Diversity of the Transient Outward Potassium Current in Somata of Identified Molluscan Neurons

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We have undertaken a quantitative study of the differences in the properties of the fast transient outward current (A-current) between identified neurons of 2 species of nudibranch mollusc. Somata from identifiable neurons of *Archidoris montereyensis* and *Anisodoris nobilis* were isolated and voltage-clamped with a 2-microelectrode voltage clamp at 11°C. We examined diversity in the expression of the time- and voltage-dependent properties of A-current by measuring the following parameters: (1) current magnitude, (2) current density, (3) inactivation kinetics, (4) the voltage dependence of steady-state activation and inactivation. We first characterized A-current in each cell type by measuring these parameters for each identified neuron in a series of animals of a given species. The results of these measurements were used to describe the A-current properties of an identified neuron in terms of a mean and SD. The SD measured diversity within the animal population for any given cell type, while the mean values could be compared to measure diversity in the expression of A-current between identified neurons. When we compared mean values for A-current properties between identified neurons of a given species, we did not detect statistically significant differences in the steady-state voltage dependence of activation and inactivation. However, there were statistically significant differences in peak A-current magnitude, density, and inactivation kinetics between identified neurons. We examined differences between the species by comparing the A-current properties of homologous neurons. The major difference between the species was that outward current magnitude and density were significantly greater in *Anisodoris* than in *Archidoris*. We conclude that the magnitude and density of A-current differ between identified nudibranch neurons. The neurons also differentially express A-current inactivation kinetics in a cell-specific manner.

Voltage-clamp studies of animal and plant cells have demonstrated the presence of numerous ionic conductances that underlie a wide variety of electrical properties expressed by cell membranes. Ion channels may be distinguished on the basis of their voltage and time dependence, their pharmacology, their ion selectivity, and, in some instances, their modulation by intracellular factors (Hille, 1984). The temporal and spatial integration of the currents flowing through these channels results in the characteristic electrical activity of a cell. Thus, in the case of excitable cells, such as neurons, the repetitive firing properties emerge from the activation or expression of multiple ion channels. Differences in the classes of channels, their relative contribution to total current flow, as well as changes in their time- and voltage-dependent properties, all contribute to the diversity observed in membrane excitability. The ionic basis for this diversity has been analyzed for individual cells such as *Loligo* giant axon (Hodgkin and Huxley, 1952) and gastropod neuron soma (Connor and Stevens, 1971b), and for classes of cells such as crustacean walking leg axons (Connor, 1975), *Drosophila* longitudinal flight muscles (Salkoff and Wyman, 1981a, b), and turtle hair cells (Art and Fettiplace, 1987). However, little systematic study has been done in quantitatively determining how a given ionic current might vary between cells of the same class within an animal population or between classes of cells (cell-type comparison). Nevertheless, a knowledge of the differences in the characteristics of ionic currents between cells is necessary in order to understand processes which regulate ion-channel expression, and ultimately, lead to the observed diversity of cell types as defined by their electrical properties. We therefore systematically studied how the expression of the time- and voltage-dependent properties of one conductance, the fast transient potassium current (A-current; I_A), varies between identified cells of 2 species of mollusc, *Archidoris montereyensis* and *Anisodoris nobilis*. Molluscan preparations provide many advantages for quantitative studies of the origins of diversity between cell types. Giant neurons in the molluscan nervous system are easily identifiable by color and location in the ganglia and due to their size, they are suitable for electrophysiological recordings with the 2-microelectrode voltage-clamp technique. Furthermore, they have the same function, electrical properties, and synaptic connections from animal to animal.

A-current was first described by Hagiwara et al. (1961) in neurons from the esophageal ganglia of *Onchidium verruculatum*, and since then it has been characterized in membranes from many phyla, including non-neuronal cells (for reviews, see Rogawski, 1985, and Rudy, 1988). The voltage-dependent properties of A-current make it possible to isolate this current by

Received Mar. 16, 1989; revised June 2, 1989; accepted June 8, 1989.

This work was supported by grants from the National Institutes of Health and the Ford Foundation. We wish to thank Drs. Richard Aldrich, Paul Fuchs, Jon Johnson, Brad Jones, and Peter Ruben for helpful discussion and criticism during the preparation of this manuscript. We are also grateful to Dr. Jon Johnson for the use of his electrophysiology setup and Dr. Stuart Thompson for the use of his laboratory during portions of this research.

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Figure 1. *A*, Common locations of identified neurons on the dorsal surface of the brain of *Archidoris montereyensis*. Right Pedal Cell One (RPE1); Right Pedal Cell Two (RPE2); Right Pleural Cell One (RPL1); Right Pleural Giant Cell (RPLG); Right Cerebral Giant Cell (RCG); Left Cerebral Giant Cell (LCG); a small white cell in the left pleural ganglion (WC-LPLG); Left Pedal Cell One (LPE1); Left Pedal Cell Two (LPE2). *B*, Input capacitance measurement for RPE1. The cell received a triangular wave command, 15 mV in magnitude and with an 8 msec wavelength. The top trace is membrane voltage (V_m), and the bottom trace is membrane current. The input capacitance of the cell (0.021 μ F) was measured using the instantaneous change in membrane current, I_a (160 nA), and the equation $C_m = I_a(W/4V)$. *C*, Illustration of the subtraction procedure used to separate A-current from other membrane currents. Left trace, A depolarizing test pulse from the holding potential of -40 mV did not activate an early transient outward current typical of I_a , because activation of A-current requires a hyperpolarizing prepulse. Right trace, A -80 mV prepulse resulted in activation of a large, transient outward current during the test pulse. The transient current reached a maximum value within 25 msec and decayed to a steady-state level by 500 msec. I_a was measured by manually subtracting the current obtained during a test pulse without a prepulse from that obtained during a test pulse with a prepulse.

voltage paradigms, thereby eliminating the need to separate currents pharmacologically (Connor and Stevens, 1971a; Neher, 1971; Thompson, 1977). The present study was designed to answer the following questions: (1) How do the A-current characteristics of an identified neuron, or cell type, differ from animal to animal? (2) How does A-current differ between identified

neurons? and (3) How does A-current differ between species? We resolved the first question by gathering statistical information about the variability in A-current properties for a given cell type within an animal population. We obtained this information by measuring the A-current of each identified neuron in a series of animals of a given species, and describing the A-current properties of the neuron in terms of a mean value with a SD (within population, class comparison). We could then answer the second question by comparing the mean values for A-current parameters between identified neurons (between populations, class comparison). Species differences were determined by comparing the mean A-current values of homologous neurons in the related marine nudibranchs *Archidoris montereyensis* and *Anisodoris nobilis* (between species, class comparison).

In this paper we quantitatively compare the time- and voltage-dependent properties of A-current between identified neurons of the doridacean nudibranchs *Archidoris montereyensis* and *Anisodoris nobilis*. We also present data comparing outward current magnitude and density between these neurons.

