Synaptogenesis and calcium current distribution in cultured leech neurons

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SUMMARY

The distribution of calcium (Ca^{2+}) currents was studied by loose-patch technique in Retzius cells isolated from the leech central nervous system. Ca^{2+} tail currents were recorded in patches held at the equilibrium potential for potassium (E_K). Measurements were made from single cells and paired cells that had formed chemical or electrical synapses in culture. In single Retzius cells, Ca^{2+} currents in the stump of the initial process were three times larger than in the middle part of the soma; intermediate amplitudes were found in the polar region of the soma. After the formation of chemical synapses, Ca^{2+} currents at the stumps of postsynaptic cells and in the polar region of the somas of presynaptic cells were reduced. The formation of electrical synapses did not affect Ca^{2+} current distribution.

1. INTRODUCTION

Ca^{2+} channels are distributed in a non-uniform manner over the surfaces of neurons. For example, presynaptic motor nerve terminals (Katz & Miledi 1965; Augustine *et al.* 1989; Stanley & Goping 1991; Cohen *et al.* 1991) and dendrites of cerebellar Purkinje cells (Ross & Werman 1986; Hockberger *et al.* 1989) show higher densities than their axons or cell bodies. Little is known about the mechanisms that determine how Ca^{2+} channels are placed in the neuronal membrane. The aim of the present experiments has been to establish whether the distribution of Ca^{2+} currents in presynaptic and postsynaptic cells changes after the formation of a synapse.

A preparation for studying this problem is provided by isolated leech neurons in culture. Neurons can be removed from leech ganglia, preserving a stump of initial process attached to the soma. In culture, they maintain their characteristic resting and action potentials, and after a few hours can establish chemical or electrical connections, depending on the targets available (Fuchs *et al.* 1981; Arechiga *et al.* 1986; Liu & Nicholls 1989). The end of the initial process (the stump) is an especially favourable region for the formation of chemical synapses (Liu & Nicholls 1989). Thus, when two Retzius cells are plated with the stump of one touching the soma of the other, a chemical unidirectional synapse is formed with the presynaptic component in the stump and the postsynaptic component in the soma (Liu & Nicholls 1989). In addition, when the stump of a Retzius cell touches the surface of a pressure sensory (P) cell, a chemical unidirectional synapse is formed in which the Retzius cell is always presynaptic. Moreover, Retzius cells can establish electrical synapses with anterior pagoda (AP) cells in less than 24 h, with no apparent chemical component. All of these combinations have the experimental advantage that the cells are large and have thick processes, and the sites of contact between the cells are identifiable.

Dyes such as Fura-2 and Arsenazo III used with optical recordings have provided information about Ca^{2+} channel distribution in individual leech neurons (Ross *et al.* 1987, 1988). Nevertheless, it is difficult to obtain quantitative information with these techniques, and difficult to observe cells for prolonged periods. An alternative approach is provided by the loose-patch clamp technique (Stühmer *et al.* 1983), which has the advantage that Ca^{2+} currents along the cell surfaces of several neurons can be recorded by using the same pipette again and again, day after day, without damage to the cells. In addition, the solution inside the recording pipette can be modified to isolate specific currents without affecting the membrane outside the region of the seal. In a previous loose-patch clamp study, it was shown that sodium (Na^+) currents are larger in the stumps of Retzius cells than in the cell bodies, but the fast and delayed potassium (K^+) currents are more equally distributed (García *et al.* 1990).

2. MATERIALS AND METHODS

(a) *Preparation*

Neurons were isolated from the central nervous system of the leech (*Hirudo medicinalis*) as described by Dietzel *et al.* (1986). In brief, the ventral nerve cord was removed from the animal and transferred to culture medium (Gibco L-15 medium supplemented with 2 mmol l^{-1} glutamine, 6 mg ml^{-1} glucose, 0.1 mg ml^{-1} Garamycin (Schering, Kenilworth, New Jersey) and 2% (by volume) Gibco foetal calf serum (FCS) heat-inactivated for 30 min at 56 °C). Ganglia capsules were opened and the exposed cells were treated with collagenase-dispase (2 mg ml^{-1}) for

