brief communication

Time course of receptor-channel coupling in frog sympathetic neurons

Stephen W. Jones
Department of Physiology and Biophysics, Case Western Reserve University, Cleveland, Ohio 44106 USA

ABSTRACT The M-type potassium current and the N-type calcium current are inhibited by several different neurotransmitters in frog sympathetic neurons. These effects seem to be mediated via G proteins, but it is not clear whether diffusible second messengers are involved. Using a rapid (~100 ms) flow tube perfusion system to apply agonists, the inhibition of calcium current develops and recovers rapidly but not instantaneously ($t_{1/2}$ = 1–2 s). M-current inhibition is considerably slower, with $t_{1/2}$ ~30 s for recovery from inhibition. At least for M-current inhibition, there appears to be sufficient time for involvement of an enzymatic cascade in receptor-channel coupling.

INTRODUCTION

Many neurotransmitters act via GTP-binding proteins (G proteins) to modulate the activity of voltage-dependent ion channels, either directly or through second messengers (Nicoll, 1988; Brown and Birnbaumer, 1990). These mechanisms interpose several steps between receptor activation and the eventual changes in channel activity. The time course of such synaptic actions are thus several orders of magnitude slower than the classical effect of neurotransmitters to directly open voltage-independent ion channels on the millisecond time scale. In cases where the G protein is thought to interact directly with the ion channel, the effect of the transmitter requires hundreds of milliseconds to develop or to recover (Nargeot et al., 1982; Breitwieser and Szabo, 1988). Where a soluble second messenger is involved, the response is even slower (Hill-Smith and Purves, 1978).

Several known or putative neurotransmitters, including acetylcholine (ACh) acting through muscarinic receptors, luteinizing hormone-releasing hormone (LHRH), substance P, and ATP, inhibit the M-type potassium current (I_M) in frog sympathetic neurons (Adams et al., 1982, 1983). In these cells, inhibition of I_M is the primary mechanism (Adams and Brown, 1982; Jones et al., 1984) underlying slow synaptic potentials which last 1–10 min (Kuba and Koketsu, 1978). The same transmitters also partially inhibit the N-type calcium current (I_{Ca}) (Jones and Marks, 1989a; Bley and Tsien, 1990; Elmslie et al., 1990; K. S. Elmslie, unpublished results), and occasionally increase the apparent leakage conductance of the cell (Katayama and Nishi, 1982; Kuffler and Sejnowski, 1983; Jones et al., 1984). The effects on I_M and I_{Ca} are mimicked by intracellular dialysis with GTP-γ-S (Pfaffinger, 1988; Brown et al., 1989; Bley and Tsien, 1990; Elmslie et al., 1990), suggesting involvement of a G protein.

To date, tests for involvement of specific second messenger systems have been negative for inhibition of I_{Ca} (Bley and Tsien, 1990) and I_M (Adams et al., 1982; Pfaffinger, 1988; Pfaffinger et al., 1988; Bosma and Hille, 1989; but see Brown and Adams, 1987). Either the G protein(s) couple directly to the ion channels, or an as yet unidentified second messenger system is involved. Because the time course of transmitter action constrains possible mechanisms, a rapid local perfusion method has been used in the present study to apply agonists to isolated frog sympathetic neurons under whole-cell voltage clamp. A preliminary report of this work has appeared (Jones, 1990).

METHODS

Neurons were isolated from bullfrog (*Rana catesbeiana*) sympathetic ganglia (Kuffler and Sejnowski, 1983; Jones, 1987*a*). Whole-cell currents were recorded with an Axopatch amplifier and pClamp software (Axon Instruments, Foster City, CA). Series resistances were <3 MΩ. Currents were sampled at twice the analog filter frequency or greater, and were filtered with a digital Gaussian filter as indicated. Values are expressed as the mean ± standard deviation, or as ranges.