Materials and Methods

Sample population of animals and cells. Specimens of *Archidoris montereyensis* and *Anisodoris nobilis* were supplied by Pacific Biomarine, Inc. (Venice, CA) and ranged in length from 4 to 14 cm. The brain was dissected from the animal and treated with a solution of 0.15% pronase at 20°C (Sigma, Grade B) for 3–5 min to assist in the removal of connective tissue surrounding the brain. After treatment, the brain was rinsed several times with artificial sea water (ASW), refrigerated at 6°C for 2–4 hr, and desheathed. A cluster of 3–6 large somata was removed from the neuropil with fine iridectomy scissors using a method similar to that developed by Connor (1977). This cluster of cells was pinned to a Sylgard surface on a Lucite dish and bathed with ASW (470 mM NaCl, 10 mM KCl, 10 mM CaCl₂, 50 mM MgCl₂, 20 mM Tris, pH 7.5) at 11°C.

The experiments were performed on identified neurons of *Archidoris* and *Anisodoris*. The common locations of the giant somata on the dorsal surface of the brain are shown in Figure 1A. Cells were identified on the basis of their position, color, size, and location relative to other cells and nerve trunks (Partridge, 1976; Blackshaw, 1979). Studies concentrated on 9 identified cell types: Right Pedal Cell One (RPE1); Right Pedal Cell Two (RPE2); Right Pleural Cell One (RPL1); Right Pleural Giant Cell (RPLG); Right Cerebral Giant Cell (RCG); Left Cerebral Giant Cell (LCG); a small white cell in the left pleural ganglion (WC-LPLG); Left Pedal Cell One (LPE1); Left Pedal Cell Two (LPE2).

Electrophysiological recordings. The voltage/current-clamp amplifier used in these experiments was identical to that used by Aldrich et al. (1979). Membrane potential was monitored differentially utilizing an intracellular and an extracellular electrode. Microelectrodes were filled with 3 M KCl and ranged in resistance from 1 to 5 M Ω . A virtual ground circuit connected to the bath via an agar bridge was used to measure membrane current which was filtered with a second-order Butterworth filter at a low-pass frequency of 2 kHz. Series resistance was between 1 and 5 k Ω . Experiments were performed without series resistance compensation. With our values for A-current, this corresponded to errors in voltage of less than 1 mV. Records were preserved on a chart recorder (Gould, Inc.) with a low-pass frequency of 50 Hz. Oscilloscope records were filmed when higher frequencies were important.

Healthy, isolated neurons had resting potentials between -35 and -45 mV and spike amplitudes of 70 to 120 mV. Under current clamp, the neurons fired repetitively in response to constant-current pulses and had input resistances ranging between 0.75 and 7.0 M Ω . Control experiments were performed on intact cells in the ganglion to test for the effect of axotomy and isolation on membrane currents. In voltage-clamp experiments conducted on unisolated WC-LPLG, it was possible to activate I_a without recruiting axon spikes. The properties of I_a were not significantly altered in isolated cells from those of WC-LPLG *in situ*.

Cells received control pulses to monitor I_a every 5 min. Data from experiments showing either (1) more than a 5% change in control current, (2) inward current notches typical of regions of unclamped axon, or (3) more than 3 mV drift in the voltage electrode, were discarded. Some experiments were designed to measure currents during depolar-

Table 1. Mean peak A-current values for 4 cell types

	Mean peak A-current (nA)				
Test voltage	WC-LPLG (n = 4)	LPE2 (n = 3)	RPE1 (n = 5)	LCG (n = 4)	Average
Activation					
−40 mV	18 ± 7 (39%)	16 ± 2 (13%)	18 ± 9 (50%)	58 ± 28 (48%)	28 ± 20 (38%)
−35 mV	42 ± 12 (29%)	38 ± 8 (21%)	46 ± 21 (46%)	127 ± 62 (49%)	63 ± 42 (36%)
−30 mV	74 ± 9 (12%)	78 ± 21 (27%)	105 ± 38 (36%)	248 ± 73 (29%)	126 ± 82 (26%)
−25 mV	118 ± 15 (13%)	123 ± 34 (28%)	178 ± 45 (28%)	420 ± 146 (35%)	209 ± 142 (26%)
−20 mV	169 ± 14 (8%)	187 ± 63 (34%)	243 ± 74 (30%)	—	—
	Mean peak A-current (nA)				
Prepulse voltage	WC-LPLG (n = 4)	LPE2 (n = 3)	RPE1 (n = 5)	LCG (n = 4)	Average
Inactivation					
−99 mV	100 ± 12 (12%)	105 ± 28 (27%)	140 ± 26 (19%)	344 ± 9 (3%)	172 ± 116 (15%)
−90 mV	95 ± 12 (13%)	98 ± 25 (26%)	131 ± 22 (17%)	315 ± 32 (10%)	159 ± 104 (17%)
−85 mV	86 ± 8 (9%)	90 ± 15 (17%)	122 ± 28 (23%)	295 ± 17 (6%)	148 ± 99 (14%)
−80 mV	74 ± 9 (12%)	78 ± 21 (27%)	105 ± 38 (36%)	248 ± 73 (30%)	124 ± 78 (26%)
−75 mV	61 ± 4 (7%)	62 ± 18 (29%)	85 ± 22 (26%)	180 ± 40 (22%)	97 ± 56 (21%)
−70 mV	42 ± 5 (12%)	44 ± 16 (36%)	60 ± 20 (33%)	130 ± 35 (27%)	69 ± 41 (27%)
−60 mV	12 ± 9 (75%)	20 ± 11 (55%)	18 ± 11 (61%)	38 ± 10 (26%)	22 ± 11 (54%)

Comparison of A-current magnitude between animals for 4 identified *Archidoris* neurons. Peak A-current magnitude was measured as a function of activation test voltage following a constant conditioning prepulse to -80 mV (top) or as a function of inactivation prepulse voltage by clamping to a standard test voltage of -30 mV (bottom). Prepulse duration was set to reach a steady-state level of inactivation at the prepulse voltage (1.5–2.5 sec). The leak-corrected peak current was recorded in *N* different animals for each cell type and the average peak A-current values for each cell are entered, together with the average of the population averages.

izations to 0 mV. These pulses were delivered from the holding potential of -40 mV and were a standard 7 sec in duration. Peak outward current measurements from these records were leak corrected prior to analysis. Leak conductances (g_L) were calculated according to standard procedures (Connor and Stevens, 1971b) and were between 0.1 and 1.4 μ mhos.

Values for current density were determined by normalizing membrane current with measurements of input capacitance (C_m), rather than cell surface area. The input capacitance of cells was measured by applying fast triangular waves under voltage clamp (Adelman and Palti, 1969; Neher, 1971). Since capacity current (I_{cm}) and membrane voltage (V_m) are related by the expression $I_{cm} = C_m(dV_m/dt)$, capacity current during a triangular wave command voltage is directly proportional to input capacitance. The equation $C_m = I_p/(W/4V)$, where I_p is the total instantaneous change in membrane current in nA, V is the peak to peak imposed voltage in mV, and W is the wavelength in seconds, can be used to measure input capacitance (Fig. 1B). All input capacitance measurements were made with triangular wave frequencies >100 Hz and peak-to-peak voltages between 0 and 30 mV.

