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Action potential broadening and frequency-dependent facilitation of calcium signals in pituitary nerve terminals

(neurohypophysis/stimulus–secretion coupling/neuropeptide/hormone secretion/synaptic plasticity)

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ABSTRACT **Hormone release from nerve terminals in the neurohypophysis is a sensitive function of action potential frequency. We have investigated the cellular mechanisms responsible for this frequency-dependent facilitation by combining patch clamp and fluorimetric Ca^{2+} measurements in single neurosecretory terminals in thin slices of the rat posterior pituitary. In these terminals both action potential-induced changes in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and action potential duration were enhanced by high-frequency stimuli, all with a frequency dependence similar to that of hormone release. Furthermore, brief voltage clamp pulses inactivated a K^+ current with a very similar frequency dependence. These results support a model for frequency-dependent facilitation in which the inactivation of a K^+ current broadens action potentials, leading to an enhancement of $[Ca^{2+}]_i$ signals. Further experiments tested for a causal relationship between action potential broadening and facilitation of $[Ca^{2+}]_i$ changes. First, increasing the duration of depolarization, either by broadening action potentials with the K^+-channel blocker tetraethylammonium or by applying longer depolarizing voltage clamp steps, increased $[Ca^{2+}]_i$ changes. Second, eliminating frequency-dependent changes in duration, by voltage clamping the terminal with constant duration pulses, substantially reduced the frequency-dependent enhancement of $[Ca^{2+}]_i$ changes. These results indicate that action potential broadening contributes to frequency-dependent facilitation of $[Ca^{2+}]_i$ changes. However, the small residual frequency dependence of $[Ca^{2+}]_i$ changes seen with constant duration stimulation suggests that a second process, distinct from action potential broadening, also contributes to facilitation. These two frequency-dependent mechanisms may also contribute to activity-dependent plasticity in synaptic terminals.**

Little is known about the mechanisms that regulate secretion from nerve terminals, despite the importance of these mechanisms to many physiological processes. For example, a presynaptic site has been proposed as an important locus for change in several forms of use-dependent synaptic plasticity, including facilitation and posttetanic potentiation at neuromuscular synapses (1) and long-term potentiation in the mammalian hippocampus (2, 3). However, in these and most other systems one can do little more than infer that some presynaptic change occurs, because technical difficulties preclude a direct physiological characterization of the nerve terminals. Although a direct physiological characterization is possible in some invertebrate systems (4, 5), until recently there was no comparable vertebrate system for the study of presynaptic physiology.

In an effort to bridge this technical gap, some investigators have made electrical and optical recordings from the nerve terminals of the vertebrate neurohypophysis (6–12). These terminals release the neuropeptides vasopressin and oxytocin and have received much attention as a model system for the study of secretion (6–12). Peptide secretion from these terminals also exhibits a striking form of use-dependent plasticity: secretion is a sensitive function of action potential frequency (11, 13–17). This frequency dependence is essential to the input–output properties of the hypothalamic-hypophyseal axis (18) and has been proposed to be due to frequency-dependent modulation of action potential duration (10, 11, 13, 14, 19–23). In the present study, we use patch clamp techniques and fluorimetric calcium indicators to test this hypothesis in individual nerve terminals in pituitary slices. A preliminary account of this work has appeared (24).

METHODS

Neurohypophysial Slices. The neurointermediate lobe of the pituitary was removed from male rats with ages ranging from 2 to 4 months and placed in carbogen (95% O_2/5% CO_2)-saturated rat Ringer's solution (125 mM NaCl, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 26 mM $NaHCO_3$, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 20 mM glucose) at 0°C for at least 1 min. Slices 70–80 μm thick were then cut with an FTB vibratome, with continued bathing in 0°C rat Ringer's solution. Slices were either kept in a 34°C bath or transferred to a recording chamber at room temperature (20–22°C) in rat Ringer's solution identical to that described above, except that the KCl concentration was 4 mM. Slices were suitable for recording immediately and were viable for up to 4 hr.

Patch clamp recordings from these slices were made at room temperature (20–22°C) following published methods (25), except that no cleaning of tissue or enzyme treatment was necessary. Patch electrodes with resistances between 2.5 and 6 MΩ were fabricated from borosilicate glass (i.d. = 1.4 mm; o.d. = 2.0 mm). Tight-seal intracellular recordings (26) were achieved with series resistances ranging from 4.5 to 15 MΩ. For experiments in which $[Ca^{2+}]_i$ was not simultaneously measured, patch pipettes were filled with a solution consisting of 140 mM KCl, 10 mM EGTA, 4 mM ATP, 4 mM $MgCl_2$, and 10 mM Hepes at pH 7.3. Signals were recorded with an EPC-7 patch clamp amplifier and digitized and stored on an Atari computer. A computer program written by Michael Pusch (University of Genoa) was used to acquire data.