I_M

For experiments on I_M, the intracellular solution was (in millimolar) KCl 74.5, $MgCl_2$ 6, Na_2ATP 5, Li_2GTP 0.3, Na-Hepes 2.5, K_4BAPTA 10, and the extracellular solution was NaCl 115, KCl 2.5, Na-Hepes 2.5, $CaCl_2$ or $MnCl_2$ 2, pH 7.2. In some cells, EGTA was used instead of BAPTA. The holding potential was −30 mV, and series resistance compensation was generally not used. Currents were not leak subtracted.

0006-3495/91/08/502/06 $2.00

I_{Ca}

For isolation of I_{Ca}, the intracellular medium was (mM) 76 N-methyl-D-glucamine (NMG) chloride, 6 $MgCl_2$, 5 $Tris_2ATP$, 0.3 Li_2GTP, 2.5 NMG-Hepes, 10 NMG_4BAPTA, and the extracellular medium was 117.5 NMG-Cl, 2 $BaCl_2$, 2.5 NMG-Hepes, pH 7.2. In some cells, EGTA was used instead of BAPTA. The holding potential was −80 mV, and series resistance compensation (nominally 80%) was used. Where noted, currents were leak subtracted using averaged and scaled hyperpolarizing voltage steps.

The predominant (>90%) calcium current in sympathetic neurons is most similar to the N-type calcium current (Nowycky et al., 1985) except that it inactivates slowly and partially (Plummer et al., 1989; Jones and Marks, 1989b; Jones and Jacobs, 1990).

Flow pipe system

Transmitters were applied from an array of five parallel stainless steel tubes (inside diameter [i. d.] 150 μm, outside diameter [o. d.] 300 μm). Gravity flow (40–50 μl/min, or a linear flow rate of 40–50 μm/ms at the mouth of each pipe) was used, and all pipes were flowing continuously during the experiment. To avoid inadvertent exposure of the cells to transmitters, the pipe array was initially outside the recording dish. After the whole-cell configuration was reached, the cell was moved off the bottom of the recording dish, the tubes were moved into place near the cell, and the cell was positioned 100–200 μm away from the opening of a tube containing control solution. Solution changes were then effected by manually moving the cell to an adjacent tube (about half a turn on an hydraulic micromanipulator [MO-103, Narishige USA, Inc., Greenvale, NY]). Because the manipulator was moved by hand, the exact time of the solution change is not known. The bath was simultaneously superfused with the normal extracellular solution. This method is based on that of Yellen (1982).

RESULTS

Speed of solution exchange

The flow tube system was calibrated primarily by changing extracellular K^+ (Fig. 1). At −30 mV, with normal intracellular K^+, the holding current is a mixture of I_M and a K^+-selective "leakage" conductance (Jones, 1989). Movement of the cell to a tube containing a five fold higher K^+ concentration produced a rapid and stable effect, seen as an inward shift in holding current at −30 mV and a reversal of I_M tail currents at −60 mV (Fig. 1 *A*). In separate experiments, changes in K^+ using either the flow tube array or bath superfusion produced equivalent shifts in the reversal potential of I_M, indicating that the solution change is complete.

Fig. 1 *B* is an expanded view of the shift in holding current during solution changes. In this case, the 10–90% exchange time was 90 ms, and comparable values (119 ± 43 ms, mean ± SD, n = 13) were found in other cells.

Other control experiments were done using 10 mM tetraethylammonium (TEA) to partially block the delayed rectifier K^+ current, and changing the Ba^{2+} concentration in experiments where I_{Ca} was measured (not shown). In either case, the effects of solution changes were complete in the 1-s interval between test pulses.