A-current isolation. Previous studies have shown that I_A can be isolated with voltage paradigms at voltages more negative than -20 mV (Connor and Stevens, 1971a; Strong, 1984). Figure 1C illustrates the subtraction procedure used to isolate I_A by voltage paradigms. Depolarizing test pulses from the holding potential of -40 mV did not activate an early transient outward current typical of I_A . Applying a prepulse to -80 mV prior to the test pulses, however, resulted in activation of a large, transient outward current during the test pulse. The transient

current rose quickly to a peak value within 25 msec and decayed to a steady-state level by 500 msec. A characteristic feature of all cells was that the steady-state level without prepulse was identical to that with prepulse (Fig. 1C). I_A was isolated from other currents by manually subtracting the current obtained during a test pulse without a prepulse from that obtained during a test pulse with a prepulse. Analysis of I_A parameters was performed on the difference current for all cells in this study. Tail reversal measurements gave A-current reversal potentials between -55 and -60 mV.

Steady-state activation and inactivation curves. Steady-state activation and inactivation curves were obtained by stimulating cells with standard pulse paradigms. Inactivation curves were obtained from values of peak current during a test pulse preceded by hyperpolarizing prepulses delivered from membrane potentials ranging between -100 and -60 mV. Prepulse duration was long enough to reach a steady-state level of inactivation at the prepulse voltage (1.5–2.5 sec). Unless otherwise indicated, the test pulse value for activation of A-current was -30 mV. Steady-state inactivation curves were normalized to the value of peak A-current activated by a prepulse to -80 mV. For steady-state activation curves, cells received a hyperpolarizing prepulse of -80 mV and 1.5–2.5 sec in duration; test voltages ranged between -50 and -20 mV. Normalized activation curves obtained from peak A-current values were not significantly different from those obtained by extrapolating the decaying phase of I_A to 0 time; therefore, activation curves were obtained by normalizing peak current values, rather than extrapolated values, relative to the activated current at -30 mV. Normalized steady-state

Table 2. Peak I_A , membrane input capacitance, and A-current density in identified *Archidoris* neurons

Cell	N	I_A (nA)	C_m (μ F)	I_A/C_m (nA/ μ F)
RPL1	3	100 $\pm$ 20 (20%)	0.038 $\pm$ 0.013 (34%)	2800 $\pm$ 450 (16%)
RPE1	5	110 $\pm$ 40 (36%)	0.026 $\pm$ 0.004 (15%)	4200 $\pm$ 880 (21%)
LPE1	3	130 $\pm$ 48 (37%)	0.026 $\pm$ 0.009 (35%)	5200 $\pm$ 1900 (37%)
RCG	3	240 $\pm$ 54 (23%)	0.016 $\pm$ 0.002 (13%)	14,000 $\pm$ 3100 (22%)

Results of experiments determining A-current density in 4 identified neurons. Peak A-current and input C_m values were obtained for each identified neuron in N animals and then used to calculate current density by normalizing peak current with input capacitance (peak I_A/C_m). These 3 numbers were averaged and their means and SDs are presented. Peak I_A values were obtained from activated A-current records at a test potential of -30 mV following a 1.5 – 2.5 sec hyperpolarizing prepotential to -80 mV; input capacitance was calculated by applying triangular waveforms to the cells under voltage clamp and measuring the instantaneous change in the current (see Materials and Methods). The variance among the population means (F statistic) for A-current density was significant at the 0.001 level; the variance in A-current magnitude was significant at the 0.02 level. There were significant differences between the mean A-current density values of RPL1 and RCG ($p < 0.01$), and RPE1 and RCG ($p < 0.01$).

activation curves were independent of prepulse voltage for prepulse voltages ranging from -99 to -75 mV. We did not examine the effect on activation curves of prepulse voltages more positive than -75 mV.

Statistics. Unless otherwise indicated, all data are presented as means $\pm$ SD. Linear relationships between variables were established by a least-squares fit to the data. Correlation coefficients were used to evaluate the significance of the linear relationship between the variables. Student's t test was used to determine the significance of the difference between the means when 2 populations were compared in an experiment. The F test was used to evaluate data from 3 or more populations and establish whether the variance among the population treatment means was significant. When a significant overall F statistic was obtained from an experiment, the Tukey honestly significant difference procedure was used to determine significant differences between pairs of means on a *post hoc* basis. We determined the degree of association between 2 variables by computing the Spearman rank correlation coefficient, r_s . We used a value of $\alpha = 0.01$ for a 1-sided alternative.

Results

A-current magnitude and density differ between identified *Archidoris* neurons

A-current was present in all *Archidoris* neurons; however, we observed dramatic differences in the magnitude of the current between neurons ($N = 104$). In order to determine the variability in A-current magnitude for an identified neuron within the animal population, we compared the magnitudes of A-current between animals for each cell type. Table 1 summarizes representative peak A-current data from 4 identified *Archidoris* neurons. These peak values were obtained as a function of test voltage (for activation) following a constant conditioning prepulse to -80 mV or as a function of prepulse voltage (for inactivation) by clamping to a standard test voltage of -30 mV (see Materials and Methods). The peak current was recorded in N different animals for WC-LPLG, LPE2, RPE1, and LCG. When we averaged peak A-current values at each test voltage, we observed that within the animal population, the average % SD of peak A-current for a given cell type was 30% about the mean value.

This approach was used to establish the average A-current magnitude of each identified neuron within the animal popu-

lation and enabled us to compare average values between identified neurons. We noted a 3-fold range in average A-current magnitude between identified neurons. The variance among the population means (F statistic) for A-current magnitude at the different test voltages was significant at the 0.02 level (Table 1).

Differences in A-current magnitude may be partially attributed to cell size. While 3 of the cells in Table 1 had approximately the same diameter (~ 250 μ m), the white cell was much smaller (~ 80 μ m). However, the magnitude of the white cell A-current was comparable to that of the other, larger cells. Furthermore, LCG, with a diameter of 250 μ m, had over $2\frac{1}{2}$ times the A-current of the other neurons. In order to normalize for differences in cell size, it was convenient to have some indication of current density, either by measuring surface area or input capacitance. The diameter of molluscan neurons cannot be used to estimate surface area because of the irregular, flattened shape of the cells and the convolution of the membrane (Coggeshall, 1967). Instead, we measured current density by normalizing A-current magnitude to input capacitance. The underlying assumption was that the specific membrane capacitance was constant among cells; cells with larger input capacitances were presumed to have larger surface areas (Adelman and Palti, 1969; Neher, 1971).

Tables 2 presents the results of experiments determining A-current density in 4 identified neurons. Since it was not possible to determine maximum conductance, we used standard pulse paradigms to activate A-current at a test potential of -30 mV following a hyperpolarizing prepotential to -80 mV. Once peak A-current and input C_m values were obtained, they were used to calculate current density by normalizing peak current to input capacitance (peak I_A/C_m). This procedure was repeated for each identified neuron in a total of N animals. Peak I_A , C_m , and A-current density were averaged and their means and SDs are presented in Table 2. For a given cell type, the average SDs in peak I_A , C_m , and A-current density were 29, 24, and 24%, respectively. Average A-current magnitude ranged from 100 to 240 nA. The variance among the population means (F statistic) for A-current magnitude was significant at the 0.02 level. Furthermore, after current was normalized to input capacitance, there was a 5-fold range in the average A-current density of the 4 identified neurons. The variance among the population means for A-current density was significant at the 0.001 level. There were also significant differences between pairs of means. For example, when the mean A-current density values were compared for cell pairs RPL1/RCG and RPE1/RCG, the differences were significant at the 0.01 level. In conclusion, these results strongly suggest that A-current magnitude and density differ between cell types.

