Electrotonic Synapses Between *Aplysia* Neurons *in situ* and in Culture: Aspects of Regulation and Measurements of Permeability

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Properties of electrotonic synapses between L14 neurons in the abdominal ganglion of the marine mollusc *Aplysia californica* were examined *in situ* and between unidentified buccal neurons maintained in tissue culture. In culture, depolarizing postsynaptic potentials in response to a train of action potentials showed apparent facilitation with increasing spike number, which was attributable to the low-pass filter properties of electrotonic transmission via gap junctions and to network properties. Gap junctional conductance (g_j), calculated from current-clamp data or measured directly under voltage clamp, indicated no significant dependence of g_j on transjunctional or inside–outside potential *in situ* or in culture. Octanol, a local anesthetic agent that reduces g_j in many other systems, had no effect on g_j between *Aplysia* neurons. The effect of intracellular acidification, a treatment that rapidly and reversibly uncouples a variety of cell types, reduced g_j between *Aplysia* neurons but did not completely abolish it. The relationship between intracellular pH (pH_i), measured with ion-sensitive microelectrodes, and g_j was steeper in cultured neurons than *in situ* and was maximally reduced by 70–80%, as compared to 50% or less *in situ* at the lowest pH_i values tested. The coupling coefficient (k) was reduced less by low pH_i than was g_j , which could be explained by a simultaneous increase in nonjunctional membrane resistance. Permeability properties of *Aplysia* electrotonic synapses to a variety of tracer molecules were also examined between identified L14 neurons *in situ* and in dissociated buccal, abdominal, and bag neurons in culture. The fluorescent dyes Lucifer yellow, 6-carboxyfluorescein, and dichlorofluorescein (1.2–1.4 nm maximal diameters) did not spread detectably from an injected neuron to its electrically coupled neighbors, regardless of the strength of electrotonic coupling. However, the smaller tetraalkylammonium ions TMA and TEA (diameters 0.66 and 0.8 nm, concentrations measured with ion-selective electrodes), could be detected in neighboring cells within minutes. In culture, transfer of the tetraalkylammonium ions was slow and not easily detectable in cell pairs where g_j was low (<20 nS). The permeability was as high as 10^{-10} cm³/sec *in situ* and 10^{-12} cm³/sec in

culture, and values were roughly correlated with simultaneously measured values of g_j . Electrotonic synapses in the nervous system of *Aplysia*, therefore, have a quantitatively different spectrum of sensitivities than has been found for gap junctions of other systems and appear to possess reduced permeability to tracer molecules.

Electrotonic synapses have been described between *Aplysia* neurons, both *in situ* and in dissociated cells in culture (Waziri, 1969; Gillette and Pomeranz, 1975; Carew and Kandel, 1976; Gardner, 1977; Kaczmarek et al., 1979; Rayport and Kandel, 1980; Dagan and Levitan, 1981; Bodmer et al., 1984), but the properties of electrotonic synapses in this species have not been compared with those of other systems. On the basis of limited freeze-fracture electron microscopic studies on *Aplysia* (Kaczmarek et al., 1979; Porvaznik et al., 1979) and more extensive studies on other molluscan species (Hall et al., 1983; Roubos et al., 1985), the gap junction has been assigned as the structural basis for electrotonic coupling in the *Aplysia* nervous system, as it is in other excitable and inexcitable tissues (see Bennett and Spray, 1985). For gap junctions in general, a variety of agents and treatments has been shown to affect junctional conductance (g_j) (see Bennett and Spray, 1985; Spray and Bennett, 1985). Stimuli that act to close (or gate) gap junction channels include voltage, applied either across the junctional membrane or between the inside and outside of the cell (see Spray et al., 1985), cytoplasmic acidification (Turin and Warner, 1978; Spray et al., 1981b), and extracellularly applied octanol (Johnston et al., 1980; Spray et al., 1985).

Intracellular pH (pH_i) can be varied by manipulation of the external media with weak acids or bases, which are membrane permeable in their undissociated forms (Roos and Boron, 1981) and by the addition of CO₂ (Thomas, 1974). CO₂, the most common treatment, has been reported to reduce electrotonic coupling in many different systems (Turin and Warner, 1978; Iwatsuki and Petersen, 1979; Giaume et al., 1980; Spray et al., 1981b; Schuetze and Goodenough, 1982; Connors et al., 1984; see also Spray and Bennett, 1985). However, in some of those reports, evidence was presented that CO₂ might have little or no effect on dye coupling (Schuetze and Goodenough, 1982; Connors et al., 1984). Preliminary results indicated a similar finding for electrotonic coupling among *Aplysia* neurons *in situ* and in culture (Bodmer, 1983).

Abdominal L14 neurons, which are involved in *Aplysia*'s inking behavior (Frazier et al., 1967; Kandel, 1976) show extensive electrotonic coupling (Carew and Kandel, 1976), as do cultured buccal neurons (Dagan and Levitan, 1981; Bodmer et al., 1984).

Received Jan. 16, 1987; revised Sept. 8, 1987; accepted Sept. 10, 1987.

Supported in part by NSF Grant BNS-8400875 (to I.B.L.) and NIH Grants NS 16524 and NS 07512 (to D.C.S.).

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In the present study, we investigate the properties of these electrotonic synapses using electrophysiological techniques, which allowed direct assessment of g_j and pH_i directly. We report here that in *Aplysia* neurons g_j is not voltage dependent and is insensitive to octanol treatment but is reduced gradually with decreasing pH_i ; block was incomplete at pH_i values as low as 5.6. The coupling coefficient was frequently insensitive to low pH_i , and this finding is attributable to effects of weak acids on the resistance of the nonjunctional membrane. The sensitivity of *Aplysia* electrotonic synapses to these treatments is therefore different from that of gap junctions in many other systems.

Gap junctional membranes are also characterized by their permeability to fluorescent tracer molecules (1.2–1.4 nm diameter). Injection of dye into one cell often leads to detectable spread to adjacent cells within minutes (e.g., Macagno et al., 1981; Schwartzmann et al., 1981; Knowles et al., 1982; Brenowitz et al., 1983; Margiotta and Walcott, 1983; Connors et al., 1984; Fraser and Bryant, 1985). Recently, junctional permeability to these and smaller probes has been assessed quantitatively in several preparations (e.g., Atkinson and Sheridan, 1985; Brink, 1985; Safranyos and Caveney, 1985; Verselis et al., 1986a, b).

