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Inward Rectification in Response to FMRFamide in *Aplysia* Neuron L2: Summation with Transient K Current

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The response of *Aplysia* abdominal ganglion neuron L2 to the molluscan neuroactive peptide Phe-Met-Arg-Phe-NH_2 (FMRFamide) was studied in voltage-clamp experiments. In all of the experiments, focal application of the peptide to the soma activated an inward rectifier current and reduced the apparent amplitude of the transient K current, I_A. In a few cells, Na and K currents were activated in addition to these effects. Voltage-jump experiments were performed to study the ionic dependence, kinetics, and voltage dependence of the inward rectifier. Inward rectification increased exponentially during hyperpolarizing pulses and recovered exponentially on return to the resting potential. The reversal potential was variable, but was near −40 mV at the beginning of experiments. Inward rectification was insensitive to changes in external Na, Ca, or K concentration, but lowering the external Cl concentration had complicated effects on current amplitude. When KCl microelectrodes were used, perfusion with low-Cl external saline increased the amplitude of the peptide-dependent inward rectifier and shifted its reversal potential to a more positive voltage. With KAc microelectrodes, perfusion with low-Cl saline reduced the amplitude of the current. Inward rectification increased when a KAc microelectrode was withdrawn and replaced with a low-resistance KCl electrode, even when there was no measurable change in reversal potential. These results suggest that the FMRFamide-dependent inward rectifier is a Cl current that, like the current described by Chesnoy-Marchais (1982, 1983), is modulated by intracellular Cl.

FMRFamide reduced the apparent amplitude of I_A without affecting the voltage dependence of I_A activation or inactivation. The reduction in I_A followed the same time course as the change in inward rectification after peptide application, but there was no evidence for a direct effect of FMRFamide on I_A. Instead, the decrease appears to result from summation of I_A with the inward tail current due to the decay of the peptide-dependent inward rectifier during depolarization.

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The neuroactive peptide Phe-Met-Arg-Phe-NH_2 (FMRFamide) elicits a variety of responses in molluscan neurons. In different cells it can activate Na currents or K currents, decrease Ca currents, or depress the amplitude of voltage-dependent and Ca-dependent K currents (Cottrell, 1982; Cottrell et al., 1984; Colombaioni et al., 1985; Ruben et al., 1986; Brezina et al., 1987a, b; Cottrell and Davies, 1987). We found that FMRFamide has a novel effect on neuron L2 in the *Aplysia* abdominal ganglion, where it activates a Cl inward rectifier. This response resembles the Cl current seen during hyperpolarizing pulses in *Aplysia* cerebral ganglion neurons after intracellular Cl loading (Chesnoy-Marchais, 1982, 1983). In the majority of experiments on L2, FMRFamide activated only the inward rectifier, while in a few preparations, Na and K currents, like those described in *Aplysia* neurons L4 and L6 (Ruben et al., 1986) were also activated. In this report we concentrate on those cells expressing only an increase in inward rectification in response to the peptide.

The voltage dependence of inward rectification, and its relatively slow response to changes in voltage, produce an interesting interaction between this agonist-dependent current and the transient outward current, I_A. The apparent amplitude of I_A is reduced and it may even appear to be abolished after applying FMRFamide. This report describes the properties of the FMRFamide-dependent inward rectifier and the interaction between this current and I_A. A preliminary report of this work has appeared (Ruben and Thompson, 1986).

Materials and Methods

Specimens of *Aplysia* were obtained from Sea Life Supply (Sand City, CA) and maintained in flowing, natural seawater. The abdominal ganglion was removed and desheathed manually without enzyme treatment. Neuron L2 (Frazier et al., 1967) was axotomized by making a cut across the left side of the ganglion and undercutting the left upper-quadrant neurons with iris scissors to isolate a cluster of cells. The cell cluster was mounted in a Lucite chamber and cooled to 11–15°C. Neuron L2 was voltage-clamped using a 2-microelectrode method (Barish and Thompson, 1983). Microelectrodes had resistances of 2–7 MΩ and were filled with 2 M KAc or 3 M KCl, as indicated in the text. The chamber was held at 0 mV by a separate voltage-clamp amplifier interfacing the bath via 2 saline–agar bridges. Membrane current was measured from the differentially recorded voltage drop across a 500 kΩ resistor in series with the bath-current electrode. This method provided good control over bath voltage and allowed rapid settling of membrane currents in response to voltage-clamp pulses.

The control saline had the following millimolar composition: 470 NaCl, 10 KCl, 10 $CaCl_2$, 50 $MgCl_2$, 10 HEPES (pH 7.8). Low-Na saline was prepared by equimolar substitution of Tris-Cl, gluconate, or *N*-methylglucamine for Na. Low-Cl saline was prepared by substituting Na-isothionate for NaCl (pH adjusted to 7.8) to yield a final Cl concentration of 130 mM, compared to the control concentration of 550 mM. Perfusion with low-Cl saline caused a change in the junction potential at the voltage electrode of the bath-voltage clamp. The magnitude of the change

was estimated by recording the bath voltage with a separate, low-resistance calomel electrode immersed in a saturated KCl solution and interfaced to the bath via a thin, porous porcelain plug that allowed KCl to weep slowly out of the electrode. The bath-voltage clamp was given a zero mV command while the solution was changed from control to low-Cl saline. After introducing the low-Cl saline, the calomel electrode reported a stable −4 mV shift in potential. Voltage-clamp command pulses were adjusted to correct for this change during perfusion with low-Cl saline. Even without correction, a voltage error of this magnitude would have little effect on the conclusions drawn from this study.