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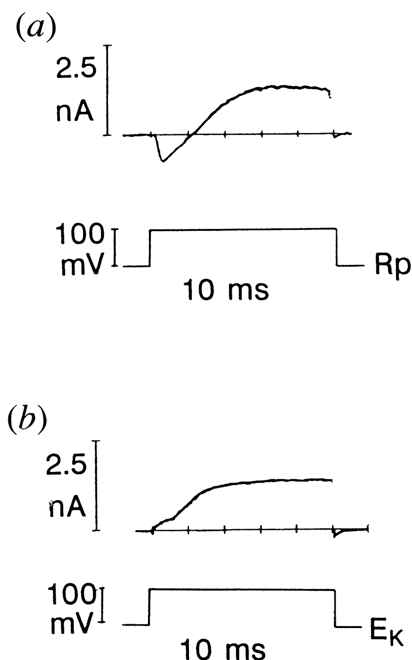

Figure 1. (*a*) Total currents recorded at the stump of a Retzius cell after 24 h in culture using normal L-15 medium inside the pipette. The holding potential of the patch was -10 mV with respect to the resting potential (Rp) of the cell. A pulse of 100 mV, lasting 10 ms, was applied to the patch. A fast inward current was followed by an outward current. The end of the depolarization was followed by an inward tail current. (*b*) Currents recorded from the same area of membrane as (*a*), but with a pipette solution containing low Na^+ (10 mM), high K^+ (20 mM) and high Ca^{2+} (10 mM). The patch was held close to E_K (-50 mV). The fast inward current at the beginning of the 100 mV depolarizing pulse was smaller and reversed in polarity. The outward current was still present, followed by an inward tail current at the end of the depolarization. The size of the tail current was enhanced by the presence of 10 mM $CaCl_2$ in the pipette.

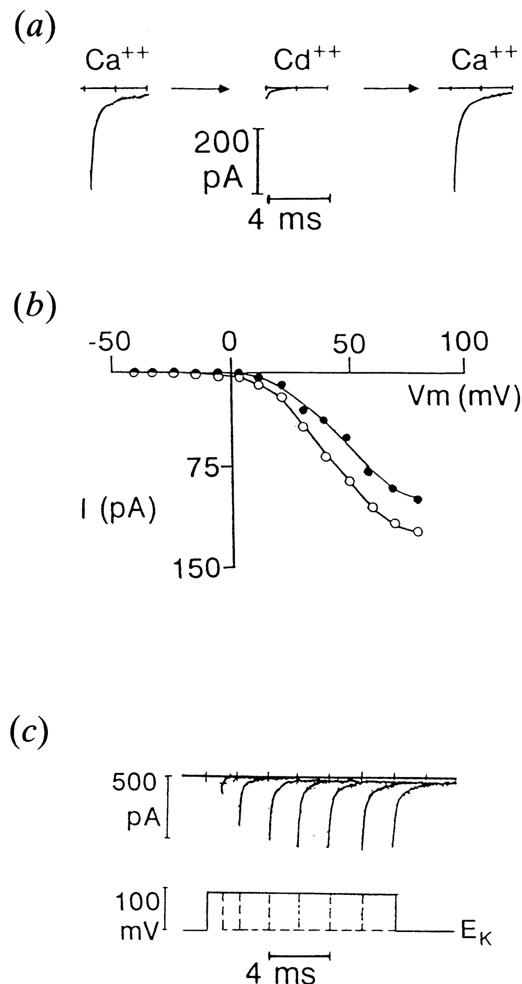

Figure 2. Characterization of Ca^{2+} tail currents. (*a*) Tail currents were reversibly blocked by the addition of 1 mM Cd^{2+} to the recording solution. For this experiment, the seal was removed and the solution inside the pipette was exchanged for each recording. (*b*) Current–voltage relation for Ca^{2+} (filled circles) and Ba^{2+} (open circles) obtained by applying increasing steps of voltage. Ba^{2+} produced larger tail currents than Ca^{2+}. Both curves were obtained from the same area of membrane by using 10 mM of the corresponding ion in the pipette. (*c*) Tail currents were measured for 100 mV depolarizing pulses with durations of 1–12 ms, using 10 mM Ca^{2+} inside the pipette.