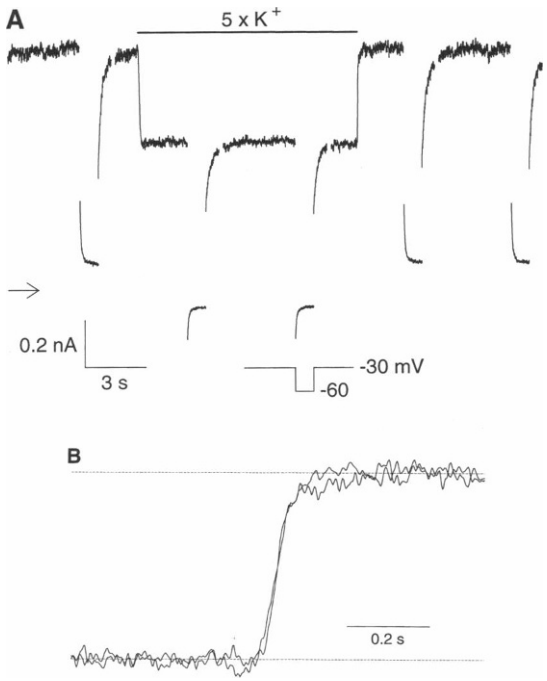

FIGURE 1 Time course of solution exchange with the flow tube system. (*A*) Extracellular K^+ was changed from 2.5 to 12.5 mM (replacing Na^+) during the time marked by the horizontal bar. 0.9 s voltage steps to −60 mV were given every 5 s from the holding potential of −30 mV. 10 ms were blanked at the start and end of each voltage step, and there is also a gap of ~0.4 s in the holding current at −30 mV at 5 s intervals. The arrow at the left indicates zero current. (*B*) The holding current at −30 mV during changes to and from high K^+, expanded from *A*. Currents were normalized to the averages before and after the change (*dashed lines*), and were aligned to the point of half maximal effect. *A* and *B* are from cell e0421, 10-mM intracellular BAPTA and 2-mM extracellular Mn^{2+}, with digital Gaussian filtering at 50 Hz.

Inhibition of I_M

I_M inhibition by teleost LHRH (T-LHRH; [Trp^7, Leu^8]LHRH) requires several seconds (Fig. 2 *A*), and recovery from inhibition is also slow. T-LHRH was used for most of these experiments because its moderate potency (50% inhibition of I_M at 0.2–0.3 μM; Jones et al., 1984; Jones, 1987*b*) suggests that binding and unbinding from the receptor would not be rate-limiting at the concentrations used (2–10 μM). Naively, with a dissociation constant of 0.25 μM and a binding rate of 3×10^7 $M^{-1}s^{-1}$, time constants for binding and unbinding would be 17 ms and 130 ms respectively at 2 μM. Half recovery from inhibition required 36 ± 14 s (mean ± SD, n = 16,

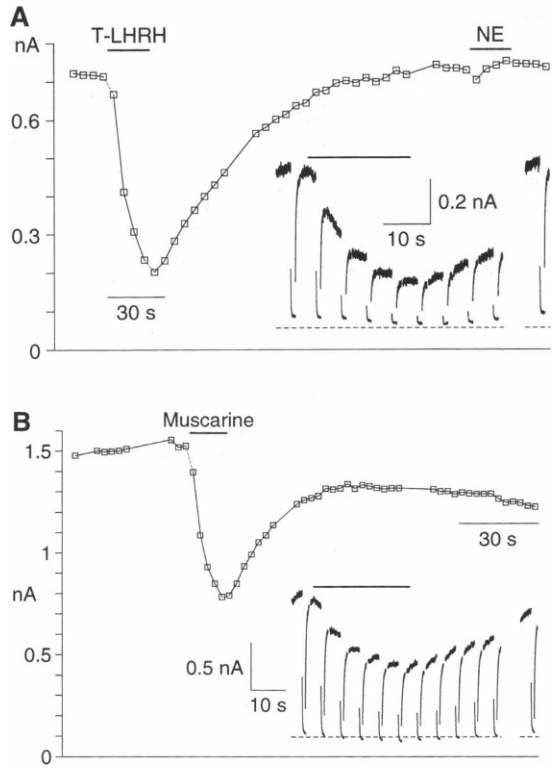

FIGURE 2 Time course of I_M inhibition. The main figures show the holding current at −30 mV, measured every 5.5 s. Transmitters (3-μM T-LHRH, 30-μM NE, and 30-μM muscarine) were applied as marked by horizontal bars. The insets are records from the T-LHRH (*A*) or muscarine (*B*) responses, showing currents at −30 mV and during voltage steps to −60 mV (as in Fig. 1 *A*). The rightmost record was recorded after a gap of ~2 min (*A*, just before application of NE) or ~30 s (*B*). Currents were filtered at 100 Hz with 5 ms blanked. The dashed lines indicate zero current. (*A*) Cell d0808, 10-mM intracellular BAPTA, 2-mM extracellular Ca^{2+}; (*B*) cell c1328, 10-mM intracellular EGTA, 2-mM extracellular Mn^{2+}.