A stock soltuion of 10 mM FMRFamide (Sigma) was prepared in distilled water and frozen until use. Peptide was applied to the cell body by a pressure pulse (20–65 psi; duration, 20–700 msec) from a micropipette containing 10 μM FMRFamide diluted in the appropriate external saline. The micropipette was postioned as close as possible to the cell without actually penetrating it. Close positioning was important to insure focal application of peptide to the soma without exposing other cell bodies or presynaptic terminals in the cell cluster. In some experiments, the bath was continuously perfused, while in others, it was flushed with 10 volumes of saline between peptide applications. These procedures were necessary to minimize desensitization of the peptide response.

Records of membrane voltage and current were stored on a laboratory computer (DEC 11/23), FM magnetic tape (Tanburg/Sangamo), or chart recorder (Gould/Brush 220). Tape and chart records were digitized before analysis. In quantifying the kinetics of currents, we used a nonlinear least-squares method to find the best fit between specified theoretical curves and the measured data points (algorithm of Marquardt, 1963, as described by Bevington, 1969). Computed curves were displayed along with the data points on the terminal to allow the accuracy of the fitting procedure to be verified by inspection.

Results

FMRFamide elicits 2 kinds of responses in *Aplysia* neuron L2. Figure 1 shows examples of membrane currents in different preparations in response to brief application of the peptide to the cell body at a holding voltage of −40 mV. In Figure 1*A*, the peptide activates an inward current that begins after a short delay, peaks in 6 sec, and recovers within 30 sec (recovery time in different preparations, 20–230 sec). This appears to be a single-component response that results from an increase in inward rectification, as described below. Figure 1*B* shows a different, multicomponent response in L2 in another preparation. This response consists of an inward current followed by a slowly developing outward current, and resembles the effect of FMRFamide on *Aplysia* neurons L4 and L6 (Ruben et al., 1986). The peptide response in L6 is shown in Figure 1*C* for comparison. The multicomponent response in L2 results from the activation of Na and K currents, in addition to the inward rectifier. It differs from the response in L4 and L6 because the peptide does not activate inward rectification in those cells (Ruben et al., 1986). The source of the variability in the response to FMRFamide in L2 is not known. It does not appear to result from differences in the spatial distributions of different FMRFamide receptor types because similar responses were obtained when the delivery pipette was moved to several locations on the cell body. In the majority of cells, single-component responses like that in Figure 1*A* were observed, and in this report we consider only those preparations.

Figure 2 shows membrane currents in L2 during a series of hyperpolarizing voltage pulses before and after application of FMRFamide. In the control (Fig. 2*A*), the hyperpolarizing pulses result in nearly square steps of inward current. FMRFamide was applied focally to the soma, and the pulses were repeated at the peak of the peptide response (Fig. 2*B*). Figure 2*C* shows difference currents made by subtracting the control current from the current in FMRFamide at each voltage. The peptide activates a voltage-dependent inward rectifier that increases in amplitude with hyperpolarization and has both a rapid, nearly instantaneous component and a time-dependent component. The simplest interpretation of these data is that the instantaneous component represents current activated by the peptide at −40 mV, while the slow increase represents voltage-dependent activation of additional current during hyperpolarization. On returning to −40 mV after the hyperpolarizing pulses, inward tail currents are seen. The tail currents show that inward rectification results from an increased conductance to ions with a reversal potential more positive than −40 mV; the tail currents result from the slow decline of that conductance on returning to the resting potential.

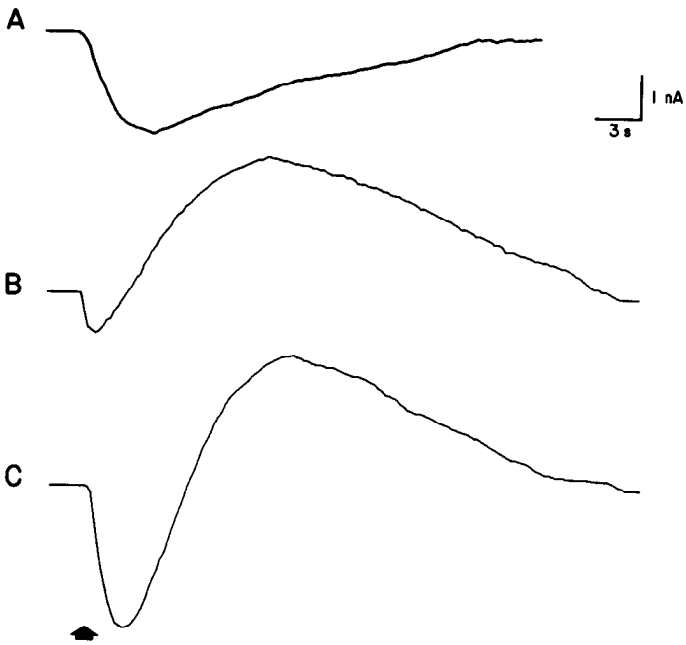

Figure 1. Membrane currents in response to FMRFamide in *Aplysia* neurons L2 and L6. The peptide was applied focally to the cell body by a short pressure pulse (40 psi, 60 msec) from a micropipette containing 10 μM FMRFamide in control saline. The time of application is indicated by the *arrow*. Currents were recorded at a holding voltage of −40 mV. *A*, Single-component, inward response in L2. *B*, Multicomponent response in L2 in a different preparation. *C*, Multicomponent response in L6. Current and voltage electrodes were filled with 3 M KCl.

