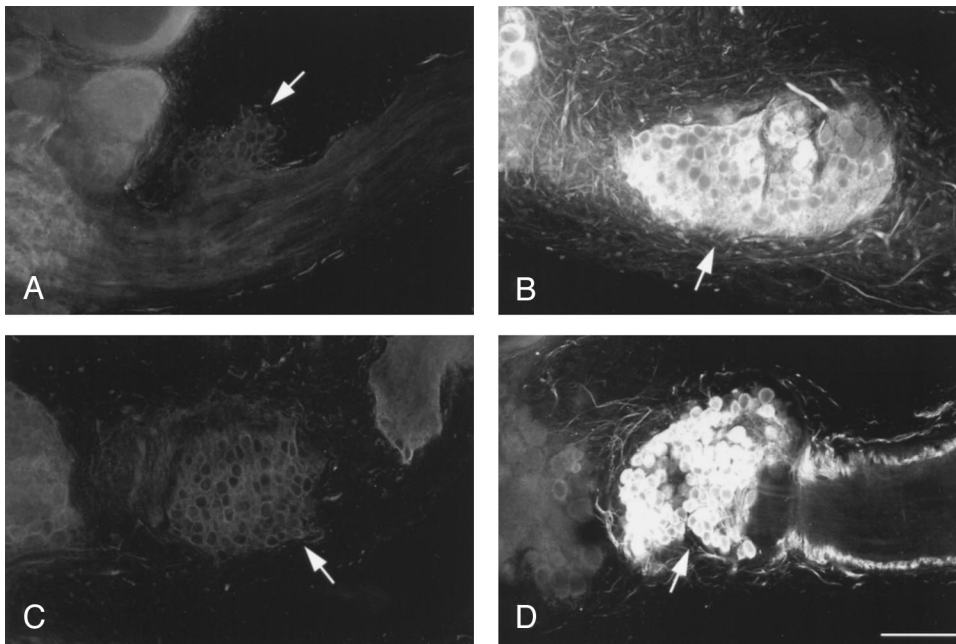


FIG. 1. Bag cell clusters of juvenile *Aplysia* strongly express the BC- α_{1D} calcium channel, but not BC- α_{1A} . Bag cell clusters (arrows) were stained in whole mounts of juvenile abdominal ganglia with antibodies to two calcium channel α_1 -subunits previously shown to be expressed by adult bag cell neurons. *A*: α BC- α_{1A} staining. *B*: α BC- α_{1D} staining. *C*: negative control, staining with anti-human glial fibrillary acidic protein antibodies. *D*: positive control, staining of bag cell neurons for *Aplysia* egg-laying hormone (ELH). Confocal images were taken at identical settings for all four samples. Scale bar is 100 μ m.

of cells on each coverslip were estimated as follows. Calcium current densities were measured in two cells before the addition of TPA and averaged to obtain I_{Ca} . Calcium current densities were then measured in two different cells (see RESULTS) ≥ 30 min after addition of 20 nM TPA. Subtraction of the current-voltage (I - V) curve obtained from the first set of measurements from the I - V curve obtained from the second set then yielded an estimate of the calcium current stimulated by TPA (I_{PKC}). The I - V relationships for both I_{Ca} and I_{PKC} peaked near +30 mV and the current density at this voltage was used for comparisons of current and calcium channel staining.

RESULTS

Whole-mount staining of bag cell clusters from juvenile *Aplysia* with antibodies directed against two calcium channels previously identified in adult bag cell neurons revealed strong differences in the levels of labeling. Affinity-purified antibodies against BC- α_{1A} , a calcium channel α_1 -subunit of the ABE subfamily, produced no significant staining of bag cell clusters above background (Fig. 1, *A* and *C*). In contrast, antibodies to BC- α_{1D} , a calcium channel α_1 -subunit of the SCD subfamily, prominently stained bag cell clusters from juvenile *Aplysia* (Fig. 1*B*). These results contrasted with those previously obtained in adult animals (200–300 g), which showed specific staining of the bag cell neurons with both antibodies (White and Kaczmarek 1997). Because previous studies have shown that juvenile bag cell neurons in culture have significantly more basal calcium current than PKC-sensitive calcium current (Nick et al. 1996), the staining results suggested that BC- α_{1A} may represent the PKC-sensitive calcium channel.