In order to assess the permeability properties of *Aplysia* neuronal gap junctions, we examined whether the fluorescent dyes Lucifer yellow (LY), 6-carboxyfluorescein (6-CF), and dichlorofluorescein (Cl_2F) passed between electrotonically coupled L14 neurons *in situ* and between dissociated buccal and bag neurons in culture. Electrophysiological measurement simultaneously assessed the strength of electrotonic coupling. We found that the fluorescent probes were not detectably permeable even when g_j was moderately high, but quantitative evaluation of very slow dye transfer was difficult because of low-level autofluorescence and cell pigmentation. We circumvented this difficulty by quantitative measurement of the intercellular flux of the smaller tetraalkylammonium ions tetramethylammonium (TMA) and tetraethylammonium (TEA) using ion-sensitive microelectrodes. Transfer of these probes was always readily detected between L14 neurons *in situ* but not between neurons in culture. This discrepancy is apparently related to g_j values and is discussed with respect to results from other systems.

A preliminary account of this work has been presented previously (Bodmer and Spray, 1985).

Materials and Methods

Tissue culture. *Aplysia* buccal neurons and bag cells were cultured as previously described (Dagan and Levitan, 1981; Bodmer et al., 1984). Briefly, the neurons were mechanically dissociated and plated under sterile conditions in 35 or 60 mm culture dishes containing 1% methyl cellulose (Methocel, DOW) and 4% fetal calf serum in L-15 medium (Gibco) supplemented with physiological salt concentrations. The culture dishes were incubated for 1–8 weeks at room temperature (19–23°C) in a humidified chamber.

Perfusion conditions. *Aplysia* saline was prepared as described previously (Levitan and Barondes, 1974), except that 10 mM HEPES was used as a buffer; pH was adjusted to 7.4. CO_2 -saturated saline was obtained by vigorously bubbling the saline solution with 100% CO_2 before (10 min) and during its application to the cultures. The pH of saline equilibrated with 100% CO_2 was about 5.0 and could be partially compensated (to pH 6.5) by raising the concentration of HEPES buffer to 50 mM. NH_4Cl (Sigma) was applied at 50 and 80 mM concentrations in saline. Octanol was prepared as a 5–10 mM suspension in saline and was shaken vigorously before adding to the preparation. Culture dishes were perfused on the stage of an inverted microscope (Nikon diaphot) at a flow rate of 0.5–2 ml/min. For *in situ* recordings of L14 neurons, ganglia were chemically desheathed using 1% dispase (Boehringer), at

21°C overnight, were then pinned out in a Sylgard-containing dish and viewed through a Wild dissecting microscope.

Intracellular recordings. Individual neurons were impaled with microelectrodes (5–20 M Ω) filled with 3 M KCl or potassium citrate. The outputs of the FET input preamplifiers were displayed on a storage oscilloscope and a 4-channel pen recorder (Gould Instruments).

Current clamp. Cellular input resistances were determined by injecting pulses of inward (hyperpolarizing) current through the recording electrode. The voltage drop across the electrode was circumvented by a chopped current injection device (Almost Perfect Electronics, Basel). For measurements of electrotonic coupling, current pulses of 0.5–5 sec were alternately delivered to each cell of the pair, and the resulting steady-state potential changes in both cells were measured.

Voltage clamp. For a single electrode voltage clamp, the electrode was alternately switched between the voltage measuring and current passing modes. I - V curves were obtained by sweeping the voltage between 2 levels at a rate (5 mV/sec) much slower than the membrane time constant (Adams et al., 1980). Junctional conductance (g_j) was measured by applying voltage command steps (V_1) to one cell and measuring the resulting current in the other cell (I_2); $g_j = -I_2/V_1$ (Spray et al., 1981a). Alternatively, the potential was swept between 2 voltage levels in the first cell and the current change in the second voltage-clamped cell was measured at a fixed potential ($dV_2 = 0$) (see Bennett, 1966).

Measurement of pH_i . Intracellular pH-sensitive microelectrodes were constructed from fiber-containing glass pulled as for conventional microelectrodes. Electrodes were silanized by filling the tip with 5% (vol/vol) dichlorodimethyl silane (Fluka) dissolved in CCl_4 . Electrodes were baked in a vacuum oven at 200°C for 30 min and allowed to cool. Liquid ion-exchange resin (5 μ l; Fluka) was used to fill the tips of the silanized electrodes and were then backfilled with 150 mM KCl buffered to pH 7.0 with 10 mM HEPES. Electrodes were calibrated in *Aplysia* physiological salines over the range 5.5–7.5 before and after experimental use; data were discarded if the calibration curves shifted by more than 0.1 pH unit and slopes were <50 mV/pH unit. Intracellular placement of the pH-sensitive electrode was verified by the criterion of similar steady-state voltage responses in the pH and voltage electrodes in response to a long current pulse delivered to the other cell (see Campos de Carvalho et al., 1984; Spray et al., 1986).

Intracellular dye injections. LY, 6-CF, and Cl_2F (2–4% wt/vol in water, fluorescein derivatives buffered to pH 7.8 with 10 mM HEPES) were intracellularly injected through separate electrodes by applying negative current pulses (–2 to –20 nA, 0.1–0.5 sec duration, for 5–30 min at about 1 Hz). TMA and TEA were intracellularly injected by applying pressure with a Picospritzer (General Valve Corp, Fairfield, NJ) to the back of electrodes filled with 1 M solutions of the tetraalkylammonium ions buffered to pH 7.6 with 10 mM HEPES. Dye injections were monitored on a Nikon Diaphot microscope with xenon arc lamp illumination using epifluorescence optics with appropriate excitation and emission filters.

Tetraalkylammonium ion measurements. Ion-selective electrodes constructed with Corning K^+ exchange resin (477317) display a high selectivity for tetraalkylammonium ions over K^+ (Ohme and Simon, 1976). Techniques for use and construction of these electrodes is detailed elsewhere (Verselis et al., 1986b). Microelectrode responses to different concentrations of TMA and TEA were linear in the range of 1–100 mM, producing an approximately 60 mV deflection per 10-fold change in concentration (see calibration curves in Figs. 10, 11). All calibration solutions contained concentrations of the appropriate tetraalkylammonium ion ranging from 1 μ M to 100 mM in 300 mM KCl (pH 7.5) to simulate the intracellular environment of the cell. Ionic strength was adjusted to 500 mM with NaCl.