Voltage dependence of inward rectification

The voltage dependence of peptide-dependent inward rectification was measured in the following way: The cell was held at −35 mV and currents were recorded during a series of 500 msec pulses to voltages between −45 and −110 mV in control saline and at the peak of the response to FMRFamide. The amplitudes of the currents were plotted against pulse voltage to produce the *I*(V) curves in Figure 3*A*. The open circles show the control *I*(V) curve. Two *I*(V) curves were obtained during the peptide response; the first represents the current measured 10 msec after beginning the pulse and approximates the instantaneous *I*(V) curve (squares), and the other represents the steady-state current measured after 500 msec (filled circles). The *I*(V) curves in Figure 3*A* show that inward rectification is increased by FMRFamide.

Difference *I*(V) curves illustrating the voltage dependence of the inward rectifier were made by subtracting the control *I*(V)

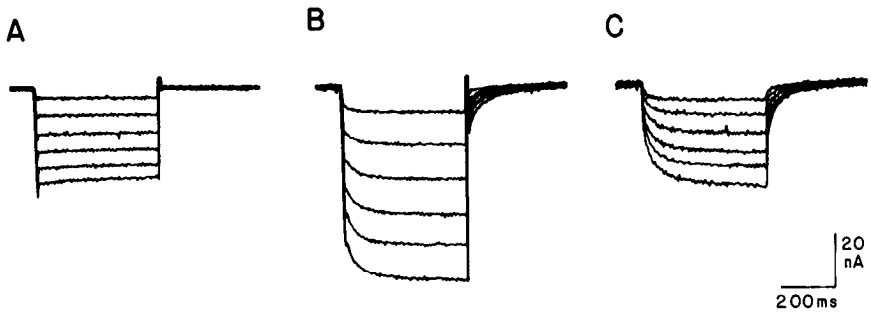

Figure 2. Membrane currents in L2 during hyperpolarizing voltage pulses in control saline and after focal application of FMRFamide to the soma. KCl electrodes were used throughout. The cell was held at −40 mV and a series of pulses to voltages between −50 and −100 mV (increasing in 10 mV steps) was applied. *A*, Control currents. *B*, Currents recorded during the same series of pulses at the peak of the peptide response. *C*, Differences currents illustrating the properties of the FMRFamide-dependent inward rectifier obtained by subtracting the currents in *A* from those in *B*.

curve from the curves measured after peptide application (Fig. 3*B*). The difference *I*(V) curve at 10 msec (squares) is nearly linear over most of the voltage range. Extrapolation of this curve provides an estimate of the reversal potential for inward rectification that has an average value of −38 ± 8 mV (n = 11) in control saline at the beginning of experiments using KCl electrodes. Comparison of the early *I*(V) curve and the steady-state *I*(V) curve (filled circles) shows that peptide-dependent inward rectification increases in a time- and voltage-dependent fashion during hyperpolarization.

Ionic dependence of inward rectification

The increase in inward rectification in response to FMRFamide does not depend on Na, Ca, or K currents. Substitution of Tris, gluconate, or *N*-methylglucamine for external Na did not have a significant, reproducible effect on the amplitude of the peptide response. Similarly, the response was not affected by substituting Mn, a calcium-current blocker, for Ca in the external saline. Several observations indicated that K current does not contribute significantly to the response: (1) the inward rectifier current reverses at a voltage that is more positive than the K equilibrium potential (about −73 mV; Kunze et al., 1971) or the reversal potential for transient K current in L2 (range, −60 to −67 mV); (2) tail currents, on returning to −40 mV after hyperpolarizing pulses in FMRFamide, are inward, whereas at this voltage any tail current due to K is expected to be outward; (3) when Rb, a blocker of K-dependent inward rectification (Standen and Stanfield, 1980), is substituted for external K, the peptide response remains unchanged.

Changes in external Cl concentration strongly affect the amplitude of the peptide response. Figure 4*A* shows peptide difference currents during a series of hyperpolarizing pulses recorded with KCl microelectrodes. FMRFamide activates an inward rectifier with an estimated reversal potential near −40 mV in this example. The same procedure was repeated 10 min after perfusing the bath with low-Cl saline, and the difference currents are shown in Figure 4*B*. Perfusion with low Cl caused a 2 nA inward shift in the holding current at −40 mV that recovered gradually during continued incubation in low-Cl saline. The amplitude of the peptide-dependent inward rectifier increased in low-Cl saline, and the reversal potential was approximately −20 mV. The change in reversal potential was close to the change expected for a Cl current (expected change, 23 mV). This result suggests that peptide-dependent inward rectification results from the activation of a voltage-dependent Cl current during hyperpolarization.

A different result was obtained with potassium acetate electrodes. Figure 4*C* shows peptide differences currents in control saline during the same series of hyperpolarizing pulses recorded with 2 M KAc microelectrodes in a different preparation. The bath was perfused with low-Cl saline and the pulses were repeated after a 10 min incubation. Peptide-dependent inward rectification was greatly reduced in low Cl (Fig. 4*D*) under these conditions, and the response recovered after washing with nor-