To more closely examine expression levels of BC- α_{1A} , we stained neurons from juvenile bag cell clusters that were triturated and cultured in ASW. We found that α BC- α_{1A} staining of the cultured juvenile neurons was low and exhibited considerable variability (Fig. 2, *A* and *B*). Many cells stained only lightly above background levels (Fig. 2, *C* and *D*), and robust staining, when observed, tended to be re-

stricted to the bag cell neuron somata, sometimes localized to one side, as seen in Fig. 2*A* (see arrow). Closer examination of this staining often showed it to consist of punctate staining of the type seen in adults with this antibody, although such staining was more easily resolved in cells where α BC- α_{1A} staining was less intense and more broadly distributed (Fig. 3). We could not determine if all the α BC- α_{1A} staining was associated with vesicles, but its specificity was evident from its inhibition by preincubation with the fusion protein against which α BC- α_{1A} was made (Fig. 2*C*). Preincubation with this fusion protein reduced staining to levels seen with secondary antibody alone (Fig. 2*D*), whereas preincubation with the fusion protein against which α BC- α_{1D} was made caused no inhibition of staining (Fig. 2*B*). Punctate staining with the α BC- α_{1A} antibody was not overtly altered by treatment with phorbol esters, as previously reported for adult bag cell neurons (White and Kaczmarek 1997).

Expression levels of BC- α_{1A} varied not only among bag cell neurons in the same cluster but also exhibited substantial differences from cluster to cluster. Of the 11 cultured bag cell neuron clusters initially examined three showed virtually no punctate staining of bag cell neurons with the α BC- α_{1A} antibody, whereas three others showed some degree of punctate staining in more than three-quarters of the bag cell neurons. Frequency of punctate staining was intermediate in the other five preparations examined. Cluster-to-cluster differences and the generally low level of staining are likely to account for our failure to detect BC- α_{1A} in the three whole mount preparations we examined. We presume that such differences derive from the variable onset of BC- α_{1A} expression in the bag cell neurons, but we did not systematically investigate the developmental time course of expression.

To determine which of the two electrophysiologically characterized channel types might be the functional correlate of the BC- α_{1A} channel we examined levels of punctate staining in populations of bag cell neurons from which basal and PKC-