Permeability values were obtained by injecting one cell of a pair with TMA or TEA and measuring changes in concentration in the injected (prejunctional) cell and recipient (postjunctional) cell over time. Transjunctional flux (J) was determined by multiplying the rate of change in TMA or TEA concentration in the postjunctional cell (dC_2/dt) by the volume (v_2) of that cell so that:

$$J = v_2 dC_2/dt \quad (1)$$

For these experiments, it was assumed that the cell volume was dominated by that of the soma, so that $v_2 = 4\pi r^3/3$, where r was measured using an eyepiece micrometer. Assuming no loss from the cells, the permeability (P) was determined by dividing the flux (J) by the driving force. For charged molecules such as TMA and TEA, the driving force is contributed by both concentration (dC) and voltage (dV) gradients,

which for a constant field assumption (Goldman, 1943; Hodgkin and Katz, 1949) yields

$$P = \frac{J[1 - \exp(zFV/RT)]}{zFV/RT[C_1 - C_2 \exp(zFV/RT)]} \quad (2)$$

where z is the valence, R is the gas constant, T is the temperature, F is the Faraday constant, V is the transjunctional voltage, and C_1 and C_2 are the concentrations in the pre- and postjunctional cells, respectively (see Verselis et al., 1986b). For cases in which the voltages of the 2 cells were the same, this equation simplifies to

$$P = J/(C_1 - C_2) \quad (3)$$

Results

Electrotonic coupling (k) and junctional conductance (g_j)

Aplysia buccal neurons in primary culture were previously shown to connect via electrotonic synapses with high frequency (Dagan and Levitan, 1981; Bodmer et al., 1984). Figure 1 demonstrates that these junctions may exhibit some apparently nonlinear properties either in pairs or in ensembles of several interconnected neurons. In a simple case (Fig. 1*B*), exciting a neuron (V_1) resulted in a one-for-one transmission to the postsynaptic neuron. In some pairs of neurons, depolarizing current injection elicited a train of action potentials that decreased in height and increased in width during the train (Fig. 1*C*, trace V_1), whereas the postsynaptic electrotonic potentials (psp) increased in width and height and only occasionally elicited action potentials (Fig. 1*C*, trace V_2). Such behavior can be explained by the low-pass filter properties of the electrotonic synapse (Bennett, 1966): a short-duration spike will be attenuated much more than a broader one. In a complex network of neurons (as depicted in Fig. 1*D*), the electrotonic transmission of spikes often exhibited a sudden increase in size of the psp superimposed on the smooth steady increase (arrow in trace V_2 , Fig. 1*E*). When the presynaptic train of spikes was examined at higher sweep speeds and superimposed, only a steady increase in width was observed (Fig. 1*F*). It was, therefore, concluded that this train of spikes eventually recruited another neuron in the network to fire, thereby contributing additively to the psp. To avoid these polysynaptic effects, only hyperpolarizing pulses were used in isolated pairs of cultured neurons with closely apposed somata (see Fig. 2*A*) analyzed to characterize electrical coupling (described below).

The strength of electrotonic transmission can be expressed by the coupling coefficient (k), which is the ratio of the voltage deflection in the coupled cell (V_1 , first pulse Fig. 2*B*) to the voltage deflection in the cell in which current was injected (V_2 , first pulse in Fig. 2*B*). The conductance of the junctional membrane (g_j) is expressed by dividing the current transferred to the coupled cell under voltage clamp (I_2 , when $dV_2 = 0$) by the

voltage applied (V_1). In Figure 2*C*, this measurement of g_j is illustrated in the case of a voltage ramp delivered to the first cell (V_1) while holding the second at a constant voltage (not shown). Alternatively, in current clamp g_j can be calculated by applying the π -t transform with k and the input resistances (r_{11} , r_{22}) of both cells as variables (Bennett, 1966):

$$k_{1,2} = V_2/V_1 = r_{12}/r_{11} \quad (r_{12}: \text{transfer resistance}) \quad (4)$$

$$k_{1,2} = 1/(1 + 1/g_j(r_{11}r_{22} - r_{12}^2)/(r_{11} - r_{12})) \quad (5)$$

$$g_j = k_{1,2}/(r_{22} - k_{1,2}^2 r_{11}) \text{ and symmetrically}$$

$$g_j = k_{2,1}/(r_{11} - k_{2,1}^2 r_{22}) \quad (6)$$

Calculating g_j from the measurements of Figure 2*B* yielded $g_j = 28$ or 30 nS when applying equation (5) to $k_{1,2}$ or $k_{2,1}$, respectively. These values are close to those obtained from measuring g_j with a voltage clamp in Figure 2*C*, where g_j was 31 nS. In Figure 2*D* the plot of I_2 versus V_1 (from the data of Fig. 2*C*) exhibits an approximately linear relationship [slope (g_j) = 31 nS], indicating that over this range of voltages g_j was largely voltage independent.

Taken together, the behavior of this electrotonic synapse in culture suggests adequate voltage control of the pre- and postsynaptic membranes. Similar results were obtained from other pairs of coupled neurons in culture, when they were sufficiently isolated from other cells and closely apposed to one another. In some pairs where the I - V curves of the individual cells were more nonlinear, the plots of I_2 versus V_1 still indicated linearity of the junctional membrane.

The results in culture were compared with those obtained from electrotonic synapses of L14 neurons *in situ*. L14 neurons (indicated schematically in Fig. 2*E*) were tightly coupled *in situ* ($0.4 < k < 0.7$); e.g., in Figure 2*F*, k was 0.5 . The value calculated for g_j under current clamp was 370 nS, and the value measured for g_j under voltage clamp was 480 nS (Fig. 2*G*). In other L14 pairs the difference between measured and calculated values was even less, indicating that the efficacy of the space clamp was comparable to the experiments on cultured neurons. No significant dependence of g_j on transjunctional potential was detectable *in situ*, as demonstrated in Figure 2*G* (compare slopes of I_1 and V_2 traces). Varying holding potential over a wide range (± 40 mV in Fig. 2, *C* and *G*) also did not change g_j appreciably, suggesting no dependence of g_j on inside-outside potential.