30 min. Retzius, P and AP cells were sucked out of the ganglion, washed and left overnight at room temperature (20 °C) in sterile L-15 medium with 2% (by volume) FCS. The following day they were plated in microwell dishes containing L-15 without FCS, which was added later to the medium. Polylysine was used as a substrate for the cells because it allows synapse formation but restricts neurite outgrowth on the dish. Cells were paired to form synapses, as described by Liu & Nicholls (1989). The configurations chosen to study chemical synapses were as follows: (i) the stump of a Retzius cell (presynaptic) touching the polar region of the soma of another Retzius cell (postsynaptic); and (ii) the stump of a Retzius cell (presynaptic) touching the stump or soma of a P cell. For electrical synapses, the stumps of Retzius and AP cells were paired. The presence of synapses was tested routinely by intracellular recordings from pairs of cells after loose-patch clamp recordings had been made.

Before each experiment, intracellular recordings were made from several cells to determine their resting potentials. Typical resting potentials found in Retzius cells ranged from -45 mV to -52 mV. The pipette solution used to record Ca^{2+} tail currents contained an increased K^+ concentration, and produced a depolarization of about 5 mV. For all the experiments, the holding potential of the patch was kept at -50 mV.

(*b*) *Loose patch set-up*

For loose-patch clamp recordings, the original circuit by Stühmer *et al.* (1983) was modified (see García *et al.* 1990). The same pipette was used to hold the potential, depolarize the patch, and record the membrane currents. Currents were amplified by an EPC-7 (List Electronics, Darmstadt) amplifier through a modified head stage with an OPA 111. Electrode series resistance and seal-leak resistance-compensation controls were housed in a separate adaptor box. Cancellation of transients and any remaining uncompensated leak currents was accomplished with a 'P/3'

protocol similar to that described by Bezanilla & Armstrong (1977), in which the currents from one depolarizing and three hyperpolarizing pulses, each one third of the amplitude of the depolarizing pulse, are added together. Signal averaging was often used to reduce noise. The traces used for illustration in figures 1–5 are averages of three traces. The adaptor box, the A/D board and the programs for storage and analysis of data were designed in the laboratory. An acquisition rate of 50 kHz was used throughout these experiments. Ca^{2+} tail currents were recorded following 10 ms depolarizing pulses.

Glass pipettes (micro-haematocrit tubing, Clay Adams) were pulled by a two-step protocol on a Kopf vertical puller to produce tip diameters of 3–7 μm with resistances of 0.3–0.5 MΩ. The shafts of the pipettes were coated with Sylgard as close as possible to the tips to decrease capacitance. Gentle suction was applied to make stable seals on the cell surfaces; typical seal resistances were in the range 3–5 MΩ. After a stable seal was formed, voltage pulses were applied to the patch, and the ionic currents were recorded. After recording from one patch of membrane, the electrode could be removed and placed in other locations on the cell surface without damage to the membrane.

(*c*) *Bath and pipette solutions*

For most purposes, cells were bathed in L-15 medium. The solutions were buffered with Trizma malate, 10 mmol l^{-1}, adjusted to pH 7.4, and the osmolarity was adjusted to 350–370 mosmol l^{-1} with *N*-methyl-D-glucamine chloride. To record Ba^{2+} currents, the bathing medium was switched to modified Eagle's medium (Gibco) buffered with HEPES to avoid Ba^{2+} precipitation.

To record total currents, the solution used inside the pipette was either L-15 or a solution consisting of (in millimoles per litre): KCl, 4; NaCl, 125; and $CaCl_2$, 10. To record Ca^{2+} or Ba^{2+} tail currents, KCl was increased to 20 mmol l^{-1} to decrease E_K from -85 to -50 mV (close to the resting potential of Retzius cells), and NaCl was reduced to 10 mmol l^{-1} to reduce Na^+ currents, as tetrodotoxin does not block leech sodium channels. $CaCl_2$ or $BaCl_2$ concentration was raised to 10 mmol l^{-1} to increase the size of the tail currents. The Ca^{2+} tail currents were blocked by adding $CdCl_2$ at 1 mmol l^{-1} to the previous solutions.