Steady-state activation and inactivation properties are conserved between *Archidoris* neurons

We examined differences in the steady-state voltage dependence of activation and inactivation, first, within the animal population for each identified neuron, then between identified neurons. Table 3 compares normalized steady-state activation and inactivation properties between 4 identified *Archidoris* neurons. Table 3 was constructed by averaging the steady-state activation and inactivation values for each identified neuron for the same animal populations characterized in Table 1. Voltage paradigms were also identical to those used in Table 1. Each entry represents the average of measurements from N animals. When successive measurements were made for a cell type in different

Table 3. Mean normalized steady-state values for 4 cell types

	Mean normalized value				
Voltage (mV)	WC-LPLG (n = 4)	LPE2 (n = 3)	RPE1 (n = 5)	LCG (n = 4)	Average
Voltage dependence of A-current activation					
−40	0.23 ± 0.07 (30%)	0.21 ± 0.05 (24%)	0.19 ± 0.04 (21%)	0.22 ± 0.06 (27%)	0.21 ± 0.03 (25%)
−35	0.55 ± 0.10 (18%)	0.48 ± 0.07 (15%)	0.44 ± 0.07 (16%)	0.50 ± 0.05 (10%)	0.49 ± 0.05 (15%)
−30	1.0	1.0	1.0	1.0	1.0
−25	1.60 ± 0.17 (11%)	1.58 ± 0.22 (14%)	1.73 ± 0.09 (5%)	1.66 ± 0.08 (5%)	1.64 ± 0.07 (8%)
−20	2.31 ± 0.42 (18%)	2.35 ± 0.50 (21%)	2.38 ± 0.10 (4%)	—	2.35 ± 0.04 (14%)
Voltage dependence of A-current inactivation					
−99	1.35 ± 0.06 (4%)	1.38 ± 0.18 (13%)	1.37 ± 0.11 (8%)	1.37 ± 0.06 (4%)	1.37 ± 0.01 (7%)
−90	1.28 ± 0.05 (4%)	1.24 ± 0.14 (11%)	1.26 ± 0.10 (8%)	1.30 ± 0.08 (6%)	1.27 ± 0.03 (7%)
−85	1.18 ± 0.15 (13%)	1.21 ± 0.10 (8%)	1.17 ± 0.02 (2%)	1.17 ± 0.05 (4%)	1.18 ± 0.02 (7%)
−80	1.0	1.0	1.0	1.0	1.0
−75	0.82 ± 0.04 (5%)	0.81 ± 0.09 (11%)	0.81 ± 0.06 (7%)	0.76 ± 0.07 (9%)	0.80 ± 0.03 (7%)
−70	0.57 ± 0.08 (14%)	0.57 ± 0.16 (28%)	0.56 ± 0.10 (18%)	0.54 ± 0.07 (13%)	0.56 ± 0.01 (18%)
−60	0.17 ± 0.13 (76%)	0.27 ± 0.06 (22%)	0.16 ± 0.08 (50%)	0.16 ± 0.04 (25%)	0.19 ± 0.05 (43%)

Comparison of normalized steady-state activation and inactivation properties among 4 identified *Archidoris* neurons. Activation values were obtained at test pulses ranging from -40 to -20 mV, following a 1.5–2.5 sec hyperpolarizing prepulse to -80 mV (top). Inactivation values were obtained at a test pulse of -30 mV, with hyperpolarizing prepulses of 1.5–2.5 sec from -99 to -60 mV (bottom). Each entry represents the average of measurements from *N* animals. The table was constructed by averaging the steady-state activation and inactivation values for each cell from the same animal populations characterized in Table 1. The average of the population averages is also included. Activation curves were normalized relative to the current at -30 mV. Inactivation curves were normalized relative to the current activated by a -80 mV prepulse.

animals, the % SD in the mean values of steady-state voltage dependence of A-current averaged 15% (Table 3). When the means for the cell types were compared, the variation in the means between cell types averaged 8% (Table 3). Application of the *F* test to these mean values did not give a significant *F* statistic. We conclude that there were no cell-specific differences in the steady-state voltage dependence of A-current between identified neurons.

Comparison of inactivation kinetics between *Archidoris* neurons

We characterized A-current kinetics by using standard pulse paradigms to measure 2 time constants: the time constant for onset of inactivation (T_A) and the time constant for removal of inactivation (T_{REM}). The voltage-clamp records in Figure 2 illustrate activated A-current in 2 *Archidoris* neurons: RPL1 (Fig. 2A) and LCG (Fig. 2B). The decaying phase of the current can be described by a single exponential (Connor and Stevens, 1971a). We determined T_A from a least-squares exponential fit of the decay of I_A . T_A was calculated from steady-state A-current records at a test pulse of -30 mV, following a hyperpolarizing prepulse to -80 mV. Correlation coefficients were always greater than or equal to 0.99. The time course of removal of inactivation can also be described by a single-exponential process. Figure 3 shows the time dependence of removal of inactivation

in RPL1. The duration of the hyperpolarizing prepulse (t_{pp}) affected the magnitude of the activated A-current (I_{A-P}) until a steady-state condition (I_{A-SS}) was achieved. The time constant for removal of inactivation, T_{REM} , was calculated from a least-squares exponential fit of the peak A-current (I_{A-P}) versus prepulse duration (t_{pp}) curve. T_{REM} was calculated from peak A-current records at -30 mV following hyperpolarizing prepulses of variable duration to -80 mV. All correlation coefficients were at least 0.98. During the course of an experiment, repeated measurements of T_A and T_{REM} varied by 5% or less. We followed our customary procedure, measuring the time constants for each cell type in several animals, then comparing the means between identified neurons.

Table 4 summarizes the means and SDs for the 2 time constants in 8 identified neurons. Within the animal population, the average % SD for T_A was 10% for each cell type. T_{REM} was slightly more variable, on the average, the % SD for T_{REM} was 18%. In contrast, there was a statistically significant, 4-fold range in A-current kinetics between identified neurons. The variance among the population means (*F* statistic) was significant at the 0.001 level. There were also significant differences between pairs of means. For example, the difference between the mean T_A values for RPL1 (240 ± 12 msec; $N = 7$) and LCG (130 ± 14 msec; $N = 4$) was significant at the 0.01 level. Similarly, the difference between the mean T_{REM} values for RPL1 (700 ± 170

Figure 2. Activated A-current in 2 *Archidoris* neurons. The decaying phase of the current can be described by a single exponential with a time constant of T_A . Inactivation was removed with a 2.0 sec hyperpolarizing prepulse to -80 mV. The current was activated by a test pulse to -30 mV. *A*, RPL1: $T_A = 230$ msec, $r = 0.99$. *B*, LCG: $T_A = 130$ msec, $r = 0.99$.

msec; $N = 7$) and LCG (270 ± 64 msec; $N = 4$) was also significant at the 0.01 level. Representative voltage-clamp records for these 2 cells are presented in Figure 2. T_A for RPL1 (Fig. 2*A*) was 230 msec in this cell, while T_A for LCG (Fig. 2*B*) was 130 msec.