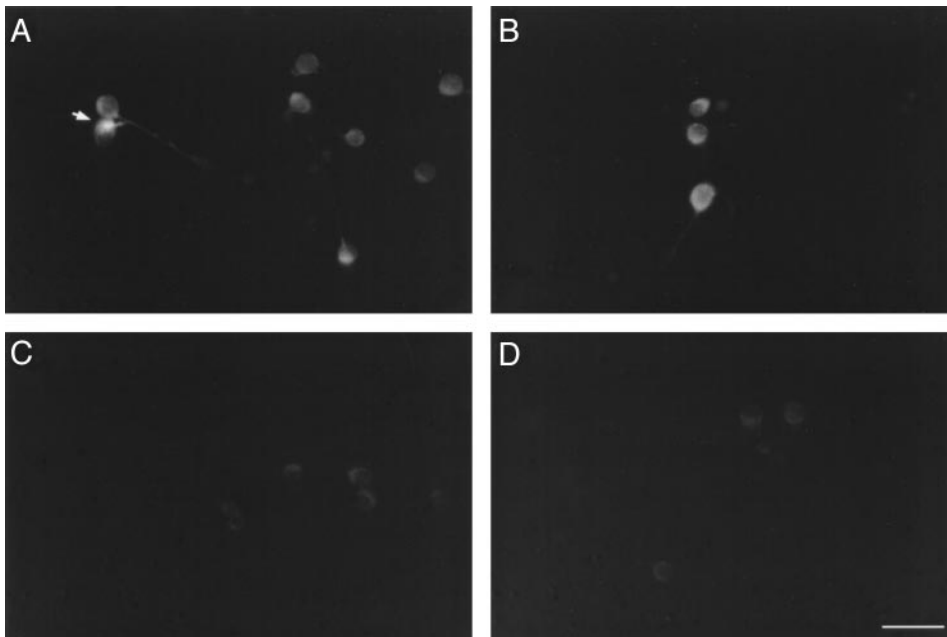


FIG. 2. $\alpha\text{BC-}\alpha_{1A}$ staining of cultured bag cell neurons from juvenile animals is specific, but variable. *A*: cultured bag cell neurons from juvenile *Aplysia* were stained with $\alpha\text{BC-}\alpha_{1A}$. The arrow indicates a "hot-spot" of specific staining in one bag cell neuron. *B*: bag cell neuron staining was not blocked by preincubation of $\alpha\text{BC-}\alpha_{1A}$ with an unrelated fusion protein, but (*C*) was blocked by preincubation with the fusion protein against which $\alpha\text{BC-}\alpha_{1A}$ was made. *D*: background staining from secondary antibody alone is shown for comparison. Scale bar is 50 μm .

sensitive calcium currents were recorded. Due to their small size, measurement of the voltage-sensitive calcium currents in juvenile bag cell neurons required use of the whole cell patch-clamp technique. Because of this, basal and PKC-sensitive currents could not be measured in the same cell since, as previously described in adults (Strong et al. 1987), addition of TPA had no acute effect when currents were recorded in the whole cell mode (data not shown). It was also not possible to examine punctate staining in the same cells from which currents were sampled because the cells did not survive patch pipette removal. Therefore, for each cluster, the average basal calcium current in the cultured bag cell neurons was estimated from whole cell currents measured in two cells before TPA addition. The average PKC-sensitive calcium current was estimated from the currents measured in two more cells after TPA addition. As a measure of the level of punctate staining, we determined the percentage of punctately stained cells in a cluster (see METHODS). Calcium current densities and frequencies of punctate staining were measured for 17 independent clusters. Scoring of cells for punctate staining was carried out blind (i.e., without knowledge of the previous current measurements).

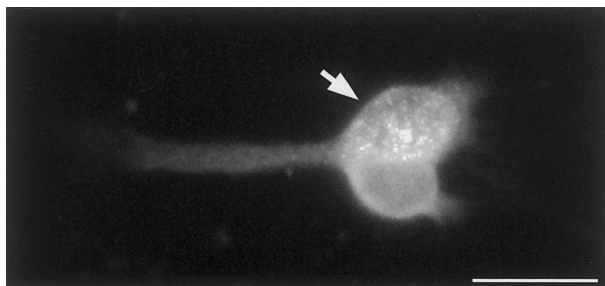


FIG. 3. $\alpha\text{BC-}\alpha_{1A}$ staining is often punctate in juvenile bag cell neurons. The $\text{BC-}\alpha_{1A}$ distribution in positively stained bag cell neurons from juvenile animals was often punctate (arrow). Note the absence of punctate staining in the adjacent bag cell neuron from the same cluster, emphasizing the variability in the staining patterns observed. Scale bar is 15 μm .

As expected from our earlier results, the 17 clusters showed considerable differences in the degree of $\alpha\text{BC-}\alpha_{1A}$ staining, with the percentage of punctately stained neurons ranging from 0 to 85%. Interestingly, the average calcium current densities for the 17 clusters also revealed cluster-to-cluster differences, with the PKC-sensitive current densities varying over twice as large a range as the basal currents. To determine whether the differences in PKC-sensitive calcium current density were likely to correlate with differences in punctate staining we sorted the 17 clusters according to their PKC-sensitive calcium current densities and divided the clusters into two groups along the median (-2.7 nA/nF). These two groups represented clusters with low and high average PKC-sensitive current densities, respectively (Fig. 4*B*, top panel), and for each group we averaged the frequency of punctate staining.