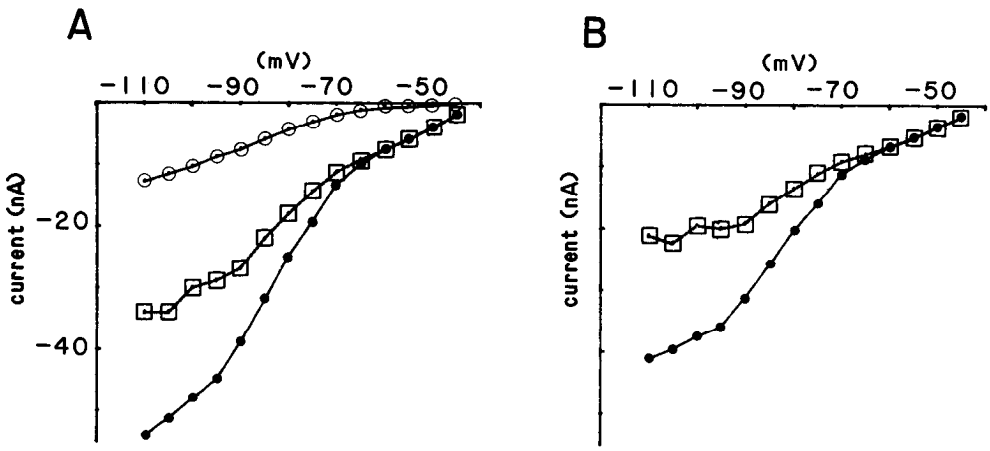

Figure 3. Current–voltage relationship of FMRFamide-dependent inward rectification. Currents were recorded during 500 msec pulses to voltages between −45 and −110 mV from a holding voltage of −35 mV. *A*, *Open circles*, *I*(V) curve in control saline. FMRFamide was applied and 2 *I*(V) curves were measured at the peak of the peptide response. The 2 *curves* represent the current 10 msec (*squares*) and 500 msec (*filled circles*) after beginning each hyperpolarizing pulse. *B*, Difference *I*(V) curves obtained by subtracting the control *I*(V) curve from the curves measured after applying FMRFamide. *Squares*, difference *I*(V) curve after 10 msec; *filled circles*, difference *I*(V) curve after 500 msec. All of the data are from the same experiment, using KCl electrodes,and were obtained during a single peptide application.

mal saline. This result is not expected if the inward rectifier is a Cl current and the intracellular Cl concentration remains constant during the external ion substitution. If it was a Cl current, lowering external Cl should shift the reversal potential to a more positive voltage and increase the current amplitude during hyperpolarization, as was seen with KCl electrodes. It is possible that the intracellular Cl concentration gradually decreases when external Cl is lowered and KAc electrodes are used. The transient inward shift in holding current that occurs during low-Cl perfusion indicates that L2 has a resting conductance to Cl that could allow the intracellular and extracellular concentrations to equilibrate. We could not measure the reversal potential in low-Cl saline using KAc electrodes because of the small amplitude of the response, and were unable to test this idea directly. A decrease in intracellular Cl concentration could have a large effect on the peptide response if internal Cl is necessary for activation of the inward rectifier, as suggested by Chesnoy-Marchais (1982, 1983). This effect could be mitigated when KCl electrodes are used if the leakage of Cl from the electrodes is sufficient to compensate in part for the loss of internal Cl.

The importance of intracellular Cl for peptide-dependent inward rectification was tested by recording peptide responses with KAc and KCl microelectrodes in the same cell. Difference currents were obtained during a series of hyperpolarizing pulses using KAc electrodes (Fig. 4*E*). The voltage microelectrode was then removed and replaced with a 2 MΩ, 3 M KCl electrode, while the current electrode and the FMRFamide delivery pipette were left in place. After 10 min, the same series of hyperpolarizing pulses was repeated and difference currents were measured as before (Fig. 4*F*). There was no attempt to load the cell with Cl by iontophoresis. The difference currents were 2 times larger after introducing the KCl microelectrode, but there was no measurable change in the reversal potential of the current.

Our interpretation of the Cl substitution and electrode replacement experiments is that FMRFamide activates a voltage-dependent Cl current in L2 that increases in amplitude during hyperpolarization and decays slowly on returning to the resting potential. Like the Cl current described by Chesnoy-Marchais (1982, 1983), the amplitude of the FMRFamide-dependent inward rectifier appears to be very sensitive to the intracellular Cl concentration, more sensitive than would be expected from the change in ion driving force. This suggests that the peptide-dependent current is modulated by internal Cl such that an increase in cytoplasmic Cl concentration favors voltage-dependent activation of the current.

Kinetics of inward rectification

Difference currents representing the increase in inward rectification during hyperpolarizing pulses are shown in Figure 5*A*. The solid lines in 5*A* are exponentials fitted to the data points. Inward rectification activates exponentially during hyperpolarization, with time constants of 112 msec at −60 mV (average, 130 ± 61 msec; $n = 10$) and 54 msec at −90 mV (average, 69 ± 25 msec; $n = 10$). The time course of decay of inward rectification on returing to the holding voltage was measured from tail currents. Tail currents recorded at −40 mV, after a series of 500 msec hyperpolarizing pulses in control saline, were subtracted from the tail currents recorded during the same series of pulses at the peak of the peptide response to obtain the difference tail currents in Figure 5*B*. Inward rectification decays exponentially at −40 mV, with an average time constant of 102 ± 30 msec.