Octanol does not effect coupling between *Aplysia* neurons

Figure 3 shows that there is no effect of octanol on k or calculated g_j of L14 neurons *in situ* ($n = 3$), even when neurons were bathed in a 10 mM octanol suspension for as long as 1 hr. Also in culture ($n = 4$), no change was found with octanol (not shown). These

Figure 1. Features of electrotonic coupling between cultured *Aplysia* neurons. *A*, Phase-contrast photograph of the cells recorded from in *B*. *B*, Current ($I = \pm 0.5$ nA) injected into cell 1 (V_1) resulted in an electrotonic potential in cell 2 (V_2). Positive current elicited a train of action potentials in cell 1, which in turn elicited one-to-one action potential transmission from cell 1 to cell 2, due to the tight coupling ($k = 0.5$, calculated from hyperpolarizing potentials in cell 1 and cell 2). *C*, Voltage recordings of another pair of coupled neurons in culture. Even though the coupling was also strong ($k = 0.5$), the electrotonic psp only reached threshold for spike generation once. This is probably attributable to high threshold in cell 2. Note that the increased action potential width in cell 1 results in an apparent facilitation of the psp in cell 2 (compare psp's early in the record with those at the end of the current pulse). *D*, Phase-contrast picture of the cells recorded from in *E* and *F*; note that the neurites from these 2 cells are probably intermingled with those of others. *E*, Train of action potentials was generated by positive current injection in cell 1, which resulted in psp's in cell 2 that were discontinuous in amplitude with spike number. *F*, Train of action potentials in *E* (V_1) was spread and superimposed, demonstrating a smooth increase in width and decrease in amplitude. Presumably additional cells in the network coupled to both of these cells reached threshold for spike generation (due to increased spike width in cell 1) and contributed to the apparent coupling potential.

Figure 2. Comparison between calculated and measured junctional conductance (g_j) in culture and *in situ*. **A**, Phase-contrast photograph of the pair of cultured neurons recorded from in **B–D**. **B**, Current injected alternately in cell 2 ($I_2 = -2$ nA) and cell 1 ($I_1 = -3$ nA) resulted in $k_{1,2} = V_2/V_1 = 0.7$ and $k_{2,1} = V_1/V_2 = 0.5$, measured at the end of the pulses. Input resistances: $r_{11} = V_1/I_1 = 26$ M Ω , $r_{22} = V_2/I_2 = 38$ M Ω . **C**, Measurement of g_j . Both cells were voltage-clamped. V_1 was swept between -20 and -120 mV (upper trace), V_2 was held at -40 mV and I_2 was recorded (lower trace), $g_j = -dI_2/dV_1 = 31$ nS. **D**, I - V plot of the measurements in **C**. The slope of I_2 versus V_1 is g_j . Note the approximately linear relationship. **E**, Schematic drawing of the ventral surface of the abdominal ganglion pointing out the L14 neurons (see Waziri, 1969). **F**, Measurement of

Figure 3. Absence of effect of octanol on electrotonic coupling. First, 5 mM and then 10 mM octanol was added to a pair of L14 cells *in situ* for more than 30 min each. In this experiment, representative of 7 others, g_j was 0.4 μ S under control conditions and 440, 404, and 460 nS in each of the other panels. The coupling coefficient was also relatively constant (about 0.6) in each panel.

results are in contrast to many other systems where octanol has been investigated for its potential to block electrotonic transmission (see Spray and Bennett, 1985).

pH dependence of electrotonic synapses

Application of CO_2 -equilibrated saline and NH_4Cl saline to *Aplysia* neurons *in situ* acidified and alkalinized the cells, respectively (Fig. 4, A, B, lowest traces, intracellular acidity upwards). No change in intracellular pH (pH_i) was observed with perfusion of saline containing impermeant acids or bases buffered to pH 5 (not shown), which is the pH of the CO_2 -saturated saline. Figure 4A (lowest trace) shows the typical range over which pH_i could be varied with these agents. pH_i changes from 7.1 (at rest) to 7.6 with NH_4Cl and to 5.9 upon NH_4Cl withdrawal and exposure to CO_2 -equilibrated saline. To investigate the influence of pH_i on electrotonic synapses we used current- and voltage-clamp methods to measure k or g_j simultaneously with pH_i .

In situ k changed very little with altered pH_i (Fig. 4A). Calculation of g_j from equation (6) and the corresponding input resistance values (r_{11} and r_{22}) revealed a 50% reduction of g_j (g_j at pH_i 7.6, 400 nS; at pH_i 5.9, 200 nS), but this effect on the coupling coefficient was offset by a decrease in nonjunctional conductance (at pH_i 7.6, $g_{nj2} = 0.25 \mu\text{S}$; at pH_i 5.9, $g_{nj2} = 0.17 \mu\text{S}$). Since k will depend on the ratio of g_j to ($g_j + g_{nj}$) (Bennett, 1966), k at pH_i 7.6 was 0.62 and at pH_i 5.9, k was 0.54, a decrease of 13%. The effects of acidification on g_j , measured directly under voltage clamp, is shown for another pair of L14 cells *in situ* in Figure 4B. The change of g_j with intracellular acidification was from 400 to 200 nS, a 50% reduction, confirming the calculated values for g_j in the previous experiment (Fig. 4A) performed under current clamp.

Figure 4, C, D, shows a similar set of experiments as in Figure 4, A, B, performed on cultured neurons. In culture, k decreased by about 50% in most cases at low pH_i (Fig. 4C), whereas non-

junctional conductances were not significantly altered. In 4 other experiments in culture, where k was only slightly decreased, nonjunctional conductance decreased as in the *in situ* cases; this might indicate that nonjunctional conductances of some neurons are differentially sensitive to low pH_i . The decrease in g_j in cultured neurons was always more pronounced [70–80% reduction is shown in a current-clamp experiment in Fig. 4C; under voltage clamp (Fig. 4D), g_j was reduced by 75%] than that obtained with L14 neurons *in situ* [approximately 50% in current clamp (Fig. 4C) and voltage clamp (Fig. 4D)].

The relation between g_j and pH_i in cultured neuronal pairs is illustrated in Figure 5 from 3 different voltage-clamp experiments. The relations between g_j and pH_i were shallow and seemed to be somewhat linear; the curves could not be well fit by simple Hill plots (see Spray et al., 1981b). A common feature of all the curves from cultured neurons was a 70–80% reduction in g_j . The differences among the curves is due to differences in maximal g_j values between these neuronal pairs.

Figure 5C presents a summary of 3 different experiments with L14 neurons and the 6 with cultured neurons from Figure 5, A, B, with g_j normalized with respect to the maximal value in each experiment. The L14 curves were also shallow, and the reductions in G_j at low pH_i were about 50%. Note that the minimal G_j values at the lowest pH_i values were larger for L14 neurons than for those obtained from cultured neurons.