We also compared the voltage dependence of T_{REM} between cells. A-current inactivation was removed more rapidly by large (more negative) hyperpolarizing prepulses. In Figure 4*A*, the absolute values for T_{REM} are plotted as a function of prepulse voltage for 3 identified neurons. The test voltage for these experiments was -30 mV; inactivation was removed by pulses of variable duration and ranging in magnitude between -60 and -100 mV. RPL1 had much slower inactivation kinetics than RCG or RPE1. However, when the curves were normalized relative to the T_{REM} at -80 mV, the voltage dependences of T_{REM} were similar for all 3 cells, despite different absolute values of T_{REM} (Fig. 4*B*).

A-current properties of *Anisodoris* neurons

We measured the steady-state voltage dependence of activation and inactivation and A-current kinetics in *Anisodoris* neurons with voltage paradigms identical to those used to study *Archidoris*. Our results revealed that steady-state properties varied on the average by 15% for cells of a given type within the animal population. A comparison of the average steady-state values showed differences of 10% between identified *Anisodoris* neurons such as RPL1 and LCG (data not shown). As with *Archidoris*, we noted statistically significant, cell-specific diversity in T_A and T_{REM} between *Anisodoris* cell types (data not shown). T_A values ranged from 80 to 350 msec and T_{REM} values were between 200 and 1000 msec. Representative traces compare features of inactivation between *Anisodoris* neurons RPL1 (Fig. 5*A*) and LCG (Fig. 5*C*). T_A was 260 msec in RPL1, 125 msec in LCG. Furthermore, *Anisodoris* neurons, like *Archidoris* neurons also exhibited cell-specific differences in current density, the LCG ($31,000$ nA/ μ F; Fig. 5*C*) had 4 times the current density of RPL1 (7100 nA/ μ F; Fig. 5*A*).

To summarize, while the steady-state voltage dependence of I_A did not vary between *Anisodoris* neurons, there was a cell-specific diversity in A-current density and kinetics. These results paralleled our previous findings comparing A-current properties between *Archidoris* neurons.

Species comparison of A-current properties

When we compared peak A-current values from *Archidoris* neurons with those from *Anisodoris* neurons, we observed pronounced differences between the 2 species in the distribution of current magnitude. We gathered steady state A-current data at -30 mV (preceded by hyperpolarizing prepulses to -80 mV) and noted that while 54% of the *Anisodoris* neurons had a peak I_A of at least 200 nA ($N = 20$), only 7% of the *Archidoris* neurons had currents of similar magnitude ($N = 84$).

In order to compare A-current density across species, peak I_A/C_m was graphed as a function of C_m for *Archidoris* and *Anisodoris* (Fig. 6). Input capacitances for cells from the 2 species extended over the same range, between 2 and 38 nF. Under the microscope, giant neurons of both species were also similar in size (100–400 μ m). We observed statistically significant ($p < 0.01$) higher A-current densities in *Anisodoris* ($14,000 \pm 2200$ nA/ μ F; $N = 11$) than in *Archidoris* (5800 ± 1100 nA/ μ F; $N =$

Figure 3. Time dependence of removal of A-current inactivation. RPL1 received prepulses to -80 mV followed by a test pulse to -30 mV. The duration of the hyperpolarizing prepulse was increased until the peak current reached a steady state. Removal of inactivation was an exponential process with a cell-specific time constant. In this cell, T_{REM} was 800 msec ($r = 0.98$).

Table 4. Diversity of I_A kinetics in identified *Archidoris* neurons

Cell	<i>N</i>	T_A (msec)	T_{REM} (msec)
RPLG	5	65 ± 7 (11%)	200 ± 30 (15%)
RPE1	5	69 ± 8 (12%)	250 ± 24 (10%)
LPE1	9	70 ± 7 (10%)	230 ± 37 (16%)
LPE2	3	73 ± 9 (12%)	210 ± 32 (15%)
RCG	6	100 ± 8 (8%)	280 ± 60 (21%)
LCG	4	130 ± 14 (11%)	270 ± 64 (24%)
WC-LPLG	5	180 ± 8 (4%)	610 ± 140 (23%)
RPL1	7	240 ± 12 (5%)	700 ± 170 (24%)

Summary of the means and SDs for T_A and T_{REM} for 8 identified *Archidoris* neurons. Time constants were measured in *N* animals for each cell type using standard pulse paradigms: T_A was calculated from A-current records at a test pulse of -30 mV, following a 1.5–2.5 sec hyperpolarizing prepulse to -80 mV; T_{REM} was calculated from peak A-current records at -30 mV following hyperpolarizing prepulses of variable duration to -80 mV. There was a statistically significant, 4-fold variance in average A-current kinetics between identified neurons (*F* test; $p < 0.001$). The difference between many pairs of means (such as the mean T_A and T_{REM} values for RPL1 and RPLG) was significant at the 0.01 level.

12). We also noted a nonlinear trend towards a decrease in current density with increasing input capacitance. Neher (1971) observed a similar correlation in ganglion cells from *Helix pomatia*. He found that while the fast transient outward currents were found regularly in small to medium-sized ganglion cells (100 – 200 μm), they were small or undetectable in giant cells.

The brain of *Anisodoris* contains identifiable neurons homologous to those presented in Figure 1*A* for *Archidoris*. We chose to compare the A-current properties of homologous identifiable neurons in order to more precisely evaluate species-specific differences. Representative steady state A-current records from RPL1 and LCG in both species are displayed in Figure 5. Cells with similar input capacitances were chosen for comparison. Following removal of inactivation by a hyperpolarizing prepulse to -80 mV, activation of I_A at -30 mV resulted in a peak I_A/C_m value of 7100 nA/ μF for RPL1 in *Anisodoris* (Fig. 5*A*) and 2900 nA/ μF for RPL1 in *Archidoris* (Fig. 5*B*). The corresponding current densities for the LCG were $31,000$ nA/ μF and $14,000$ nA/ μF for *Anisodoris* (Fig. 5*C*) and *Archidoris* (Fig. 5*D*), respectively. The A-current densities of homologous neurons were about $2\frac{1}{2}$ times as large in *Anisodoris* as in *Archidoris*, confirming the observations from the species comparison in Figure 6. When the average steady-state properties of homologous neurons were compared between the 2 species (i.e., RPL1, *Anisodoris* and *Archidoris*), we noted differences of 10% or less between the means. We conclude that with our protocol, there were no detectable, statistically significant differences in the steady-state voltage dependence of activation and inactivation either between identified neurons or species. Therefore, the steady-state voltage dependence of I_A was conserved between as well as within the species. Finally, we did not detect statistically significant differences in A-current kinetics

Figure 4. Voltage dependence of removal of inactivation in 3 identified *Archidoris* neurons. A-current inactivation was removed more rapidly by large (more negative) hyperpolarizing prepulses. The test voltage for these experiments was -30 mV. Inactivation was removed by pulses of variable duration and ranging in magnitude between -60 and -100 mV. *A*, Absolute values for T_{REM} are plotted as a function of prepulse voltage for 3 identified neurons. RPL1 had much slower inactivation kinetics than RCG or RPE1. *B*, Normalized T_{REM} values (data from Fig. 4*A*) were normalized to the value at -100 mV had a similar voltage dependence in all 3 neurons. RPE1 (●), RCG (○), RPL1 (X).

when we compared T_A and T_{REM} values from homologous *Archidoris* neurons with those from *Anisodoris*. Therefore, time-dependent features of A-current were preserved between the species, with the corresponding result that A-current kinetics were faster in LCG than in RPL1 in both species.