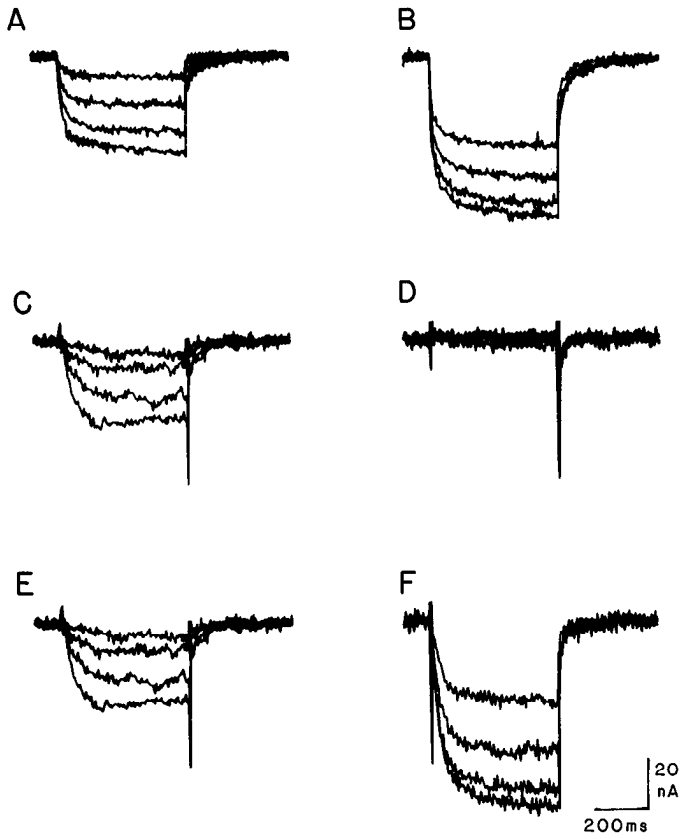

Figure 4. Peptide-dependent inward rectification is sensitive to the extracellular and intracellular Cl concentrations. *A–F*, Difference currents during hyperpolarizing pulses to −70, −80, −90, and −100 mV obtained by subtracting the currents in the control from the currents recorded at the peak of the response to focally applied FMRFamide. *A*, Difference currents in normal saline, recorded with 3 M KCl electrodes. *B*, Currents measured 10 min after perfusing the bath with low-Cl saline. The parameters of the peptide application were the same as in *A*. *C*, Difference currents in normal saline in a different preparation, recorded with 2 M KAc electrodes. *D*, Currents measured 10 min after perfusion with low-Cl saline in response to the same peptide application. *E*, Difference currents in normal saline recorded with 2 M KAc electrodes. *F*, Currents measured in the same cell 10 min after exchanging the KAc voltage microelectrode for one containing 3 M KCl. The FMRFamide delivery pipette was left in place and the parameters of the peptide application pulse were the same as in *E*. *C–F* are from the same cell.

A complication arises when measuring tail currents with this method. A pulse to −40 mV from a hyperpolarized conditioning voltage can cause partial activation of the transient potassium current I_A, which could sum with the inward rectifier tail current and obscure its time course. At this voltage, the subtraction will give the true time course of the tail current only if the pepetide has no effect on I_A. The evidence suggests that this is the case, and that I_A is accurately subtracted. It was found that the time constant of the inward rectifier tail current at −50 mV, a voltage that does not activate I_A, is about the same as the time constant measured at −40 or −30 mV, voltages at which I_A is partially activated. The simplest interpretation is that the subtraction procedure successfully separates the inward rectifier tail current from I_A, but this interpretation would be invalid if the peptide had a direct effect on the potassium current.

Summation of the inward rectifier with I_A

The effect of FMRFamide on the apparent amplitude of I_A is illustrated in Figure 6. This experiment was conducted more

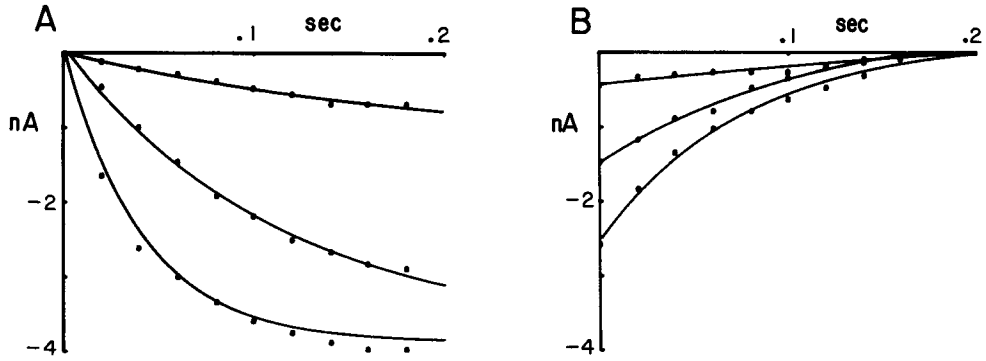

Figure 5. Kinetics of FMRFamide-dependent inward rectification. *A*, Activation of inward rectification during hyperpolarizing pulses to −50, −60, and −80 mV in the same cell. The pulses were applied at the peak of the response to FMRFamide. Only the time-dependent increase in inward rectification is shown, after subtracting the instantaneous current and the control current. *Solid lines*, single exponentials fitted to the data points by the method of least-squares. *B*, Inward rectifier tail currents in the same cell on returning to −40 mV after each pulse. The points show difference tail currents obtained by subtracting the control current from the current at the peak of the peptide response. *Solid lines*, single-exponentials fitted to the data points. KCl electrodes were used throughout.