Dye injections

Attempts to detect transfer of LY and the fluorescein derivatives 6-CF and Cl_2F between electrotonically coupled neurons in culture and between L14 neurons *in situ* 0.5–2 hr after injection are illustrated in Figures 6 and 7. Figure 6 (top panels) shows a pair of cultured neurons whose somata were not directly apposed but were electrotonically coupled (coupling coefficient, $k = 0.3$), presumably via interconnected neurites. LY diffused rapidly into many of the neurites within the injected cell, but

electrotonic coupling between two L14 neurons. $k_{1,2} = k_{2,1} = 0.5$, $r_{11} = r_{22} = 1.8 \text{ M}\Omega$. G, Same two L14 neurons as in F were voltage-clamped. V_1 was held at -60 mV with -20 mV hyperpolarizing command voltage steps, while V_2 was swept between -10 and -120 mV . The resulting junctional slope conductance was $0.48 \mu\text{S}$. The current deflection in I_1 resulting from the step pulses in V_1 remained the same regardless of the potential in V_2 , indicating isopotentiality, and the current deflections in I_2 were also invariant, suggesting no dependence of g_j on inside-outside potential.

Figure 4. Comparison of pH_i dependence of k and g_j in culture and *in situ*. In order to measure pH_i , one neuron of a pair was impaled with a pH-sensitive microelectrode. *A* and *B*, Recordings from 2 pairs of L14 neurons; *C* and *D*, recordings from 2 pairs of cultured buccal neurons. *A* and *C*, Current-clamp records; *B* and *D*, voltage-clamp records. The up- and downward deflection of the pH records are transients of the relatively slow response characteristics of these electrodes. *A*, Cells 1 and 2 receive alternate current injections of -15 nA, 0.6 sec duration pulses at 0.2 Hz (only I_2 is shown), resulting in voltage deflections in V_1 and V_2 , respectively, as well as the corresponding coupling potentials ($k_{1,2} = k_{2,1} = 0.6$). Consecutive addition of 80 mM NH_4Cl , CO_2 -saturated saline, and 50 mM NH_4Cl caused the pH_i of cell 2 to change between 7.6 and 5.9 . The coupling coefficient was only slightly altered: k (pH_i 5.9) = 0.55 . However, the input resistances changed over this pH range: r_{11} (pH_i 7.6) = 2.5 M Ω , r_{11} (pH_i 5.9) = 4.5 M Ω , r_{22} (pH_i 7.6) = 2.2 M Ω , r_{22} (pH_i 5.9) = 4 M Ω . The calculated junctional conductances are g_j (pH_i 7.6) = 400 nS and g_j (pH_i 5.9) = 200 nS. *B*, Cell 1 and 2 were stepped alternately between -60 and -80 mV command voltage (only V_2 is shown), and the resulting current changes I_1 and I_2 (transjunctional currents are the upward current deflections) were recorded. Upon addition of CO_2 -saturated saline, the pH_i changed from 7.3 to 6.0 . The corresponding junctional conductances are g_j (pH_i 7.3) = 400 nS and g_j (pH_i 6.0) = 200 nS. Note that these measured g_j values are quite similar to those of the previous pair of L14 neurons (*A*), where the g_j 's were calculated from current-clamp records. *C*, Experiment similar to that shown in *A* except cultured neurons were impaled and CO_2 -saturated saline was added to alter pH_i . Transients of the pH_i trace are truncated at a low pH_i by the chart recorder. Calculation of junctional and nonjunctional parameters showed that k measured from cell 1 to 2 or cell 2 to 1 decreased from 0.3 – 0.4 at pH 7.2 to 0.15 – 0.2 at pH 5.6 (50% change), whereas g_j changed from 75 to 20 nS between pH 7.2 and 5.6 (70% decrease). *D*, Experiment similar to that shown in *B* but recorded from cultured neurons, with pH_i decreased from 7 to 6 ; g_j at pH_i 7 = 90 nS, g_j at pH_i 6 = 25 nS. Note that the relative decrease in g_j at low pH_i is comparable to g_j changes obtained in the current-clamp experiment (*C*). pH_i in this case was simultaneously recorded on another chart recorder with a different time scale and is replotted in this figure.

no dye was detected in the electrotonically coupled neighboring cell (arrow in Fig. 6, top) or in other nearby neuronal somata (partially visible at the periphery of the phase photograph). In the cell pair shown in Figure 6 (middle panels), the cell bodies were closely apposed and coupling was strong ($k = 0.6$), but no diffusion of LY into the neighboring cell was detectable up to 2 hr after injection (arrows indicate location of the neighboring cell). In several instances LY appeared to spread into adjacent cells as illustrated in Figure 6, bottom panels, for a pair of closely apposed bag cells. Immediately after the injection a portion of the postjunctional cell directly overlying the injected cell was apparently fluorescent. Closer inspection of the distal margin of this cell (arrows), however, showed no staining, even after 1–2 hr; apparent dye coupling was attributable to refractile material beneath the cells.

LY has a maximum diameter from CPK models of approximately 1.4 nm, whereas the fluorescein derivatives 6-CF and

Cl_2F are slightly smaller (maximum diameters of 1.3 and 1.2 nm, respectively). In order to determine whether visualization of dye coupling would improve with the use of a slightly smaller probe, coupled neurons *in situ* and in culture were injected with 6-CF and Cl_2F (not shown). Although coupling was moderately strong ($k = 0.2$ – 0.4), no dye coupling was observed. Occasional faint staining of cross-ramifying neurites and of adjacent and nonadjacent neurons was attributable to cell autofluorescence.

In summary, we injected 27 pairs of cultured neurons with LY, 11 with 6-CF, and 12 with Cl_2F . All pairs had coupling coefficients >0.2 . Except for 2 cases, no dye transfer was detected between electrotonically coupled neurons; in 2 cases, transfer with LY was visible only after the cells became depolarized and were no longer excitable.

A similar lack of dye transfer was observed *in situ* as long as 2 hr after injection of LY ($n = 8$), 6-CF ($n = 3$), or Cl_2F ($n = 3$) into L14 neurons. The coupling coefficients were between 0.4

Figure 5. Dependence of g_j and normalized g_j (G_j) on pH_i . *A*, Plots of g_j calculated from 3 different current-clamp experiments on cultured neurons versus pH_i . *B*, Plots of g_j measured from 3 different voltage-clamp experiments on cultured neurons versus pH_i (see text). *C*, Cumulative plot of normalized conductance G_j (maximal conductance = 1) of 3 different experiments *in situ* (L14) and the above 6 experiments in culture. Note that the pH_i - G_j relations for cultured neurons are steeper than for L14 neurons *in situ*. *D*, Comparative pH_i versus G_j plots in different systems (from Spray and Bennett, 1985). Note that the curve for rat heart junctions is closest to *Aplysia* buccal neurons in culture, but none resembles the behavior of the junctions in L14 neurons *in situ*.

and 0.7. Typical LY and Cl_2F injections into L14 neurons with confinement of dye to the injected cell are illustrated in Figure 7, top and bottom, respectively. In these experiments the apparent dye diffusion was shown to be artifactual. Figure 7 (top) shows an injected L14 neuron that fluoresced brightly, with an apparent silhouette above it (due to reflection from injected cell), but there was no dye in the L14 neuron to which the injected cell was well coupled (arrow, electrical recording below). Subsequent impalement of the apparently silhouetted cell with a recording electrode showed no detectable electrotonic coupling to the injected cell. Figure 7 illustrates another example of the lack of dye (Cl_2F) transfer between L14 neurons with the iris on the epi-illuminator closed down to achieve spot excitation and thereby reduce reflection artifact. Spot detection within the neighboring cell revealed no detectable dye transfer.