range 12–51 s) following removal of T-LHRH. Similar results were obtained with 100 μM ATP (n = 3) and 30 μM muscarine (21 ± 5 s, n = 5; Fig. 2 *B*). The time course of development of I_M inhibition was not measured systematically, as the effect usually did not reach a steady-state during brief (10–30 s) test applications. The magnitude of I_M inhibition was variable, possibly due to calcium buffering (Beech et al., 1991), but strong I_M inhibition was occasionally observed with 10-mM intracellular BAPTA (Fig. 2 *A*).

Norepinephrine (NE) was generally ineffective on I_M (Fig. 2 *A*), with >10% inhibition in only 1 cell of 10 tested at 10–100 μM.

Inhibition of I_{Ca}

Transmitters do not simply reduce the amplitude of I_{Ca}, as the kinetics of channel gating are affected (Bean, 1989; Elmslie et al., 1990). In particular, some of the current activates slowly, but normal gating can be restored by strong depolarization (Fig. 3 *A*). The effects of all of the transmitters that inhibit I_{Ca} (ACh, LHRH, NE, ATP, and substance P) are kinetically indistinguishable (Elmslie, K. S., unpublished results).

The effect of transmitters on I_{Ca} is considerably faster than the effect on I_M (Fig. 3 *B*). The time course is shown most clearly by the ratio of currents recorded before and after each prepulse to +80 mV ("*Post/pre*" in Fig. 3 *B*; see the illustration of the protocol in Fig. 3 *A*).

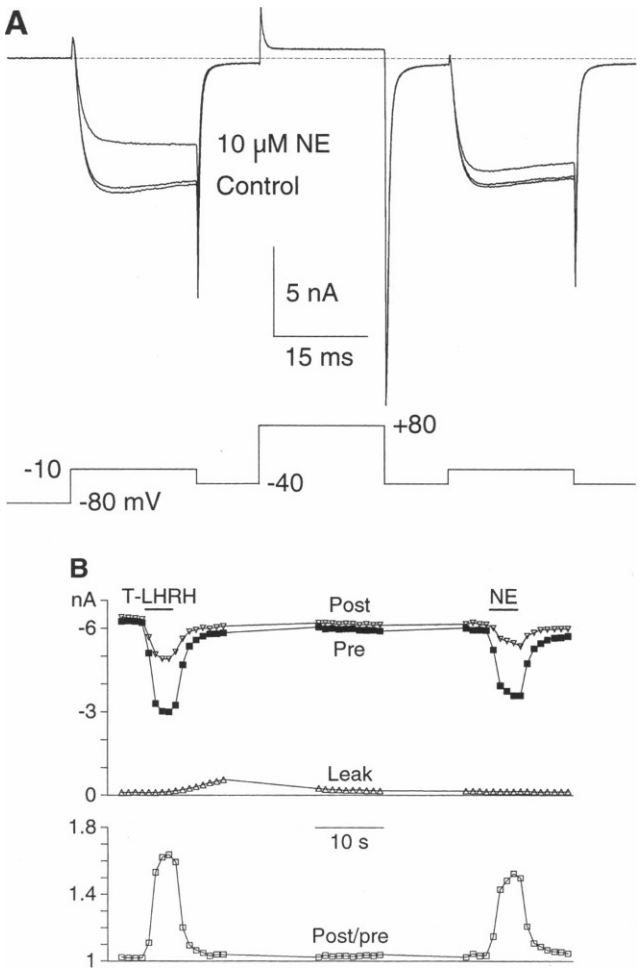

FIGURE 3 Time course of I_{Ca} inhibition. (*A*) Superimposed records recorded before, during, and after a brief (4 s) application of 10-μM NE. The currents, from the response shown in *B*, were leak subtracted and filtered at 2 kHz. (*B*) The time course of responses to T-LHRH (10 μM) and NE. Note the more rapid time scale than Fig. 2. Currents were measured as the average between 2–3 ms during steps to −10 mV (postpulse, ▽; prepulse, ■), from the protocol illustrated in *A*. The holding current at −80 mV ("Leak", △) was reversibly increased by T-LHRH but not NE. Cell b0805, 10-mM intracellular BAPTA, 2-mM extracellular Ba^{2+}.