We found that the group of clusters with the high PKC-sensitive calcium current density also had a strikingly higher proportion of neurons with vesicular $\alpha\text{BC-}\alpha_{1A}$ staining (Fig. 4*B*, bottom panel). Indeed, the approximately sixfold difference in peak current densities observed for the two groups was accompanied by a nearly fourfold difference in the frequency of punctate staining. This difference was found to be very significant by the two-tailed *t*-test ($P < 0.01$). To determine whether basal calcium currents might vary together with the PKC-sensitive calcium currents, we also calculated the average basal calcium current densities for the two groups of clusters (data not shown). In contrast with sixfold difference in peak current density exhibited by the PKC-sensitive currents (Fig. 4*B*, top panel), the peak basal current densities were virtually the same for the groups with low and high PKC-sensitive current densities (-3.8 ± 0.6 nA/nF and -4.3 ± 0.6 nA/nF at +30 mV, respectively). This suggested that the basal current densities were correlated with neither the PKC-sensitive currents nor with levels of $\alpha\text{BC-}\alpha_{1A}$ staining.

We confirmed that there was no correlation between basal calcium current densities and levels of punctate staining by

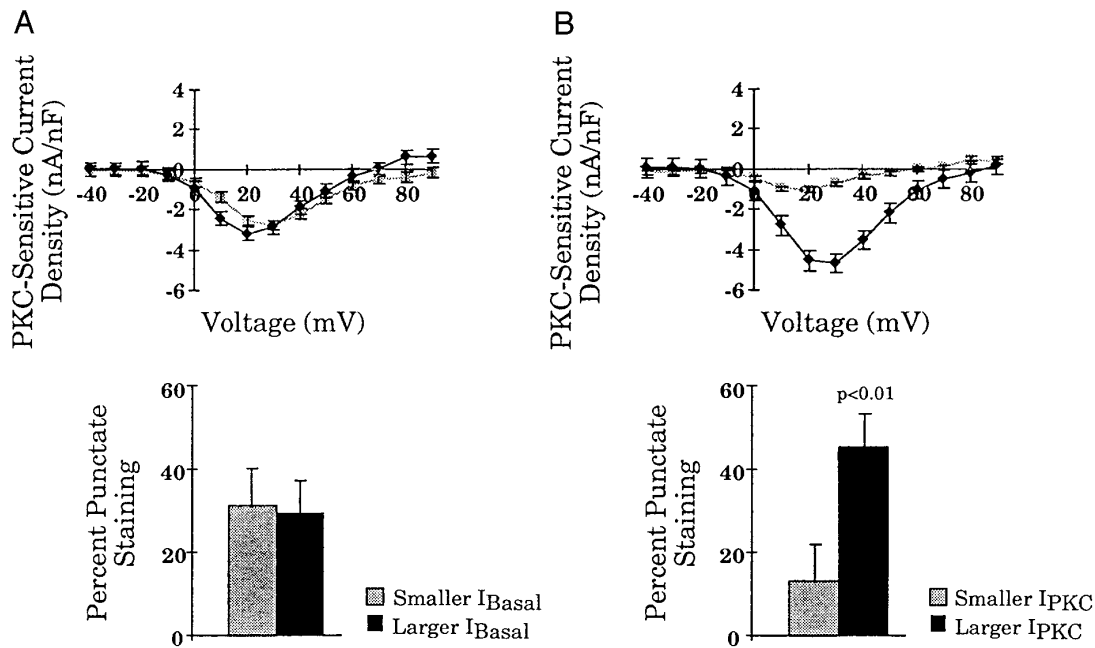


FIG. 4. Clusters with low protein kinase C (PKC)-sensitive currents have lower frequencies of punctate staining than clusters with high PKC-sensitive currents. Bag cell clusters were sorted according to the magnitude of their (A) basal or (B) PKC-sensitive calcium current densities, and the average PKC-sensitive calcium current densities and average percentages of punctately stained neurons were calculated for clusters above and below the median current density in each case. *Top panels*: the average PKC-sensitive calcium current densities as a function of voltage for the clusters above (squares) and below (diamonds) the median current density in each case. *Bottom panels*: average percentage of punctately stained neurons for clusters above (stippled) and below (solid) the median current density in each case. Significance was evaluated by the 2-tailed *t*-test. Error bars represent standard errors.