To estimate the lower limit of dye detectability we injected LY and Cl_2F solutions, serially diluted with extracellular solution, into cultured cells. Fluorescence was detectable in the injected cells using concentrations in the injection electrode as low as 0.1%, which was used in the experiments illustrated in Figures 6, 7. Although the proportionality between the dilution factor and the amount of dye electrophoresed into the cells was not assessed, the concentrations of dye in the neighboring coupled cells 30–120 min after injection must have been at most 0.1% of that in the injected cell. We conclude that the dye passage between *Aplysia* neurons must be considerably restricted compared with other systems in which dye coupling is readily observed.

Permeation of tetraalkylammonium ions between electrotonically coupled neurons

Because fluorescent dye molecules did not transfer detectably between electrotonically coupled *Aplysia* neurons we undertook a quantitative study of permeability with the aid of smaller ions. Results of an experiment demonstrating transfer of TEA between L14 neurons *in situ* is shown in Figure 8. The upper 2 traces show the voltages recorded in the cells (V_1 , V_2) as a result of current pulses of constant amplitude alternately applied in each cell. The 2 lower traces show the voltages of ion-selective electrodes in the injected cell (TEA_2) and the coupled recipient cell (TEA_1). All records are illustrated as short segments (ca. 1–2 min) taken at specified times over a duration of 2 hr. Representative action potentials in the recipient cell (V_1) are shown as insets for each record segment to illustrate the effects of intracellular TEA on action potential duration. TEA was repeatedly injected into the presynaptic cell (TEA_2 trace, arrows) in order to achieve a large concentration gradient and sufficient driving force to overcome detection problems in the postsynaptic cell. The threshold for detection is about 0.1 mM TEA, due to interference from intracellular K^+ , estimated to be at concentrations of about 0.35–0.4 M in these cells. Transfer to the postsynaptic cell 2 was detected about 10 min after the third injection and continued until the end of the experiment (2 hr), at which time the measured TEA concentration was about 20 mM in the postsynaptic cell and 300 mM in the injected cell. Transfer of TEA to the postsynaptic cell was also evident be-

Figure 6. Dye injection into cultured neurons. *Top panels*, Separate cells with interconnecting neurites: At *left* is a phase-contrast picture showing 2 impaled neurons; the corresponding epifluorescence picture (*right*) shows that LY stained most neurites, but dye did not detectably transfer to the electrotonically coupled cell (*arrow*; $k = 0.3$). *Middle panels*, Closely apposed cells. At *left* a Nomarski picture of apposed neurons is shown.

Figure 7. Injections of fluorescein derivatives into cultured neurons. *Left panels*, Dichlorofluorescein injection into 1 of 2 coupled cells ($k = 0.3$). No dye transfer was detected to the uninjected cell (*arrow*). *Right-hand panels*, 6-CF injection into 1 of 2 electrotonically coupled neurons ($k = 0.2$ – 0.4). No fluorescence was detected in the other cell except for the neurites as in Figure 1 (bottom). Calibrations: 50 mV, 0.6 sec, current 2 μ A.

cause of broadening of the action potential induced by pharmacological blockade of delayed rectifier K^+ current (cf. Stanfield, 1983). Only after 50–70 min was an increase in the duration of the action potential evident in the postsynaptic cell

(trace *d*); the TEA concentration was more than 1 mM. After sufficient transfer of TEA, durations of action potentials evoked directly in the postsynaptic cell were consistently longer than those evoked by transsynaptic stimulation (cf. traces *d* and *f*,

←

LY was injected into one cell depicted by the fluorescence photo at *right*. Note that many neurites originating from the cluster are fluorescent, but cell 1 to which cell 2 is electrotonically coupled ($k = 0.6$, not shown) is not dye-coupled (*arrow*). *Bottom panels*, Apparent dye coupling. At *left* is a phase-contrast photograph of 2 closely apposed bag cells, right cell having been injected with LY. The *arrow* points to the left margin of the electrotonically coupled cell ($k = 0.5$). Photograph in the *center* is of the same field with epifluorescence; at *right*, the same field is shown with both transmitted light and epifluorescence. Although part of the left cell appears fluorescent, dye coupling must be ruled out because the left side of the cell at the left is unstained. The fluorescent halo under the left cell was probably due to neurites originating from the dye-injected cell ramifying under the other. No dye coupling was observed in these cells as long as 30–120 min after LY injection. Calibrations: *Top*: V_1 , 100 mV; V_2 , 20 mV; 0.2 sec; currents, ± 1 , -2 , -3μ A; *middle*: V_1 , V_2 , 100 mV; 1 sec currents ± 1 , -2 , -3μ A; *C*: V_1 , 100 mV; V_2 , 40 mV; 0.1 sec; currents $\pm 2 \mu$ A.

Figure 8. TEA transfer between two L14 neurons *in situ*. Current pulses of -15 nA (traces not shown) were alternately injected into cell 1 (V_1) and cell 2 (V_2) to monitor junctional conductance (which changed only toward the end of the experiment). V_1 electrode was filled with 0.5 M KCl and V_2 with 1 M TEA. Three TEA injections (arrows) into cell 2 resulted in an intracellular TEA concentration of about 100 mM. Ten minutes after the TEA injections in cell 1, transfer of TEA to cell 2 became detectable and progressively increased to 20 mM after 2 hr. *Inset*, Fast sweep voltage traces show the shape of the action potential at approximately the times indicated above V_1 . Note the action potential width increase in cell 2 after the first 2 injections and an even greater increase after the third (V_2). The action potential of cell 1 has been elicited transjunctionally by stimulating cell 2 in the traces b – d and f and by direct stimulation in the traces e and g . The width was increased only when the TEA concentration of cell 1 exceeded 1 mM. Note that the width increase was more pronounced when cell 1 was directly stimulated (traces e and g compared with trace f). Trace a is an action potential elicited in cell 2 (with direct stimulation) before TEA injection. TEA concentrations were calculated as described in Materials and Methods. Note the short latency in trace b (<5 msec), indicating a short distance from cell body to the junction.

which are evoked transsynaptically, with e and g , which are evoked by direct stimulation). This result is opposite that for action of TEA along the expected concentration gradient from axon to soma of the postsynaptic cell and may suggest that TEA-sensitive channels are differentially distributed between the axon and the soma. The threshold for TEA action was at least 1 mM and the variability in spike width is considerable. Estimation of TEA concentration from action potential duration was therefore much less reliable than measuring TEA concentration directly with K^+ -sensitive electrodes.