The times for half maximal onset of inhibition and recovery from inhibition are each ~2 s (range 1–5 s for recovery from inhibition, $n = 15$ for T-LHRH and $n = 15$ for NE). The currents were measured only once per second (Fig. 3 *B*), and the precise timing of the solution change is not known, so these values are undoubtably inaccurate. However, it is clear that the effect is considerably slower than the solution exchange time (Fig. 1) and considerably faster than I_M inhibition (Fig. 2).

Other effects

T-LHRH also caused a reversible increase in the apparent leakage conductance in about half of the cells (Jones et al., 1984). The leak increase develops more slowly than the inhibition of I_{Ca} (Fig. 3 *B*) or I_M (Fig. 2 *A*, *inset*). NE had little effect on the leak in most cells.

Occasionally, there appeared to be a small rapid effect on I_{Ca} or I_M (not shown), which was effectively complete in the ~100 ms exchange time of the perfusion system. (For I_{Ca}, this was tested by applying NE or T-LHRH briefly during a 2–3 s depolarization.) In different cells, this rapid component was 0–20% of the total current. In several cases, this effect was reversible and reproducible for a given cell. However, the inconsistency of the rapid effect among cells made it difficult to determine conclusively whether it was genuine, or an artifact of the change in flow.

DISCUSSION

The primary result of this study is that neurotransmitter-induced inhibition of I_M is ~10-fold slower than inhibition of I_{Ca} in frog sympathetic neurons. Lopez (1990) and Bernheim et al. (1990) have recently reported similar data for the time course of I_M and I_{Ca} inhibition (respectively), although a slow component of I_{Ca} inhibition can be seen under some conditions in rat sympathetic neurons (Beech et al., 1991). These results do not identify the receptor-channel coupling mechanisms for the two effects, but do suggest that the mechanisms differ significantly at some stage. This is true even when inhibition of I_M and I_{Ca} are triggered by what may be the same receptor (for T-LHRH).

In addition to the major (seconds to minutes) component of the transmitter effects, in some cells there was a suggestion of a smaller, more rapid (<100 ms) effect. Although it is not absolutely clear in the present study that the more rapid effect is real, it is reminiscent of the report of a fast component to the action of β-adrenergic agonists on cardiac calcium channels, possibly due to a direct coupling between G proteins and channels (Yatani et al., 1989). Bean (1989) has reported a rapid effect of NE on calcium current in sensory neurons.

Different transmitters (ACh, LHRH, ATP, and substance P) usually have similar effects on I_M and leak conductance in frog sympathetic neurons (Katayama and Nishi, 1982; Kuffler and Sejnowski, 1983; Jones et al., 1984). However, NE strongly inhibits I_{Ca} (Lipscombe et al., 1989) with little or no effect on I_M or leak (Figs. 2–3). Inhibition of I_M by adrenergic agonists has been reported previously (Akasu, 1988), but the effect seems to be inconsistent (Selyanko et al., 1990).

Comparison to synaptic potentials

How do the effects reported here compare to synaptic potentials in bullfrog sympathetic ganglia? The slow excitatory postsynaptic current (EPSP) peaks in ~2 s, and lasts ~1 min (Kuba and Koketsu, 1978; Adams and Brown, 1982). This slow muscarinic EPSP presumably results from the same ACh as the fast nicotinic EPSP recorded from the same cells. The fast EPSP lasts only tens of milliseonds (Kuba and Koketsu, 1978), as the receptor is itself an ion channel. The rapid time course of the fast EPSP suggests rapid removal of ACh from the synapse. Although the location of the muscarinic receptors responsible for the slow EPSP is not yet known, it seems most plausible that the slow EPSP results from an effectively instantaneous pulse of ACh, so that its time course reflects processes subsequent to binding of ACh. A similar interpretation has been proposed for a slow muscarinic EPSP in myenteric neurons (North and Tokimasa, 1984). In contrast, the time course of the late slow EPSP reflects continued presence of an LHRH-like peptide in the extracellular space for up to several minutes (Jan and Jan, 1982).