than an hour after introducing 2 low-resistance KCl microelectrodes into the cell at a time when the reversal potential of the inward rectifier was estimated to be about −10 mV. In Figure 6, *A–D*, membrane currents are shown during test pulses to −20 mV from 2 conditioning voltages, −40 and −90 mV. I_A is completely inactivated at −40 mV, but the pulse to −20 mV causes partial activation of delayed outward currents (see Adams et al., 1980). At a conditioning voltage of −90 mV, I_A inactivation is removed and I_A activates together with the delayed outward currents during the test pulse (trace I_a, Fig. 6*A*). The difference between the 2 records at −20 mV in *A–D* represents I_A. Figure 6*A* shows control currents, while Figure 6*B* shows the currents recorded at the peak of the response to FMRFamide. Figure 6, *C* and *D*, shows currents recorded during the decay of the peptide response, 45 sec after application (Fig. 6*C*), and after washing with normal saline (Fig. 6*D*). FMRFamide decreases the apparent amplitude of I_A and alters its apparent time course. These effects recover as the peptide response decays, and are reversed by washing. The decrease in I_A follows the same time course as the peptide-dependent current. This is illustrated in Figure 7, where the peak amplitude of I_A during a standard test pulse is plotted as a function of time after peptide application, together with the inward current activated by FMRFamide at a holding voltage of −40 mV in the same cell. The maximum decrease in I_A amplitude was scaled to the peak of the inward current to illustrate the similarity in time course.

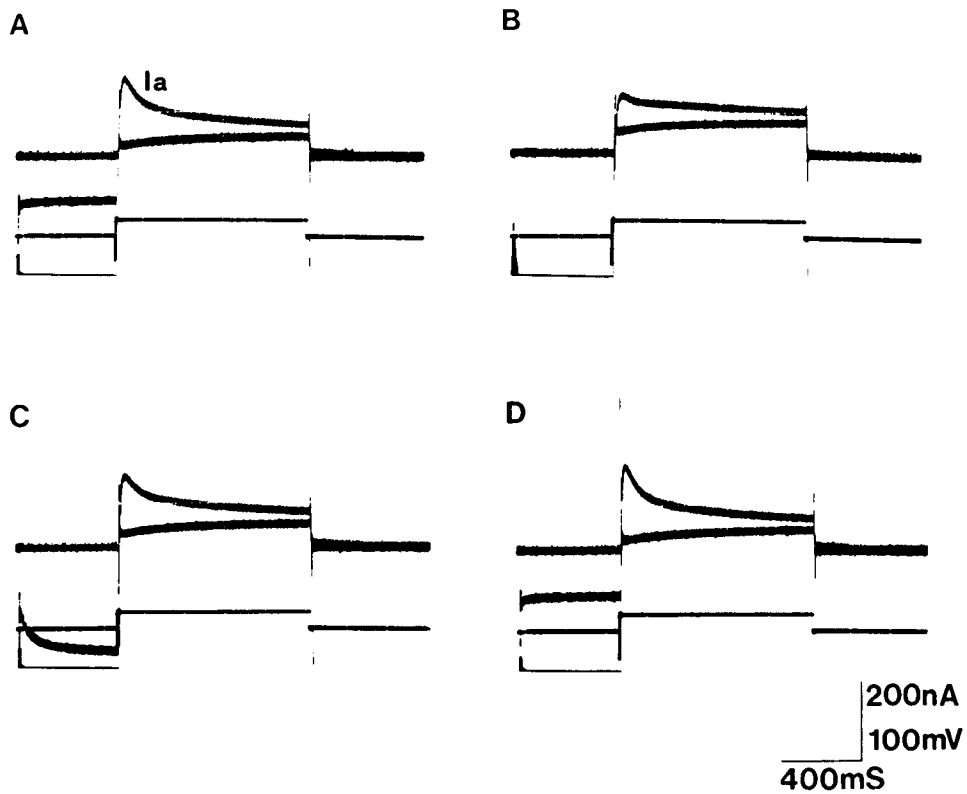

Figure 6. Decrease in I_A after applying FMRFamide. Membrane currents were recorded during pulses to −20 mV from 2 conditioning voltages, −40 and −90 mV. I_A is inactivated at −40 mV, and the pulse to −20 mV from this voltage causes partial activation of delayed outward currents. The inactivation of I_A is removed by a 500 msec conditioning pulse to −90 mV, and during the subsequent pulse to −20 mV, I_A activates, in addition to the delayed outward currents (I_d). The difference between the 2 currents at −20 mV in *A–D* represents I_A. *A*, Control currents. *B*, Pairs of currents recorded at the peak of the response to FMRFamide. The inward rectifier current during the conditioning pulse to −90 mV is off scale. *C*, Pairs of currents recorded during the decline of the peptide response, 45 sec after application. *D*, Currents recorded after washing with control saline. All the records were taken during a single peptide application. KCl electrodes were used throughout.

FMRFamide had no effect on the voltage dependence of I_A activation or inactivation. The I_A activation curve was measured from the peak amplitude of the current during a series of depolarizing pulses from a holding voltage of −90 mV under control conditions and at the peak of the peptide response. Although the apparent amplitude of I_A was reduced by peptide application, the voltage dependence of activation was not changed. The voltage dependence of I_A inactivation was measured using a prepulse method. The peak current during a test pulse to −30 mV, minus the steady-state current at that voltage, was measured after a series of 1 sec conditioning pulses to more negative voltages and plotted against conditioning voltage. It was found that, although the apparent amplitude of I_A was reduced by FMRFamide, the voltage dependence of I_A inactivation was not affected.

The steady-state voltage dependence of I_A inactivation in normal saline (Fig. 8, dotted line) and the voltage dependence of the FMRFamide-dependent inward rectifier (Fig. 8, solid line) are compared in Figure 8. Both curves were normalized to a value of 1 at −110 mV. Removal of I_A inactivation and activation of the inward rectifier both increase with hyperpolarization. The curves are somewhat different in shape, but extend over approximately the same voltage range.