In conclusion, we found that the major difference between the species was an average higher A-current density in *Anisodoris* than in *Archidoris*, based on a comparison of homologous neurons.

Diversity of outward currents of Anisodoris and Archidoris

Do *Anisodoris* neurons have larger currents in general or simply more I_A than *Archidoris* neurons? In Figure 7, the voltage-clamp records obtained from 7 sec depolarizations to 0 mV from -40

Figure 5. Species comparison of A-current density and kinetics in homologous, identifiable neurons of *Archidoris* and *Anisodoris*. Steady-state A-current records from RPL1 and LCG in the 2 species are displayed. Cells with similar input capacitances were chosen for comparison. I_A was activated at -30 mV following removal of inactivation by a hyperpolarizing prepulse to -80 mV. The A-current densities of homologous neurons were at least twice as large in *Anisodoris* as in *Archidoris*. Both species exhibited cell-specific differences in current density; the LCG had 4 times the current density of RPL1. The arrows indicate the time required for the current to inactivate to 67% of the peak value. A-current inactivated twice as fast in LCG as in RPL1. *A*, *Anisodoris*, RPL1, $C_m = 0.035$ μ F, peak $I_A = 250$ nA, peak $I_A/C_m = 7100$ nA/ μ F, $T_A = 260$ msec. *B*, *Archidoris*, RPL1, $C_m = 0.038$ μ F, peak $I_A = 110$ nA, peak $I_A/C_m = 2900$ nA/ μ F, $T_A = 230$ msec. *C*, *Anisodoris*, LCG, $C_m = 0.012$ μ F, peak $I_A = 370$ nA, peak $I_A/C_m = 31,000$ nA/ μ F, $T_A = 125$ msec. *D*, *Archidoris*, LCG, $C_m = 0.014$ μ F, peak $I_A = 190$ nA, peak $I_A/C_m = 14,000$ nA/ μ F, $T_A = 130$ msec.

mV are compared for homologous cells of the 2 species. At 0 mV the outward current is a mixture of currents and contains contributions from both I_K , the inactivating outward current, and I_C , the Ca-activated outward current (Aldrich et al., 1979). The data is for the same neurons presented in Figure 5. Peak outward current density (I_p/C_m) was 71,000 nA/ μ F in *Anisodoris* RPL1 (Fig. 7A), and 24,000 nA/ μ F in *Archidoris* RPL1 (Fig. 7B). Similarly, I_p/C_m was 140,000 nA/ μ F in *Anisodoris* LCG (Fig. 7C) and 40,000 nA/ μ F in *Archidoris* LCG (Fig. 7D). While the shape of the delayed outward current trajectory was similar in the homologous cells, the magnitude of the current was much larger in *Anisodoris*.

We also noted that the outward current trajectories differed dramatically in shape between the cell types in both species. Aldrich et al. (1979) made similar observations, which they attributed to differences in the relative contributions of I_K and I_C to delayed outward current. In particular, I_p contains contributions from both I_C and I_K . In Figure 7, not only did the homologues share similar outward current trajectories, but the current recorded from RPL1 (Fig. 7, A, B) showed features characteristic of cells with a pronounced contribution from I_C to the total outward current (Aldrich et al., 1979). In contrast, the recording from LCG (Fig. 7, C, D) inactivated in a manner typical of a cell whose outward current is predominantly I_K . Thus, I_C may contribute more to total current density in RPL1 relative to I_K , while I_K may have a larger relative current density

Figure 6. A-current density as a function of input capacitance. In order to compare A-current density between species, peak I_A/C_m was graphed as a function of C_m for *Archidoris* and *Anisodoris*. Peak I_A was obtained from test pulses to -30 mV following removal of inactivation by a 1.5–2.5 sec hyperpolarizing prepulse to -80 mV. There was a statistically significant trend ($p < 0.01$) in *Anisodoris* ($14,000 \pm 2200$ nA/ μ F, $N = 11$) towards higher A-current densities than in *Archidoris* (5800 ± 1100 nA/ μ F, $N = 12$). *Archidoris* (○), *Anisodoris* (●).

in LCG. Indeed, these observations would support the prediction that I_K density and I_C density will differ significantly between identified neurons, in a manner analogous to our findings for A-current density (Table 2).

The differences in delayed outward current density and A-current density between the 2 species are presented in Figure 8. First, peak outward current density (I_p/C_m) was determined for *Archidoris* and *Anisodoris* neurons by normalizing peak outward currents from 7 sec depolarizations to 0 mV (delivered from a holding potential of -40 mV), with membrane input capacitance. The resulting outward current contains no I_A , since it was previously inactivated by holding the membrane potential at -40 mV. These values were then plotted against peak I_A/C_m for the same cell. The majority of the *Anisodoris* cells fell at a greater distance from the origin than the *Archidoris* neurons, indicating a larger average current density. The difference between I_p densities for *Archidoris* ($33,000 \pm 12,000$ nA/ μ F; $N = 8$) and for *Anisodoris* ($72,000 \pm 11,000$ nA/ μ F; $N = 8$) was significant at the 0.01 level. Therefore, on the average, *Anisodoris* cells had a larger peak outward current density at 0 mV than *Archidoris*, suggesting that other currents, not just I_A , were larger in this species.

Figure 8 also illustrates the wide range in the relative amounts of I_p to I_A . In this graph, the slope of a line from the origin to a given point is equivalent to the ratio I_p/I_A ; the points are dispersed within a 60° arc. In both species, the ratios varied between 18 and 2.6. Furthermore, certain pairs of cells had similar A-current density levels and disparate I_p density levels, while other pairs of cells had comparable I_p densities and different I_A levels. We tested the hypothesis that I_p density and I_A density varied between cells independent of one another by computing the Spearman rank-correlation coefficient for the 2 species. The r_s value was not in the critical region, and we concluded that these were independent variables. Since I_p reflected the amount of I_C and I_K present in the cell (Aldrich et al., 1979), this observation would be consistent with differences in the relative contribution of I_K , I_C , and I_A to total current available to the cell at a given voltage. Furthermore, when taken