(*d*) *Statistical analysis*

Non-parametric statistics were used for all analyses. For the data in table 1, and the comparisons of currents in pre- and postsynaptic Retzius–Retzius pairs, the measurements were paired, for example, the amplitude of a tail current in the stump was compared with the amplitude of the tail current in the soma of the same cell. In these cases, a two-tailed Wilcoxon paired sample test, essentially a binomial distribution test, was used to detect differences between measurements. For comparison of group data, as in tables 2 and 3, a one-tailed Wilcoxon rank sum test was used to look for increases or decreases in the ratios of currents. The means of the measurements in tables 1–3 are provided only for information, and were not used in the statistical analysis.

3. RESULTS

(*a*) *Isolation of Ca^{2+} tail currents*

After 24 h in culture, application of depolarizing pulses through the loose-patch pipette elicited characteristic currents in patches of Retzius cells (García *et al.* 1990). These currents showed three components: an inward (Na^+) current with a maximum peak at 700 μs, an outward current (composed of three main types of K^+ current) with a peak after 3 ms, and, following the end of the pulse, an inward (mostly Ca^{2+}) tail current (figure 1 *a*). Ca^{2+} currents are too small to be measured in isolation during the depolarizing pulse (García *et al.* 1990); for that reason, the amplitudes of the Ca^{2+} tail currents that followed the pulse were analysed in this study. The fast Na^+ current was suppressed with the use of low Na^+ (10 mmol l^{-1} NaCl) inside the pipette. The amplitude of the Ca^{2+} tail currents was increased by raising the concentration of Ca^{2+} to 10 mmol l^{-1}.

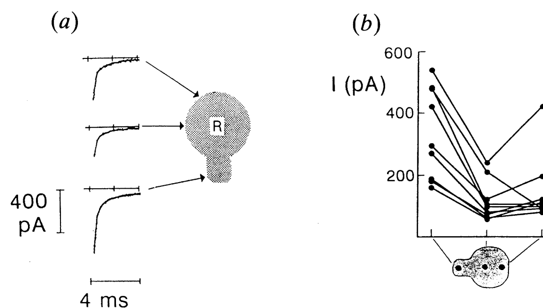

Figure 3. Ca^{2+} tail currents recorded from three different locations on a single Retzius cell after 24 h in culture. (*a*) Recordings obtained from the stump, middle soma, and polar region of the soma of a Retzius cell. The largest amplitudes of Ca^{2+} tail currents were consistently recorded from the stumps of the cells, whereas the central region of the soma showed the smallest amplitudes. (*b*) Amplitudes of Ca^{2+} tail currents in nine Retzius cells obtained from the same three locations as shown in (*a*).

Table 1. *Ca^{2+} tail current amplitudes from isolated Retzius cells* ($n = 9$)

(Means, medians and ranges are given for the stumps, middle somas and polar region of the somas. The p values give the significance level for differences between groups (two-tailed paired sample test).)

location	mean pA	median pA	range pA	p	
stumps	335	293	160–540	< 0.004 (stumps vs middle)	< 0.004 (stumps vs polar)
middle somas	115	96	56–240	0.125 (middle vs polar)	
polar somas	145	110	78–420		