During depolarizing pulses to voltages between −40 and −25 mV from a conditioning voltage of −90 mV, I_A reaches a peak in 10–15 msec and then inactivates exponentially, with a time constant of 129 ± 27 msec (n = 11), in normal saline. The time course of I_A is similar to that of the inward tail current in FMRFamide in this voltage range. Also, I_A and the tail current are about equal in absolute amplitude. The apparent decrease in I_A after application of FMRFamide, therefore, appears to result from summation of I_A with the inward tail current due to the decay of peptide-dependent inward rectification. There was no evidence that FMRFamide directly modulates I_A.

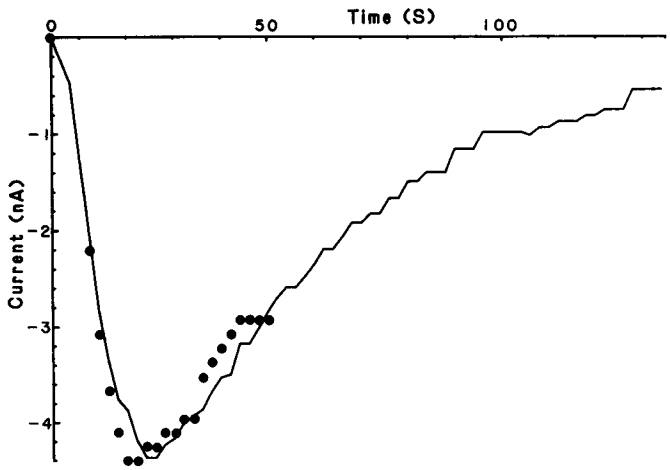

Figure 7. The decrease in I_A follows the same time course as the inward current in response to FMRFamide. FMRFamide was applied to the cell body by a 100 msec pressure pulse (20 psi), beginning at time zero. The holding voltage was −40 mV. *Solid line,* inward current in response to FMRFamide. *Filled circles,* peak amplitude of I_A at various times after a second, identical application of FMRFamide. I_A was activated by a pulse to −30 mV after a 500 msec conditioning pulse to −90 mV. The maximum decrease in I_A was scaled to the peak of the inward current in order to illustrate the similarity in time course. KCl electrodes were used throughout.

Discussion

Several examples of the modulation of inward rectifier currents by neurotransmitters or peptides have appeared. Serotonin increases a K inward rectifier and decreases a Cl inward rectifier in *Aplysia* neuron R15 (Benson and Levitan, 1983; Lotshaw et al., 1986). Stanfield et al. (1985) found that substance P decreases K inward rectification in neonatal rat neurons. In the present experiments, FMRFamide appears to activate a voltage-dependent Cl inward rectifier in *Aplysia* neuron L2. This interpretation is based on the observation that the estimated reversal potential is close to the Cl equilibrium potential (Chesnoy-Marchais, 1982, 1983) and shifts to a more positive voltage when the external Cl concentration is lowered, provided that KCl microelectrodes are used.

The peptide-dependent inward rectifier in L2 has several features in common with the Cl current studied by Chesnoy-Marchais (1982, 1983) in *Aplysia* cerebral ganglion A cells after intracellular Cl injection. The 2 currents are similar in voltage dependence, and they activate and deactivate exponentially with similar kinetics. In both cases, the time constants for activation and deactivation are only weakly dependent on voltage, suggesting that the 2-state model for voltage-dependent gating presented by Chesnoy-Marchais (1983) may apply equally well to the peptide-dependent current. Also, in both cases the amplitude of the current increases when the intracellular Cl concentration is raised by leakage of Cl from a low-resistance KCl microelectrode. Because of these similarities, it is likely that the current activated by FMRFamide in L2 is the same voltage-dependent Cl current that was described by Chesnoy-Marchais (1982, 1983). One important difference is that, in L2, FMRFamide is necessary for expression of the Cl current even when KCl microelectrodes are used, whereas in cerebral A cells the current is expressed after intracellular Cl loading without applying agonists. The Cl inward rectifier in *Aplysia* neurons also has several properties in common with Cl channels isolated from *Torpedo* electroplaques (White and Miller, 1979).

The amplitude of peptide-dependent inward rectification increases when a KAc microelectrode is replaced by a low-resistance KCl microelectrode, even when there is little change in reversal potential. This suggests that intracellular Cl may play an important role in Cl-current activation. Chesnoy-Marchais (1983) suggested that binding of Cl to a site associated with the channel, and accessible from the inner face of the membrane, potentiates Cl-channel activation during hyperpolarization. The mechanism by which internal Cl modulates the current may be similar to the potentiation of K inward rectification in starfish egg by internal Na (Hagiwara and Yoshii, 1979), or to the effect of increased external K on the egg inward rectifier (Hagiwara et al., 1976; Chiani et al., 1978; Hagiwara and Yoshii, 1979).