The decay phase of the slow EPSP agrees well with the ~30 s half-time for reversal of M-current inhibition observed here, but the onset of T-LHRH or muscarine action was generally slower than the rising phase of a slow EPSP. Repetitive stimulation of the presynaptic inputs is necessary to produce full inhibition of I_M, which might mean that repetitive or maintained activation of receptors is necessary for a maximal effect. Alternatively, if spare receptors exist, 2–10-μM T-LHRH or 30-μM muscarine may not fully saturate receptors, resulting in a less than maximal rate of I_M inhibition.

Comparison to cardiac cells

What is the time course of effects where the mechanism of receptor-channel coupling is known? Perhaps the best studied cases are the actions of ACh and NE on the heart, mediated respectively by direct coupling of G

proteins to channels and by cAMP-dependent phosphorylation (Brown and Birnbaumer, 1990). It has long been known that the vagal (parasympathetic) inhibition of the heart is more rapid than the sympathetic excitation (Stewart, 1892). Rapid direct application of adrenergic agonists, or stimulation of the sympathetic innervation, produces an effect that peaks after ten seconds, and recovers after a minute or more, following a latency of 5–15 s (Hill-Smith and Purves, 1978). The latency appears to be due to processes prior to generation of cAMP, as it is not observed when cAMP is delivered rapidly by photolysis of caged cAMP (Nargeot et al., 1983), but the time course of agonist-mediated and cAMP-mediated responses are thereafter similar. The effect of muscarinic agonists or vagal stimulation lasts ~10 s after a latency of only ~250 ms (del Castillo and Katz, 1955; Hartzell et al., 1977; Hill-Smith and Purves, 1978).

The implication seems to be that second-messenger mediated effects on ion channels are considerably slower than direct G protein mediated effects, but it is not obvious that this need be a general result. In particular, lipid-soluble second messengers (e. g., diacylglycerols) might act more locally and rapidly than water-soluble second messenger such as cAMP.

How do the effects reported here compare? Although drug applications were not precisely timed, the latency of actions on I_M was clearly less than 2 s, and probably even shorter, in contrast to the ~10-s latency for cAMP-mediated enhancement of I_{Ca} in the heart. But, except for the latency, the time course of I_M inhibition (Fig. 2) is comparable in speed to cAMP-mediated effects. The actions of T-LHRH and NE on I_{Ca} were more rapid than cAMP-mediated effects on I_{Ca} in the heart, but were slower than direct G protein mediated effects there. The time course of the leak increase, when apparent, was generally slower than effects on I_M or I_{Ca} (see Katayama and Nishi, 1982; Jones et al., 1984).

It is impossible to reach definitive conclusions about receptor-channel coupling mechanisms from the present results. However, the slow time course of I_M inhibition suggests that the search for a second messenger pathway should continue.

We thank Dr. Keith S. Elmslie for helpful discussions, and Dr. Jose Whittembury for construction of the flow tube array.

This work was supported by National Institutes of Health grant NS24471.

Received for publication 21 January 1991 and in final form 4 April 1991.

REFERENCES

Adams, P. R., and D. A. Brown. 1982. Synaptic inhibition of the M-current: slow excitatory post-synaptic potential mechanism in bullfrog sympathetic neurones. *J. Physiol.* 332:263–272.

Adams, P. R., D. A. Brown, and A. Constanti. 1982. Pharmacological inhibition of the M-current. *J. Physiol.* 332:223–262.

Adams, P. R., D. A. Brown, and S. W. Jones. 1983. Substance P inhibits the M-current in bullfrog sympathetic neurones. *Br. J. Pharmacol.* 79:330–333.