repeating the sorting of the 17 clusters, this time according to the magnitude of their basal calcium current densities. The clusters were divided, as before, into two groups, which now represented clusters with low and high basal current densities. In addition to the average basal calcium current density of each group, the average PKC-sensitive calcium current density and the average level of punctate staining of each group were again determined. The basal calcium current densities for the groups above and below the median (data not shown) differed by a factor of two with peak values at +30 mV of -2.5 ± 0.3 nA/nF and -5.3 ± 0.3 nA/nF, respectively. There was, however, no significant difference in the average PKC-sensitive calcium current densities for the two groups (Fig. 4A, *top panel*) and, more importantly, no difference in the average percentage of punctately stained neurons (Fig. 4A, *bottom panel*).

To confirm the correlation results with the entire range of data, we plotted the frequency of punctate staining for each cluster as a function of its basal and PKC-sensitive calcium current densities and analyzed the resulting scatterplots by linear regression (Fig. 5). As expected, the regression analysis found no significant correlation between the basal calcium current densities of the clusters and their frequencies of punctate staining ($r = -0.11$). In contrast, the observed cluster-to-cluster differences in PKC-sensitive calcium current densities correlated significantly with their differences in punctate staining ($r = 0.55$, $P = 0.02$). It is instructive to note that some of the clusters with current density and staining values that correlated poorly may reflect necessary methodological biases more than an absence of correlation between $\alpha BC-\alpha_{1A}$ staining and PKC-sensitive currents. For

example, the cluster with 4% punctate staining and -6.4 nA/nF PKC-sensitive current density (Fig. 5, *right panel*) exhibited robust $\alpha BC-\alpha_{1A}$ staining, but because the staining could not generally be resolved as punctate the cluster scored low for the percentage of neurons punctately stained.

DISCUSSION

It has been known for nearly a decade that PKC potentiates calcium currents in *Aplysia* bag cell neurons by recruiting a voltage-sensitive calcium channel that is not normally observed in the plasma membrane. The molecular identity of this channel and the mechanism of recruitment have, however, remained unknown. The evidence presented here suggests that $BC-\alpha_{1A}$, a member of the ABE subfamily of voltage-sensitive calcium channels whose localization in bag cell neurons is predominantly intracellular, is the carrier of the PKC-sensitive current. Expression of both $BC-\alpha_{1A}$ and PKC-sensitive calcium currents is attenuated in the bag cell neurons of juvenile *Aplysia*, and the presence of vesicular $BC-\alpha_{1A}$ channels in cultured juvenile bag cell neurons correlates significantly with the presence of PKC-sensitive currents. In addition, vesicular $BC-\alpha_{1A}$ expression fails to correlate with basal calcium current densities in juvenile bag cell neurons.

Previous physiological characterization of bag cell neuron calcium currents indicates that the basal and PKC-sensitive calcium currents derive from only two types of voltage-sensitive calcium channel, a 12-pS channel constitutively present on the membrane surface and thought to be responsible for the basal current and a 24-pS channel recruited by