Figure 9*A* shows the time course of the rise in TEA concentration in the recipient cell from the *in situ* experiment illustrated in Figure 8. The inset shows the calibration measurement of the ion-selective electrodes prior to the experiment (calibrations did not change appreciably after the experiment). Figure 9*B* shows the result of a similar experiment with TMA injected into one cell of a pair of L14 neurons. The maximal TMA concentration obtained in the postsynaptic cell after 50 min was about 20 mM. (The limit of detection in the recipient cell was also 0.1 mM TMA.) Electrode calibration appears in the inset (slope, 60 mV/ 10 -fold change in TMA). Figure 10 illustrates examples of tetraalkylammonium ion transfer experiments between neurons in culture. For TEA (Fig. 10*A*) and TMA (Fig. 10*B*), injection resulted in large transfers to recipient cells within 60 min.

A summary of tetraalkylammonium ion transfer data for neurons in culture and *in situ* is presented in Table 1. Transjunctional flux (J) and permeability (P) were calculated from records such as those shown in Figures 9 and 10 using Eqs. 1 and 3 (see

Materials and Methods). Junctional conductance (g_j), which was measured simultaneously, is included for comparison.

Junctional permeability (P_j) values for either TMA or TEA and corresponding g_j 's obtained *in situ* are larger than for cells in culture. Several pairs of weakly coupled neurons in culture exhibited little or no TMA transfer (Table 2). The low value of g_j in these cases may have reduced the flux of TMA to below detection threshold.

Discussion

We show here that nonlinearities in electrotonic transmission in cultured *Aplysia* neurons are attributable to properties of the nonjunctional membrane as described previously in *Aplysia* (Carew and Kandel, 1976; Rayport and Kandel, 1980). The adequacy of voltage-clamp techniques for studies of synaptic events in *Aplysia* has been demonstrated for situations in which the synaptic site is located near the isopotential region of the voltage-clamped cell (Shapiro et al., 1980). L14 neurons are located next to each other in the ganglion (Fig. 2*E*), and the strong coupling (Fig. 2*F*) suggests that the synapse lies within short electrotonic distance from the somata. In culture, cell pairs chosen for voltage clamp were close together (e.g., Fig. 2*A*); since cultured neurons can couple well without visible neurites (Bodmer, 1983), we conclude that the electronic synapses in culture were located close to the soma and were therefore controllable by the voltage-clamp circuits.

We found that there was no appreciable voltage dependence of the electrotonic synapses themselves. This is the case for most

Figure 9. TEA (*A*) and TMA (*B*) transfer between L14 neurons. *A*, Time course of TEA transfer (data from Fig. 8) beginning after the third TEA injection (time zero). TEA concentrations measured with K^+ -sensitive electrodes were calculated from equations (4) and (6) (Materials and Methods) using the calibration curve (*inset*), obtained immediately after the experiment. Final TEA concentration in injected cell 2 and recipient cell 1 were 0.3 mM and 20 mM, respectively. Intracellular K^+ concentrations were estimated to be 0.3 mM. *B*, TMA transfer between two L14 neurons. Time course of transfer in an experiment similar to that shown in *A* but using TMA as a probe. *Inset*, calibration curve for both K^+ -sensitive electrodes obtained immediately after the experiment. Estimated intracellular K^+ concentration was 0.3 mM. Final TMA concentrations: recipient cell, 20 mM; injected cell, 0.25 mM.

gap junctions studied in detail so far (cf. Spray and Bennett, 1985); exceptions include a few cases of rectifying electrotonic synapses (Furshpan and Potter, 1959; Auerbach and Bennett, 1969; Nicholls and Purves, 1972; Margiotta and Walcott, 1983)

Table 1. Transfer of TMA and TEA between coupled *Aplysia* neurons

Experiment	Flux (mol/sec)	Permeability (cm ³ /sec)	g_j (μ S)
TMA			
(L14)	$3-7 \times 10^{-14}$	$1-3 \times 10^{-10}$	0.2
(Culture)	$0.5-1.2 \times 10^{-15}$	$1.3-3 \times 10^{-12}$	0.017
	$1-2 \times 10^{-16}$	$2-4 \times 10^{-13}$	0.021
TEA			
(L14)	$0.7-2 \times 10^{-14}$	$2-8 \times 10^{-11}$	0.22
	2.4×10^{-14}	$6-12 \times 10^{-11}$	0.25
(Culture)	$0.3-1 \times 10^{-16}$	$2-5 \times 10^{-13}$	0.033

Figure 10. TEA (*A*) and TMA (*B*) transfer between cultured neurons. Experiments similar to those shown in Figure 10 but with cultured neurons. *A*, Final TEA concentration in the recipient cell was 3 mM. *B*, Final TMA concentration in the recipient cell was 90 mM; final TMA concentration in the injected cell, 0.4 mM. TEA and TMA concentrations were calculated from equations (1) and (3) (Materials and Methods). *Insets*, Calibration curves obtained as in Figure 9.

and all early embryos thus far examined (Spray et al., 1985). Considering their presumed function in impulse transmission, the voltage independence of *Aplysia* electrotonic synapses has apparent value because L14 neurons mediate the inking behavior in *Aplysia*, which requires fast, synchronous transmission (Kandel, 1976).

The junctional conductance (g_j) of all the coupled cell pairs we investigated showed a significant dependence on cytoplasmic acidification. The relation between pH_i and g_j in all cases exhibited a shallow slope and large residual g_j at low pH_i (Fig.

Table 2. TMA transfer, k and g_j between cultured neurons

No.	k (mean)	g_j (nS)	TMA (mM) ^a
1	0.6	17	1.5
2	0.4	21	2.2
3	0.3	18	0.1-0.5
4	0.15	11	0.1-0.5
5	0.15	10	0 ^b
6	0.1	10	0.1-0.5

^a Concentration of TMA in the recipient cell 20 min after injection.

^b No transfer detected up to 40 min after injection.