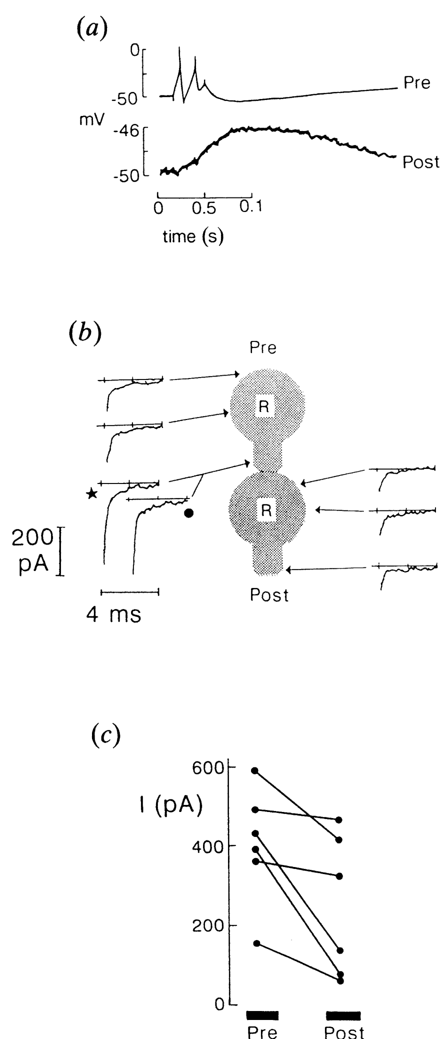

Figure 4. The formation of a chemical synapse is accompanied by a reduction in the Ca^{2+} tail-current amplitude in the postsynaptic cell stump. (*a*) In the Retzius–Retzius stump to soma configuration, after 24 h in culture, a synaptic potential was generated in response to intracellular stimulation of the presynaptic cell. The inward chloride synaptic potential was reversed by using electrodes filled with 3 M KCl. (*b*) Ca^{2+} tail currents recorded from the stumps, middle soma and polar region of the soma of two Retzius cells that had formed a chemical synapse. Ca^{2+} tail currents from both cells were recorded using the same pipette under identical experimental conditions. Moreover, pipettes with similar resistances were used to record from the six pairs. Stump currents from the presynaptic cell were recorded before (star) and after (filled circle) recording from the postsynaptic stump. (*c*) Comparison of the amplitudes of the Ca^{2+} tail currents at the stumps of pre- and postsynaptic Retzius cells ($n = 6$) that had formed chemical synapses. Current amplitudes from paired cells are linked.

Contamination by K^+ tail currents was minimized by measuring the tail currents at a holding potential equal to E_K. Under these conditions, depolarization of the membrane produced an outward current followed by an inward tail current at the end of the depolarization (figure 1*b*).

Tail currents were blocked reversibly by the addition of 1 mmol l^{-1} cadmium (Cd^{2+}) to the pipette solution (figure 2*a*). Substitution of barium (Ba^{2+}) for Ca^{2+} produced larger tail currents and faster deactivation (Bookman & Liu 1990). Figure 2*b* shows current–voltage relations for Ca^{2+} and Ba^{2+}. Addition to the pipette solution of 4-aminopyridine, tetraethylammonium or caesium, which selectively block K^+ currents in leech cells, affected neither the amplitudes nor the kinetics of the Ca^{2+} tail currents. Moreover, in the presence of Cd^{2+} in the pipette, no K^+ tail current was detected when the holding potential was changed by 10 mV in depolarizing or hyperpolarizing directions. These observations show that the charge carrier of the tail current is Ca^{2+}, and that any contamination by K^+ currents is minor.

The activation of Ca^{2+} currents was analysed by applying depolarizing pulses of 100 mV with durations ranging from 1 ms to 12 ms, and measuring the amplitudes of the tail currents (figure 2*c*). After 8 ms the amplitudes of the Ca^{2+} tails reached a plateau with a half-time of 1.6 ms, as has been previously shown in Retzius cells (Stewart *et al.* 1989; García *et al.* 1990).

Deactivation of Ca^{2+} tail currents did not follow a single exponential. Most tail currents showed at least three components: a fast deactivating component with a time constant of 200 μs, a slow deactivating component with a time constant of 1.82 ms (both of these were similar to those described already in leech Retzius cells (Bookman & Liu 1990)), and a third, very slowly deactivating component.