Figure 7. Species comparison of outward currents in homologous, identifiable neurons of *Archidoris* and *Anisodoris*. The voltage-clamp records obtained from 7 sec depolarization to 0 mV from a holding potential of -40 mV are compared for homologous cells of the 2 species. The data are for the identical neurons (i.e., the same animals) presented in Figure 5. While the shapes of the outward current trajectories were similar in the homologous cells, the magnitudes of the currents were much larger in *Anisodoris*. For measurements of peak outward current density (I_p/C_m), peak outward current (I_p) was normalized with cell input capacitance (C_m). *A*, *Anisodoris*, RPL1, $C_m = 0.035$ μ F, $I_p = 2500$ nA, $I_p/C_m = 71,000$ nA/ μ F. *B*, *Archidoris*, RPL1, $C_m = 0.038$ μ F, $I_p = 900$ nA, $I_p/C_m = 24,000$ nA/ μ F. *C*, *Anisodoris*, LCG, $C_m = 0.012$ μ F, $I_p = 1640$ nA, $I_p/C_m = 140,000$ nA/ μ F. *D*, *Archidoris*, LCG, $C_m = 0.014$ μ F, $I_p = 560$ nA, $I_p/C_m = 40,000$ nA/ μ F.

together with our other findings, these results predict that the ratio $I_K:I_C:I_A$ should be characteristic of a cell type.

Discussion

Population differences in the A-current properties of identified neurons (within population, class comparison)

Our experiments were aimed at establishing the degree of diversity in A-current properties between cell types, based on a comparison of the mean values and SDs obtained from identifiable neurons. The mean and SD were calculated from the results of experiments on the same identifiable neuron in many different animals of a given species. In particular, the SD of a parameter measured diversity within the animal population for any given cell type and could be used to evaluate population differences in the A-current properties of identified neurons. A-current was present in all dorid neurons voltage-clamped in this study.

Certain parameters systematically had a larger % SD than others. Table 5 summarizes the average % SD in A-current parameters within the animal population for the identified neu-

Figure 8. Relationship of peak outward current density to A-current density. Peak outward current was measured from voltage-clamp records obtained from 7 sec depolarizations to 0 mV from a holding potential of -40 mV, and included a correction for leak conductance. Peak outward current density (I_p/C_m) was determined for *Archidoris* and *Anisodoris* neurons by normalizing peak outward currents with membrane input capacitance. These values were then plotted against A-current density (peak I_A/C_m at -30 mV) for the same cell. The sample included 8 different identified neurons from each species. The majority of the data for the *Anisodoris* cells were at a greater distance from the origin than the *Archidoris* neurons, indicating a larger average peak current density. The difference between mean peak outward current densities for *Archidoris* ($33,000 \pm 12,000$ nA/ μ F, $N = 8$) and for *Anisodoris* ($72,000 \pm 11,000$ nA/ μ F, $N = 8$) was significant at the 0.01 level, suggesting that other currents, not just I_A , were larger in *Anisodoris* neurons. *Archidoris* ($\circ$), *Anisodoris* ($\bullet$).

rons. The voltage- and time-dependent properties of A-current showed the least differences within the animal population. When the steady-state voltage dependence of A-current and the time constants for inactivation were measured for the same cell type in different animals of a given species, they showed average % SD of 15 and 11%, respectively. In contrast, when A-current was activated in the same cell type in a series of animals of a

Table 5. Comparison of % SD within and between populations

Parameter	% SD	
	Average within population	Between populations
Peak I_A	29	82
A-current density	24	77
Steady-state voltage dependence	15	8
T_A	11	56
T_{REM}	20	55

The average % SD within the population was calculated by averaging the % SDs for all cell types for each parameter using the data in Table 2 (Peak I_A , A-current density), Table 3 (steady-state voltage dependence), and Table 4 (T_A , T_{REM}). The % SD between populations was obtained by calculating the mean and SDs of each parameter using the mean values for the cell types. For example, on the average, for each identified neuron, peak I_A varied 29% from animal to animal in the population. However, when we compared the means of the identified neurons, peak I_A varied by 82% between populations.

given species, there were substantial differences in the magnitudes of peak I_A and A-current density. On the average, A-current density varied by 24% about the mean for a given cell type as compared with 29% variability in peak I_A .

Comparison of A-current properties between identified neurons (between populations, class comparison)

A comparison of mean peak current values established statistically significant differences in the magnitude and density of A-current between identified neurons (Tables 1, 2). While mean peak I_A values varied 2-fold between cell types, A-current density ranged 5-fold, from 2800 to 14,000 nA/ μ F, between identified neurons. The difference between the mean values of current density of pairs of cells, such as RPE1 and RCG, was significant at the 0.01 level. One important conclusion from this result is that cell types differ in the amount of I_A (or A-current density) available to the cell.

We did not observe statistically significant differences in the steady-state voltage dependence of A-current between cell types (Table 3). In contrast, the time course of conductance changes differed dramatically between dorid neurons. There was a 4-fold range in A-current kinetics as characterized by the inactivation time constants T_A and T_{REM} (Table 4). We did not measure activation kinetics because of the difficulties in measuring the exact value of the time constant for the rising phase of the current (Neher, 1971). However, the observation that the time to peak varied between identified neurons in parallel with the time course of inactivation (Fig. 2) is consistent with the possibility of cell-specific differences in A-current activation kinetics. The time to peak in *Archidoris* RPL1 (Fig. 2A) was approximately 50 msec, as compared with 30 msec in *Archidoris* LCG (Fig. 2B). The corresponding values for T_A (A-current inactivation) in these 2 cells were 230 and 130 msec, respectively. The finding that the time constants for activation and inactivation of A-current covary in dorid neurons with changes in temperature (Connor and Stevens, 1971a) is also in accord with the prediction that cells with relatively slow inactivation kinetics will have slow activation kinetics as well.

It is important to recognize that, although the inactivation voltage dependence of A-current was well characterized in these experiments, activation was only examined in a restricted voltage domain. The presence of additional conductances at potentials more positive than -20 mV made it possible to isolate A-current in only a limited range of voltages, and thus precluded measurements of maximum A-conductance. It is conceivable that if activation were measured at more positive voltages, there could be differences in the steady-state voltage dependence of A-current activation between identified neurons. In our experiments, we also did not examine the foot of the activation/inactivation curves in detail because as the test voltage approached reversal potential, current amplitudes were both reduced and more sensitive to small fluctuations in reversal potential, making such experiments technically more difficult. Nevertheless, Partridge and Connor (1978) have shown that characteristics of the foot of the curves, such as the degree of overlap and the steepness of the voltage dependence of inactivation, are important in modulating firing frequency, and consequently this deserves further study.

It may seem surprising that the steady-state voltage dependence of A-current was invariant between cells, while the kinetics were not. However, there is evidence for this independent behavior from other experiments. Partridge and Connor (1978)

studied the temperature dependence of A-current in *Archidoris* and *Anisodoris*. Their results show that as temperature increases, A-current inactivates more rapidly while its magnitude simultaneously increases. There are no concomitant changes in the steady-state voltage dependence of A-current or cell repetitive firing. They conclude that in these neurons, features of the temperature dependence of the A-current system function as an effective buffer to conserve repetitive firing patterns when the organism is exposed to fluctuations in temperature. In addition, Strong (1984) showed that the application of forskolin and R020-1724 to *Aplysia* bag cell neurons accelerated inactivation time constants for A-current. The steady-state voltage dependence of A-current remained unaltered by these drugs.