Akasu, T. 1988. Adrenaline depolarization in paravertebral sympathetic neurones of bullfrogs. *Pfluegers Arch. Eur. J. Physiol.* 411:80–87.

Bean, B. P. 1989. Neurotransmitter inhibition of neuronal calcium currents by changes in channel voltage dependence. *Nature (Lond.).* 340:153–156.

Beech, D. J., L. Bernheim, A. Mathie, and B. Hille. 1991. Intracellular Ca^{2+} buffers disrupt muscarinic suppression of Ca^{2+} current and M current in rat sympathetic neurons. *Proc. Natl. Acad. Sci. USA* 88:652–656.

Bernheim, L., D. J. Beech, and B. Hille. 1990. Muscarinic and α-adrenergic suppressions of calcium current (I_{Ca}) are blocked only partially by pertussis toxin in rat sympathetic neurons. *Soc. Neurosci. Abstr.* 16:793.

Bley, K. R., and R. W. Tsien. 1990. Inhibition of Ca^{2+} and K^+ channels in sympathetic neurons by neuropeptides and other ganglionic transmitters. *Neuron.* 4:379–391.

Bosma, M. M., and B. Hille. 1989. Protein kinase C is not necessary for peptide-induced suppression of M current or for desensitization of the peptide receptors. *Proc. Natl. Acad. Sci. USA.* 86:2943–2947.

Breitwieser, G. E., and G. Szabo. 1988. Mechanism of muscarinic receptor-induced K^+ channel activation as revealed by hydrolysis-resistant GTP analogues. *J. Gen. Physiol.* 91:469–493.

Brown, D. A., and P. R. Adams. 1987. Effects of phorbol dibutyrate on M-currents and M-current inhibition in bullfrog sympathetic neurons. *Cell. Mol. Neurobiol.* 7:255–269.

Brown, A. M., and L. Birnbaumer. 1990. Ionic channels and their regulation by G protein subunits. *Annu. Rev. Physiol.* 52:197–213.

Brown, D. A., N. V. Marrion, and T. G. Smart. 1989. On the transduction mechanism for muscarine-induced inhibition of M-current in cultured rat sympathetic neurones. *J. Physiol.* 413:469–488.

del Castillo, J., and B. Katz. 1955. Production of membrane potential changes in the frog's heart by inhibitory nerve impulses. *Nature (Lond.).* 175:1035.

Elmslie, K. S., W. Zhou, and S. W. Jones. 1990. LHRH and GTP-γ-S modify calcium current activation in bullfrog sympathetic neurons. *Neuron.* 5:75–80.

Hartzell, H. C., S. W. Kuffler, R. Stickgold, and D. Yoshikami. 1977. Synaptic excitation and inhibition resulting from direct action of acetylcholine on two types of chemoreceptors on individual amphibian parasympathetic neurones. *J. Physiol.* 271:817–846.

Hill-Smith, I., and R. D. Purves. 1978. Synaptic delay in the heart: an ionophoretic study. *J. Physiol.* 279:31–54.

Jan, L. Y., and Y. N. Jan. 1982. Peptidergic transmission in sympathetic ganglia of the frog. *J. Physiol.* 327:219–246.

Jones, S. W. 1987*a*. Sodium currents in dissociated bull-frog sympathetic neurones. *J. Physiol.* 389:605–627.

Jones, S. W. 1987*b*. Chicken II luteinizing hormone-releasing hormone inhibits the M-current of bullfrog sympathetic neurons. *Neurosci. Lett.* 80:180–184.

Jones, S. W. 1989. On the resting potential of isolated frog sympathetic neurons. *Neuron.* 3:153–161.

Jones, S. W. 1990. Time course of neurotransmitter effects on voltage-dependent currents of bullfrog sympathetic neurons. *Soc. Neurosci. Abstr.* 16:623.

Jones, S. W., and L. S. Jacobs. 1990. Dihydropyridine actions on calcium currents of frog sympathetic neurons. *J. Neurosci.* 10:2261–2267.