5C). Neurons *in situ* showed a reduced pH sensitivity. Possible explanations include pH sensitivity of the series resistance (see below) and failure of the somatic pH_i recording to measure pH_i at the site of coupling. The g_j - pH_i relations could not be fit well to simple Hill plots, implying that g_j is not a simple function of pH_i . Interference of pH dependence of the nonjunctional membrane can only partially account for these results since extreme decreases in nonjunctional conductance would be required to compensate for a substantial decrease in g_j . (Since the equivalent resistances are in series, reducing g_j by 90% would produce an apparent reduction of 50% only if the shunting along the coupling pathway were reduced 5-fold.) For effects of pH_i on coupling coefficient, however, nonjunctional membrane properties are likely to play a major role. With shunting resistances (r_j) symmetrically interposed between soma and site of electrotonic connection, the coupling coefficient, k , is determined by $k = r_2 / (r_2 + 2r_s + r_j)$, where r_2 is the nonjunctional resistance of the cell in which coupling is measured and r_s is the parallel sum of shunts to extracellular medium along the coupling pathway (which presumably have a pH dependence similar to that of somatic nonjunctional membrane). Consider the case in which $k = 0.5$ and $r_j = 2r_s$. If r_2 at pH 6 is twice that at pH 7 and r_s has the same pH dependence, reducing r_j by 70% would decrease k by less than 0.01 (see equation 2). Such a contribution of nonjunctional membranes to promoting conduction under reduced pH_i has been pointed out previously in studies on crayfish septate axon (Giaume et al., 1980; Campos de Carvalho et al., 1984) and should be kept in mind when k is measured without assessing g_j directly.

Most systems seem to have pH_i -dependent gap junctions (cf. Spray and Bennett, 1985); and those studied in detail all close sigmoidally with various $\text{pK}'s$ (see also Fig. 5D). Thus, the gap junctions of L14 cells *in situ* and buccal neurons in culture have a quantitatively different regulation by pH_i compared with any other system known, and possibly also a qualitatively different pharmacology as indicated by their insensitivity to octanol.

Recently, Connors et al. (1984) reported that dye coupling between pyramidal neurons in neocortical slices was relatively insensitive to CO_2 , while dye passage between astrocytes was blocked by CO_2 treatment. Previously, Schuetze and Goodenough (1982) found decreased CO_2 sensitivity of dye transfer in later developmental stages of a non-neuronal system (chick lens). Although the extent to which g_j was affected and the minimal pH_i values reached in these experiments are unknown, these findings suggest that there might be classes of gap junctions less sensitive to pH_i . The possibility that there are several different gap junctional proteins is supported by many reports (e.g., Nicholson et al., 1983; Revel et al., 1985) where polypeptide maps and partial amino acid sequences of gap junctions from different tissues in a single species were substantially nonhomologous. Using a polyclonal antibody to rat liver gap junctions (Hertzberg and Skibbens, 1984), gap junctional protein was not detected in immunoblots of *Aplysia* ganglia (Hertzberg and Spray, 1985), again supporting the possibility of gap junction heterogeneity.

Injection of the fluorescent dyes LY, 6-CF, and Cl_2F into L14 neurons *in situ* as well as into cultured neurons resulted in no detectable spread to electrotonically coupled neighboring cells (Figs. 6, 7). The lack of dye coupling is in contrast to many other systems where one criterion used to assess coupling via gap junctions is rapid dye spread to neighboring cells (Spray and Bennett, 1985). There are, however, several systems where

electrotonic coupling is present, whereas dye coupling is not (Muller, 1981; Warner and Lawrence, 1982), including neurons of other molluscs (Audesirk et al., 1982; Murphy et al., 1983). One possible explanation for the absence of dye coupling is that the size of the gap junction channel is smaller in some tissues of some species, at some developmental stages or by partial closure induced by a change in functional state. Apparent diversity of channel size has been reported where gap junctions between salivary gland cells in arthropods were found to be more permeable than gap junctions connecting mammalian cells (Schwartzmann et al., 1981). Nevertheless, initial suggestions that size of the gap junction channel might be developmentally regulated (see Warner and Lawrence, 1982) have not been substantiated by subsequent studies (see Bennett et al., 1978; Safaryos et al., 1986), and constrictive closure of the gap junction channel by physiological stimuli (see Loewenstein, 1981) has not been supported in quantitative studies on voltage-dependent gating in arthropod salivary gland and amphibian embryonic cells (Zimmerman and Rose, 1985; Verselis et al., 1986b).

It is clear that the permeabilities of the fluorescent dye molecules used in the present study were considerably less than to the small ions that carry current between coupled cells. Two molecules (TMA and TEA), which are considerably smaller in size than the dyes, were permeable between electrotonically coupled neighboring cells both *in situ* and in culture. In some instances, transfer was quite rapid when cells were well coupled (Table 1). Permeabilities were often difficult to measure between cells in which g_j was low (<20 nS) (see Table 2). Factors contributing to the difficulty in measuring low permeabilities to TEA and TMA could include slight nonjunctional permeability to the probes causing leakage out of the cells and interference from K^+ leaking into the cells from the microelectrodes.

The high coupling coefficients we report here were due to high input resistances of the cells relative to the junctional membrane and obscure the rather low values of junctional conductance we generally encountered (10–100 nS). The fluxes of the tetraalkylammonium compounds measured in *Aplysia* neurons were slightly less than those found in fish and amphibian embryos when scaled for g_j (Verselis et al., 1986a, b). It follows that in electrotonic synapses of *Aplysia*, the ratio of permeability to conductance may be somewhat lower than for other tissues. Taking into consideration the geometry of the synapse, we cannot conclude unambiguously that the gap junction channels in *Aplysia* are smaller. Extrapolating the low permeabilities of the smaller tetraalkylammonium ions to the dye molecules would put dye permeabilities at very low values. This, together with the high detection threshold caused by somatic autofluorescence in the *Aplysia* ganglion, could obscure a low junctional dye permeability.

Aplysia neuronal gap junctions combine a characteristic set of responses to physiological stimuli (compare the present report with the summary in Spray et al., 1985) with a definable range of permeabilities to tracer molecules. Frictional models for diffusion through restricted pores predict that permeability decreases rapidly as molecules approach the diameter of the channels (Renkin, 1955). The low conductance of the junctional membrane in these cells and the finding that ratios of P_j to g_j in *Aplysia* are somewhat lower than in other preparations raises the possibility that the apparent junctional impermeability to larger molecules may represent junctional channels of distinctly smaller aperture or with charge selectivity (see Brink, 1985). Unambiguous resolution of this issue, however, will require

quantitative studies of junctional permeability to nonreactive tracer molecules of a greater size and charge range, or single-channel measurements, where unitary events can be viewed directly.

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