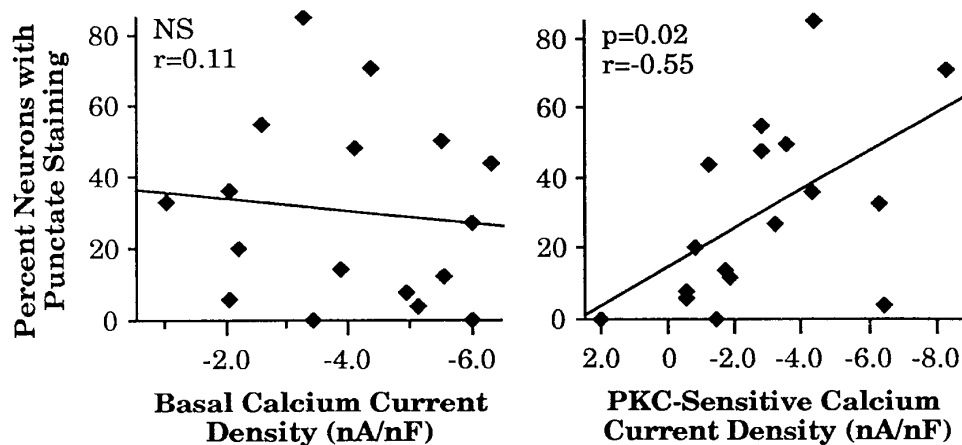


FIG. 5. Punctate BC- α_{1A} staining correlates significantly with the magnitude of PKC-sensitive, but not basal, calcium currents. Scatter plots show the percentage of cells staining punctately as a function of the average basal (*left*) and PKC-sensitive (*right*) calcium current densities for the 17 bag cell clusters analyzed. Linear regression was performed with the Origin graphics and data analysis program and regression lines are shown for the PKC-sensitive and basal current densities. r (coefficient of correlation) and P (probability that r is 0) values are indicated. NS: not significant.

PKC (Strong et al. 1987). These observations constrain the interpretation of the correlation data presented here. In particular, our observation that BC- α_{1A} expression fails to correlate with basal calcium current densities in juvenile bag cell neurons appears to preclude identification of BC- α_{1A} with the 12-pS channel. Conversely, the positive correlation of α BC- α_{1A} staining with PKC-sensitive currents strongly supports the conclusion that BC- α_{1A} is the 24-pS calcium channel of bag cell neurons recruited by PKC.

It was previously suggested that the PKC-sensitive channels of bag cell neurons are translocated to the plasma membrane from an intracellular pool (Strong et al. 1987). Our finding that a vesicular class of calcium channels correlates with the presence of PKC-sensitive calcium currents in bag cell neurons is consistent with this hypothesis. Our inability to directly detect redistribution of BC- α_{1A} in response to phorbol ester treatment of juvenile neurons is not surprising given that only 1,000 24-pS channels may be sufficient to produce a 3-nA/nF change in calcium current density, which is the mean change we observe on PKC stimulation. Such a small number of channels would be difficult to resolve by immunohistochemical techniques, particularly if the translocatable pool is large compared with the number of channels translocated. Although we cannot rule out other mechanisms of modulation, the vesicular localization of BC- α_{1A} and its correlated presence with PKC-sensitive currents in bag cell neurons make translocation a plausible mechanism of channel modulation by PKC.

It also has been suggested that the PKC-sensitive channels serve to potentiate secretion of the peptide hormone ELH from bag cell neurons (Loechner et al. 1992). Secretion of ELH is associated with a prolonged period of electrical activity known as the afterdischarge, which is characterized by potentiation of calcium currents, reflected in enhancement of bag cell neuron action potentials. Inhibition of PKC during the afterdischarge has been shown to block both action potential enhancement (Conn et al. 1989) and reduce ELH release (Loechner et al. 1992), suggesting a plausible role for BC- α_{1A} in these processes. This possibility is particularly interesting because BC- α_{1A} is a member of the ABE subfamily of voltage-sensitive calcium channels. Two mammalian members of this subfamily (α_{1A} and α_{1B}) have been implicated in the release of classical neurotransmitters, but less is known about their possible role in peptide release (Dunlap et al. 1995).

It is also possible, however, that BC- α_{1A} plays a different role in peptide release, independent of possible translocation in response to PKC stimulation. Recent evidence suggests that calcium entry through voltage-gated calcium channels may play a limited role in sustaining ELH release in mature bag cell neurons because release can outlast the period of the afterdischarge (Wayne and Wong 1994). Moreover, insulin, which stimulates calcium release from a novel intracellular store in bag cell neurons, can stimulate ELH secretion from mature bag cell clusters in the absence of an afterdischarge (Jonas et al. 1997). These results suggest that calcium release from intracellular stores plays an important role in sustaining ELH release, and it is possible that the intracellular calcium channel BC- α_{1A} may reside on calcium-containing vesicles and mediate release of calcium from that pool. Further work is clearly necessary to determine the role of BC- α_{1A} in bag cell neuron function, but the evidence presented here strongly suggests that the physiological correlate of this channel is the previously identified, 24-pS channel recruited by PKC.

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