(*b*) *Distribution of Ca^{2+} tail currents in single Retzius cells*

To study the distribution of Ca^{2+} tail currents in single Retzius cells, we recorded from many locations along the surface of the cells. A characteristic distribution of Ca^{2+} tail-current amplitudes was observed from one cell to another. The largest amplitudes were found at the stump of the cell. From here, a gradual decrease was observed toward the middle of the soma. Moving farther towards the polar region of the soma (i.e. the soma surface opposite the stump), amplitudes tended to increase to values intermediate between those recorded from the stump and the middle soma. Owing to this trend, we chose for further analysis the stump close to its tip, the middle soma and the polar region of the soma (figure 3*a*).

There was considerable variability in the measurements of the Ca^{2+} tail-current amplitudes from one cell to another (figure 3*b*). For example, the range of current amplitudes recorded from the stumps covered more than a threefold range (160–540 pA). Our experiments show that the variability arises from several sources. Clearly, recording from larger areas of membrane, by using larger pipettes, results in larger currents. However, data in figure 3*b* were all collected by using electrodes with resistances between 0.35 MΩ and 0.5 MΩ. When the currents were normalized with respect to electrode conductance (presumably a measure of lumen size), the range of normalized amplitudes was not decreased. The amplitudes of the Ca^{2+} tail currents at the tip of the cells were also normalized against the size of the outward K^+ current at the end of the pulse, again presumably a measure of effective membrane area; the range of normalized

Table 2. *Comparisons of style Retzius cells* ($n = 9$) *with Retzius cells that had formed chemical synapses* ($n = 6$)

(Means, medians and ranges are given for the ratios of stump/middle soma Ca^{2+} tail-current amplitudes. The p values give the significance level for differences between groups (one-tailed rank sum test).)

stump/middle soma	mean	median	range	p	
presynaptic cells	3.8	3.7	1.0–6.1	0.48 (presynaptic vs single)	< 0.04 (presynaptic vs postsynaptic)
single cells	3.1	2.9	2.3–4.6		
postsynaptic cells	2.1	2.1	0.8–3.4	< 0.03 (single vs postsynaptic)	

current amplitudes was still threefold. Considerable variability in Ca^{2+} currents of Retzius cells was also observed when recordings were made with two-electrode voltage clamp or whole-cell perfusion (R. R. Stewart & J. G. Nicholls, personal communication). Together these results suggest that total Ca^{2+} currents may differ widely in their amplitudes from cell to cell in culture. Thus at least part of the variability seen with loose patch may reflect genuine differences rather than a procedural artefact.

Data in figure 3*b* support qualitative observations about tail-current amplitudes. The differences were confirmed by non-parametric statistical analysis of the group data (table 1). The currents at the stumps (mean 335 pA) were larger than those at the middle soma (mean 115 pA, $p < 0.004$, paired-sample test) and those at the polar region of the soma (mean 145 pA, $p < 0.004$), but the currents at the polar region of the soma were not significantly larger than those at the middle soma ($p = 0.125$).

(*c*) *Ca^{2+} tail currents after the formation of chemical synapses*

When the stump of a Retzius cell touches the soma of another Retzius cell or the soma of a P cell, a unidirectional chemical synapse is formed after 6–8 h. The presynaptic component is the stump of the Retzius cell. Dozens of Retzius–Retzius synaptic pairs were tested with similar results. In six pairs of cells (figure 4), the same pipette was used to record the Ca^{2+} tail currents from the stumps, middle somas and polar region of the somas of both cells. Only those pairs of cells in which similar seal resistances and stable seals were obtained at all locations were chosen for analysis. After recording the tail currents, synaptic transmission between cells was verified. Figure 4*a* is an example of postsynaptic potentials produced by two action potentials in the presynaptic cell.

(*d*) *Decrease of the Ca^{2+} currents at the postsynaptic stump*

Figure 4*b* illustrates tail currents recorded from a typical Retzius–Retzius synaptic pair. The Ca^{2+} tail current on the stump of the postsynaptic cell (mean 261 pA) was smaller than the corresponding current in the presynaptic cell (mean 422 pA, $p < 0.04$, paired-sample test, figure 4*c*). The amplitudes of the currents recorded from the middle somas of pre- and postsynaptic cells were the same (both means 116 pA), and were essentially the same as for the other groups of cells. For example, in single cells the mean was 115 pA. To compensate for possible differences in the overall Ca^{2+} channel densities from cell to cell, the amplitudes of the currents at the stump and polar region of the soma were normalized to the amplitude of the middle soma current of the same cell. Again the normalized stump current amplitudes were larger in the presynaptic cells (mean ratio 3.1) than in the postsynaptic cells (mean ratio 2.1, $p < 0.04$, paired-sample test).