Jones, S. W., and T. N. Marks. 1989*a*. Calcium currents in bullfrog sympathetic neurons. I. Activation kinetics and pharmacology. *J. Gen. Physiol.* 94:151–167.

Jones, S. W., and T. N. Marks. 1989*b*. Calcium currents in bullfrog sympathetic neurons. II. Inactivation. *J. Gen. Physiol.* 94:169–182.

Jones, S. W., P. R. Adams, M. J. Brownstein, and J. E. Rivier. 1984. Teleost luteinizing hormone-releasing hormone: action on bullfrog sympathetic ganglia is consistent with role as neurotransmitter. *J. Neurosci.* 4:420–429.

Katayama, Y., and S. Nishi. 1982. Voltage-clamp analysis of peptidergic slow depolarizations in bullfrog sympathetic ganglion cells. *J. Physiol.* 333:305–313.

Kuba, K., and K. Koketsu. 1978. Synaptic events in sympathetic ganglia. *Prog. Neurobiol. (OXF).* 11:77–169.

Kuffler, S. W., and T. J. Sejnowski. 1983. Peptidergic and muscarinic excitation at amphibian sympathetic synapses. *J. Physiol.* 341:257–278.

Lipscombe, D., S. Kongsamut, and R. W. Twien. 1989. α-Adrenergic inhibition of sympathetic neurotransmitter release mediated by modulation of N-type calcium-channel gating. *Nature (Lond.).* 340:639–642.

Lopez, H. S. 1990. Kinetics of G protein-mediated modulation of M current. *Soc. Neurosci. Abstr.* 16:539.

Nargeot, J., H. A. Lester, N. J. M. Birdsall, J. Stockton, N. H. Wassermann, and B. F. Erlanger. 1982. A photoisomerizable muscarinic antagonist. Studies of binding and of conductance relaxations in frog heart. *J. Gen. Physiol.* 79:657–678.

Nargeot, J., J. M. Nerbonne, J. Engels, and H. A. Lester. 1983. Time course of the increase in the myocardial slow inward current after a photochemically generated concentration jump of intracellular cAMP. *Proc. Natl. Acad. Sci. USA* 80:2395–2399.

Nicoll, R. A. 1988. The coupling of neurotransmitter receptors to ion channels in the brain. *Science (Wash. DC).* 241:545–551.

North, R. A., and T. Tokimasa. 1984. The time course of muscarinic depolarization of guinea-pig myenteric neurones. *Br. J. Pharmacol.* 82:85–91.

Nowycky, M. C., A. P. Fox, and R. W. Tsien. 1985. Three types of neuronal calcium channel with different calcium agonist sensitivity. *Nature (Lond.).* 316:440–443.

Pfaffinger, P. 1988. Muscarine and t-LHRH suppress M-current by activating an IAP-insensitive G-protein. *J. Neurosci.* 8:3343–3353.

Pfaffinger, P. J., M. D. Leibowitz, E. M. Subers, N. M. Nathanson, W. Almers, and B. Hille. 1988. Agonists that suppress M-current elicit phosphoinositide turnover and Ca^{2+} transients, but these events do not explain M-current suppression. *Neuron.* 1:477–484.

Plummer, M. R., D. E. Logothetis, and P. Hess. 1989. Elementary properties and pharmacological sensitivities of calcium channels in mammalian peripheral neurons. *Neuron.* 2:1453–1463.

Selyanko, A. A., P. A. Smith, and J. A. Zidichouski. 1990. Effects of muscarine and adrenaline on neurons from *Rana pipiens* sympathetic ganglia. *J. Physiol.* 425:471–500.

Stewart, G. N. 1892. The influence of temperature and of endocardiac pressure on the heart, and particularly on the action of the vagus and cardiac sympathetic nerves. *J. Physiol.* 13:59–164.

Yatani, A., K. Okabe, and A. M. Brown. 1989. Time course of cardiac Ca^{2+} current after a concentration jump of isoproterenol. *Biophys. J.* 55:36*a*. (Abstr.)

Yellen, G. 1982. Single Ca^{2+}-activated nonselective cation channels in neuroblastoma. *Nature (Lond.).* 296:357–359.