The amplitude of the peptide-dependent current increases after perfusion with low-Cl saline when KCl electrodes are used, but decreases when the experiment is repeated with KAc electrodes. One possible explanation for this difference is that the intracellular Cl concentration gradually decreases during perfusion with low-Cl external saline in the absence of a source of internal Cl. A small decrease in internal Cl could dramatically reduce the peptide response if internal Cl were necessary for activation. The internal Cl concentration does appear to be labile. Ascher et al. (1976) showed that internal Cl decreases in *Aplysia* neurons during perfusion with low-Cl external solutions, and Chesnoy-Marchais (1983) showed that the Cl reversal po-

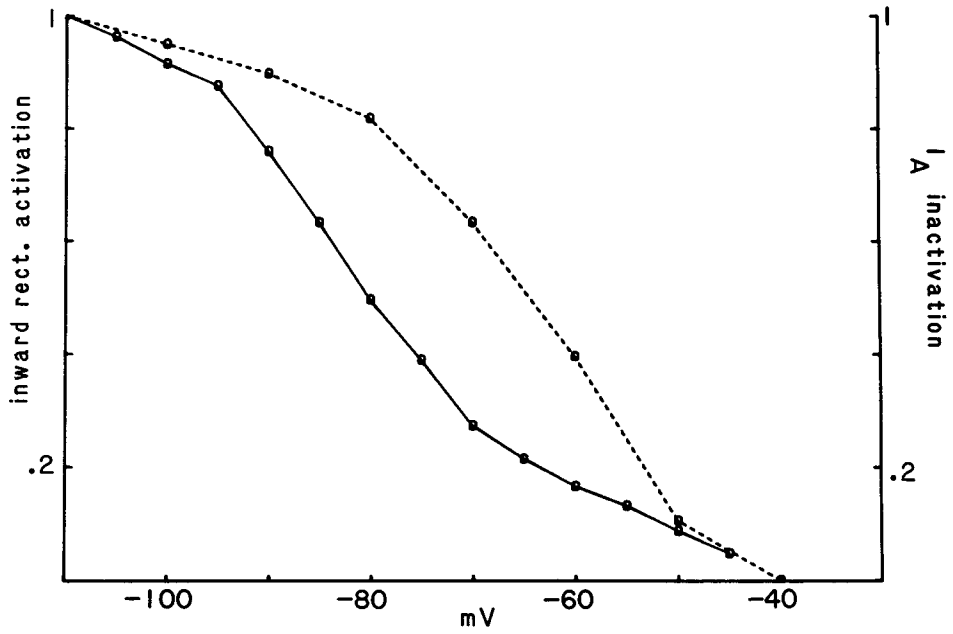

Figure 8. Comparison of the steady-state voltage dependence of I_A inactivation (*dotted line*) and FMRFamide-dependent inward rectification (*solid line*) in the same cell. The voltage dependence of I_A inactivation was measured using the prepulse method described in the text. The voltage dependence of inward rectification was determined as in Figure 5. Both curves were normalized to a value of 1 at −110 mV.

tential changes when cerebral ganglion A cells are held hyperpolarized while the Cl conductance is active. A low-resistance KCl electrode can act as a source of intracellular Cl, and it has often been observed that the reversal potential for the Cl-dependent response to ACh in *Aplysia* neurons gradually shifts to more positive voltages when recorded with KCl electrodes, presumably because of Cl leakage. The leakage of Cl from the electrode may be sufficient to compensate in part for the loss of Cl during perfusion with low-Cl saline, allowing the peptide-dependent current to persist. We note that inward rectification in crayfish muscle also results from the activation of a Cl current during hyperpolarization. In that tissue, the inward rectifier is abolished by prolonged exposure to low-Cl solutions, probably because of a decrease in intracellular Cl concentration (Ruben et al., 1962; Ozeki et al., 1966).

Mechanism of action

FMRFamide may activate an agonist-dependent current in a manner similar to the activation of endplate current by ACh in vertebrate muscle. By analogy to the ACh channel, the voltage dependence of inward rectification might be explained by a direct effect of voltage on the rates of channel opening and closing (Magleby and Stevens, 1972; see Ascher et al., 1978). Chesnoy-Marchais (1983) applied this kind of model in describing the Cl current in *Aplysia* A cells. The long duration of the response to FMRFamide might be due to persistence of the peptide in the vicinity of the receptor or to a slowly reversible agonist–receptor interaction. Alternatively, the activation of inward rectification by FMRFamide might involve a cytoplasmic second messenger, and there are examples in the literature of the importance of second messengers in the gating of inward rectifier currents. Madison et al. (1986) showed that phorbol esters decrease the amplitude of the Cl inward rectifier in hippocampal neurons, and Adams and Levitan (1982) found that activation of the K inward rectifier in *Aplysia* cell R15 by serotonin is mediated by cAMP-dependent protein kinase. Brezina et al. (1987b) suggest that another effect of FMRFamide on molluscan neurons, specifically the suppression of Ca current, might involve a GTP-binding protein. The role of cytoplasmic second messengers and G proteins in the activation of FMRFamide-dependent inward rectification in L2 has not been fully investigated, but the experiments do show that Ca influx is not required (see also Chesnoy-Marchias, 1983).

Summation of inward rectification with I_A

The FMRFamide-dependent inward rectifier and I_A inactivation are similar in voltage dependence and kinetics in the subthreshold voltage range. During a depolarizing step to voltages between −40 and about −25 mV from a hyperpolarized conditioning voltage, the 2 currents are nearly equal in amplitude. The apparent decrease in I_A, therefore, appears to result from summation of I_A with the inward tail current due to the decay of inward rectification during depolarization. The response to FMRFamide is well suited to influencing the excitability of L2, especially during the approach to threshold during repetitive firing. FMRFamide increases the membrane conductance and hyperpolarizes the cell away from threshold. Also, I_A is an important determinant of the repetitive firing rate (Connor and Stevens, 1971; Byrne, 1980), and summation of I_A with the inward rectifier tail current is expected to modify its influence. This interaction between an agonist-dependent current and I_A may be important to neuronal function in the subthreshold voltage range.

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