As the site of synaptic contact is located at the tip of the stump of the presynaptic cell, it was clearly of interest to determine whether the differences in pre- and postsynaptic cells resulted from an increase in the stump current of the presynaptic cell or from a decrease in the stump current of the postsynaptic cell as compared with the control group of single cells. This analysis is not straightforward because of the variability of the data and the necessity for comparing group data rather than paired data. The results are given in table 2. Although the currents in the stumps of the presynaptic cells were larger than those in the controls, the difference was not significant. In contrast, the normalized current amplitudes in the stumps of postsynaptic cells were significantly smaller ($p < 0.03$, rank sum test) than those in the controls.

(*e*) *Decrease in Ca^{2+} currents at the polar region of the soma of presynaptic cells*

In addition to the changes occurring at the stumps of postsynaptic cells after the formation of chemical synapses, a consistent change was observed in the presynaptic cells: calcium currents in the polar region of the soma became smaller than those in single cells ($p < 0.05$, rank sum test; table 3). Reduced currents were observed in presynaptic Retzius cells whether they formed a chemical synapse on another Retzius cell (figure 4*b*) or on a P cell (figure 5*a*). Owing to the technical difficulty of obtaining P cells with long stumps, no analysis was made of their Ca^{2+} current distribution.

Retzius and AP cells establish electrical synapses in less than 24 h with no obvious chemical component. Ca^{2+} tail currents recorded from Retzius cells that formed electrical synapses ($n = 7$) were indistinguishable from those in single Retzius cells (see figure 5*b*). In addition, no changes were observed in the distribution of Ca^{2+} tail currents in AP cells that had formed synapses. Moreover, in several instances, it was possible to follow pairs of cultured Retzius cells over a period of several days during which, in addition to the chemical synapse, an electrical synapse is formed (Liu & Nicholls 1989). In five cell pairs, the amplitudes of the Ca^{2+} tail currents increased all along the surfaces of the cells but without significant changes in the relative amplitudes at different locations. We were unable to determine

Table 3. *The amplitudes of the Ca^{2+} currents at the polar region of the soma of presynaptic Retzius cells (either to other Retzius cells ($n = 6$) or to* P *cells ($n = 10$) were grouped and compared with the corresponding currents in single Retzius cells ($n = 9$)*

(Means, medians and ranges are given for the ratios of the polar region of the soma to middle soma Ca^{2+} tail-current amplitudes. The p value gives the significance level for the difference between presynaptic ($n = 15$) and single cells (one-tailed rank sum test).)

polar soma/middle soma	mean	median	range	p
all presynaptic	1.0	1.0	0.5–2.0	< 0.05
single cells	1.3	1.3	0.4–2.0	

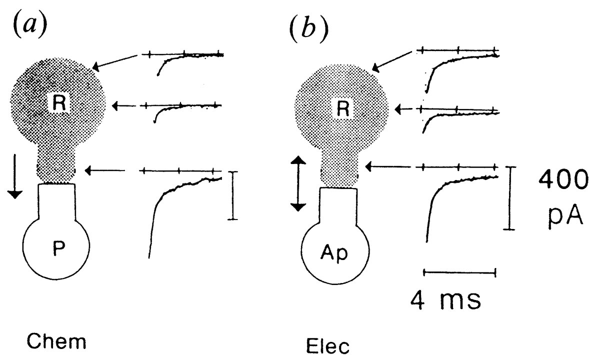

Figure 5. Characteristic Ca^{2+} tail currents in Retzius cells that had formed synapses with (*a*) P and (*b*) AP cells. In Retzius cells that formed chemical synapses with P cells, Ca^{2+} currents in the polar region of the somas were reduced, whereas Ca^{2+} tail currents in Retzius cells that established electrical synapses with AP cells were similar to those found in single cells.

whether the overall increase in tail-current amplitudes was a biological effect, or whether it was caused by cell membranes becoming cleaner after longer periods in culture.