Outward currents in Archidoris and Anisodoris (between species, class comparison)

Features of A-current diversity were comparable in the 2 dorid species. In both, the steady-state voltage dependence of A-current did not exhibit statistically significant differences between cells, and in both there were dramatic, significant differences in A-current density and kinetics between cell types. A comparison of homologous identified neurons from *Archidoris* and *Anisodoris* revealed that the steady-state voltage dependence of A-current, A-current kinetics (Fig. 5) and even the shape of outward current voltage-clamp records at 0 mV (Fig. 7) were similar in the 2 species. The major difference between the species was that A-current density was $2\frac{1}{2}$ times greater in *Anisodoris* than in *Archidoris* (Fig. 5). Furthermore, in addition to A-current, other outward currents were larger in *Anisodoris* than in *Archidoris*. Peak outward current density at 0 mV was, on the average, over twice as large in *Anisodoris* as in *Archidoris* (Figs. 7, 8).

A comparison of steady-state activation and inactivation curves for dorid neurons with those from other species suggests that the voltage dependence of A-current can vary between preparations (Rudy, 1988). Dekin and Getting (1987) present evidence for 3 classes of neurons in brain-stem slices from the ventral part of the nucleus tractus solitarius of the guinea pig, based on the presence of 3 kinds of firing patterns. The patterns of repetitive activity correlate with differences in the voltage dependence of A-current. In addition, the A-channels characterized by Solc et al. (1987) in cultured *Drosophila* myotubes are very different from those of cultured *Drosophila* neurons. The myotube channels are faster than the neuronal channels, have a larger steady-state component, and have right-shifted activation/inactivation curves with a greater overlap at the foot of the curves. The voltage dependence of the A-current of dorid neurons resembles that of cultured *Drosophila* neurons rather than the *Drosophila* myotubes. Indeed, Iverson et al. (1988) propose that differences in the voltage dependence of activation and inactivation may prove useful in categorizing A-channel types. Finally, Connor (1978) observed that the time course of A-conductance changes in crustacean axon was faster than in molluscan somata. Since our experiments were confined to measurements of A-current in molluscan soma, they may not reflect the properties of the channel in the axon.

What are the possible sources of diversity for A-current properties?

The mechanisms underlying the differences in A-current properties between dorid neurons are unknown at present. However, the diversity we observed in A-current properties could be the result of processes which contribute to cell differentiation during

development. Such diversity would also be influenced by genetic differences, the developmental stage of the animal and environmental adaptation. Some of these contributions to diversity could be reduced with the use of an isogenic population, raised in a constant, controlled environment and examined at precisely the same stage of development, criteria not fulfilled in these experiments; our sample population of animals was collected over a 1½ year period from different marine habitats and contained animals of varying size and age.

Diversity in A-current density between identified neurons could be explained by differences in unit A-channel conductance or A-channel density between cells. The unit A-channel conductance has been determined only in a few preparations, with various results. Experiments have given values of 5–8 and 12–16 pS in cultured neurons and myotubes from wild-type Canton-S *Drosophila* (Solc et al., 1987), 14 pS in *Helix aspersa* neurons (Taylor, 1987), 22 pS in rat nodose neurons (Cooper and Shrier, 1985) and 40 pS in *Lymnaea* neuron soma (Kazachenko and Geletyuk, 1984). Although these values extend over a 5-fold range, there is no report of large differences in unit channel conductance within a preparation. While changes in unit channel conductance alone could account for differences in current density, it may be more plausible to expect the large differences in current density to be due to the numbers of channels inserted, or a combination of both factors. If the differences in current density are due to variable channel density, regulation of channel formation or insertion into the membrane may be important underlying mechanisms.

A possible explanation for the differences in A-current density between the species is that they are an environmental adaptation to different ambient temperatures. Partridge and Connor (1978) observed that A-current magnitude increased with temperature. Divers from the supplier (Pacific Biomarine, Inc.) noted that *Archidoris* preferred habitats with slightly warmer temperatures than *Anisodoris*. Thus, the actual current densities of the 2 species may be similar, if measured at their habitat temperature.

Recent advances in our understanding of the processes which affect channels and membranes may have important implications for the striking, 4-fold differences we observed in A-current kinetics between neurons. First, differences in kinetics may reflect immediate membrane environment, unique to each cell. Thus, the channel may be responding to local membrane fluidity or interacting with adjacent molecules. Second, channel properties may be modulated by cytoplasmic factors. Calcium (Salkoff, 1983; Alkon, 1984) and cAMP (Strong, 1984) have been shown to have effects on A-current. Finally, the results of experiments on neurons and muscle of *Drosophila* combining the techniques of genetics, electrophysiology, and molecular biology point to another explanation: the diversity may be attributed to intrinsic differences in the A-channels and may be the result of mechanisms that determine how genes for the channel are expressed in the membrane. Voltage-clamp studies of *Drosophila* flight muscle show the early appearance of A-current in developing muscle (Salkoff and Wyman, 1981a). *Drosophila Shaker* mutations affect A-current properties (Salkoff and Wyman, 1981b; Salkoff, 1983; Wu et al., 1983) and specifically, inactivation kinetics (Salkoff and Wyman, 1981b). The *Shaker* locus has been successfully cloned (Baumann et al., 1987; Kamb et al., 1987; Papazian et al., 1987; Tempel et al., 1987) and recently, Timpe et al. (1988a, b) and Iverson et al. (1988) have shown that *Xenopus* oocytes injected with mRNA transcribed from *Drosophila Shaker* cDNA clones produce functional A-type

potassium channels with different inactivation kinetics but with the same voltage dependence of activation and inactivation. The results of our analysis of A-current diversity *in situ* parallel these findings, because identified neurons differ in kinetics but not in steady-state properties, suggesting that there may be a correlation between mechanisms underlying A-channel diversity in *Drosophila* and in the molluscan nervous system. The physiological significance of the results of the molecular analysis of potassium channels may perhaps be elucidated by examining A-current expression in these giant molluscan neurons.

In conclusion, during differentiation, the kinds, numbers, properties, and locations of membrane constituents would be under regulation and ultimately result in the characteristic features of a cell. In the case of neurons, differences in membrane excitability based on a unique complement of channels, receptors, and transporters, can lead to diverse firing patterns and synaptic activity. Emerging properties of channel and receptor genomes suggest gene duplication as an important mechanism for the establishment of different channel types, based on the level of structural homology between the sodium channel and the AChR (Salkoff and Tanouye, 1986). Posttranscriptional modification and alternative splicing could also result in differences by altering channel proteins at crucial sites, thereby generating ion-channel diversity (Leff et al., 1986; Iverson et al., 1988; Schwarz et al., 1988). With the present technology, such issues as how cells differentiate during development may be resolved at multiple levels, from the genome to the membrane and processes governing channel formation and diversity may be better understood. In particular, identifiable molluscan neurons such as those found in *Archidoris montereyensis* and *Anisodoris nobilis* may provide a useful model system for understanding the origins of systematic, regulated differences between neurons.

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