4. DISCUSSION

Leech neurons offer a number of advantages for measuring the distribution of Ca^{2+} currents, in that they are large, have thick processes, form specific types of synapses, and the sites of contact are well defined. A disadvantage is that Ca^{2+} currents during a depolarizing pulse are small, and masked by outward K^+ currents which cannot be easily avoided (García *et al.* 1990). However, it appears that the tail currents that follow the end of the pulse are carried purely by Ca^{2+}, as they are blocked by Cd^{2+} and are unaffected by K^+ channel blockers. In addition, the characteristics of the Ca^{2+} tail currents obtained in this study with loose patch are similar to those reported in previous studies using two-electrode and whole-cell mode voltage clamp (Stewart *et al.* 1989; Bookman & Liu 1990).

Loose-patch clamp has provided a high-resolution map of the distribution of Ca^{2+} currents along the surface of isolated Retzius cells. A similar distribution has been described by using optical recordings with Arsenazo III (Ross *et al.* 1987). The stump close to its tip, from which processes grow and establish synapses, had the largest Ca^{2+} currents. The amplitude of the Ca^{2+} currents in the middle soma was very small; surprisingly, in most cells the polar region of the somas of single cells showed larger Ca^{2+} tail currents than the middle somas. This distribution seemed to be graded along the surface of Retzius cells, although it was not rare to find variations within a small area. We do not know whether these variations reflect the presence of 'hot spots' of Ca^{2+} channels (Thompson & Coombs 1988; Silver *et al.* 1990; Lipscombe *et al.* 1988) or different degrees of membrane folding.

One curious aspect of our findings is that the changes in Ca^{2+} tail-current amplitudes occurred distant from the site of synapse formation, at the polar region of the soma of the presynaptic cell and at the stump of the postsynaptic cell. At present, we cannot distinguish whether these decreases reflect a reduction in the density of channels, modulation of their properties, or the appearance of different types of channels with lower conductances. These changes, however, are reminiscent of the effect observed after reinnervation of muscle, where there is a reduction in the number of acetylcholine receptors in regions far from the developing end plate (Diamond & Miledi 1962).

There is no *a priori* reason to believe that similar mechanisms mediate the reductions in Ca^{2+} tail currents in pre- and postsynaptic cells, nor to expect these reductions to have similar functional significances. That decreases of Ca^{2+} current occurred distant from the site of synapse formation suggests the mediation by second-messenger systems. In leech neurons, an example of such modulation is the reduction in the response of serotonergic receptors on P cells after they contact Retzius cells. This effect is mediated by protein kinase C (Drapeau 1990; Sánchez-Armass *et al.* 1991).

Amplitudes of the Ca^{2+} currents at the stump, close to the synapse, were preserved after the formation of synapses. This may be analogous to the accumulation of Ca^{2+} channels in presynaptic terminals at the neuromuscular junction of the frog (Katz & Miledi 1965; Cohen *et al.* 1991; Robitaille *et al.* 1991) and in the giant synapse of the squid (Augustine *et al.* 1989), where Ca^{2+} is required for transmitter release. In contrast, Ca^{2+} current reduction at the postsynaptic stump may participate in the control of cell sprouting. During early development of the nervous system there is an excess in the number of neurons produced and in the amount of sprouting. Eventually, mechanisms are triggered that result in cell death and neurite retraction. Ca^{2+}, which is necessary for transmitter release and many other cellular processes, may trigger neurotoxic reactions at high intracellular concentrations (Gofraind & Govoni 1989), and it has been proposed that the entrance of Ca^{2+} into the cell may induce retraction of neurites in Retzius cells

(S. Grumbacher & J. G. Nicholls, unpublished observations), as well as in other types of cells (for review see Kater & Mills 1991).

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