Intracellular Ca^{2+} Measurements. For intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) measurements from single terminals, the fluorescent calcium indicator fura-2 (27) (100 or 300 μM) was added to a patch pipette filling solution with the composition 135 mM KCl, 5 mM NaCl, 0.2 mM EGTA, 4 mM ATP, 4 mM $MgCl_2$, and 10 mM Hepes at pH 7.3. The ratiometric technique used to measure $[Ca^{2+}]_i$ was identical to that described by Neher (28). In brief, terminals were

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Abbreviation: TEA, tetraethylammonium.

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alternately excited with UV light, filtered at 360 or 390 nm with a wheel rotating at 5 Hz, and the emitted fluorescence was measured with a Hamamatsu photomultiplier tube (model R928). $[Ca^{2+}]_i$ was then computed from the ratio of emission at the two wavelengths. Such measurements of $[Ca^{2+}]_i$ could be made from single terminals for up to 30 min.

RESULTS

Nerve terminals were visible in thin slices of the neurohypophysis when viewed under Nomarski optics (Fig. 1). These terminals are round in appearance, with diameters ranging from 1 to 20 μm. The identity of these structures as terminals has been established (S. A. DeRiemer, R. Schneggenburger, M.B.J., and A.K., unpublished results) by the following criteria: (*i*) absence of nuclei, as judged by the lack of staining with a nuclear dye; (*ii*) orthograde transport of dye from hypothalamic neurons; and (*iii*) axonal stimulation generates action potentials. Furthermore, as shown below, electrical stimulation leads to increases in $[Ca^{2+}]_i$ in these structures. The terminals observed on the upper surface of a slice were often exposed and accessible to patch pipettes. An example of such a terminal, filled with the fluorescent dye Lucifer yellow, is shown in Fig. 1.

Calcium Signaling in Single Nerve Terminals. Loading of the fluorescent Ca^{2+} indicator fura-2 (27) from the patch pipette solution was accomplished in 2 min or less. The resting $[Ca^{2+}]_i$ in these terminals was 110 ± 10 nM (mean ± SEM; n = 16), which is comparable to the resting $[Ca^{2+}]_i$ of many cell types (29). The value of 350 nM reported for secretosomes prepared from the posterior pituitary is considerably higher (30), but this discrepancy can be explained by leakage of dye from secretosomes.

Since secretion from the nerve terminals of the posterior pituitary is triggered by Ca^{2+} entry (8, 9, 31), we were interested in asking whether the frequency dependence of secretion was a result of the frequency dependence of $[Ca^{2+}]_i$ changes. Repetitive stimulation of current-clamped terminals with current pulses large enough to evoke action potentials caused transient increases in $[Ca^{2+}]_i$ (Fig. 2). These increases were rapid, with a rate of rise that depended on the duration and frequency of stimulation. The peak in $[Ca^{2+}]_i$ was followed by an exponential decay back to the resting level. The half-time for decay was in the range of 10 to 20 s. Ca^{2+} buffers such as fura-2 are known to slow $[Ca^{2+}]_i$ recovery (ref. 32; G.J.A. and E. Neher, unpublished results), so that the fura-2 may have slowed the decay of $[Ca^{2+}]_i$ signals. However, lowering the fura-2 concentration from 300 to 100 μM had little effect on the time course of recovery, which suggests that such effects were relatively minor in our experiments.

Stimulation with action potentials at high frequencies increased $[Ca^{2+}]_i$ much more than stimulation with action potentials at low frequencies (Fig. 2). At 1 Hz the change in $[Ca^{2+}]_i$ induced by 100 action potentials was barely detectable, but following 100 action potentials at frequencies of 10 Hz or higher, $[Ca^{2+}]_i$ increased to 300 nM or more (Fig. 2*A*). High-frequency stimulation also enhanced the rate of increase of $[Ca^{2+}]_i$ during the stimulus train. The frequency dependence of action potential-evoked increases in $[Ca^{2+}]_i$ is shown in Fig. 2*B*. This frequency dependence resembles that reported for vasopressin release from isolated neurointermediate lobes (13, 14). Both signals are maximal at ≈10 Hz, although the frequency of the half-maximal response is roughly 3 Hz higher for vasopressin release than for $[Ca^{2+}]_i$ signals (Fig. 2*B*). This difference could be due to a nonlinear relationship between $[Ca^{2+}]_i$ and the rate of secretion (33–35) or to minor differences in experimental conditions (vasopressin release was measured in a similar saline but with a bovine serum albumin concentration of 1.5 mg/ml at 37°C). Nonetheless, the similar frequency dependences of $[Ca^{2+}]_i$ and

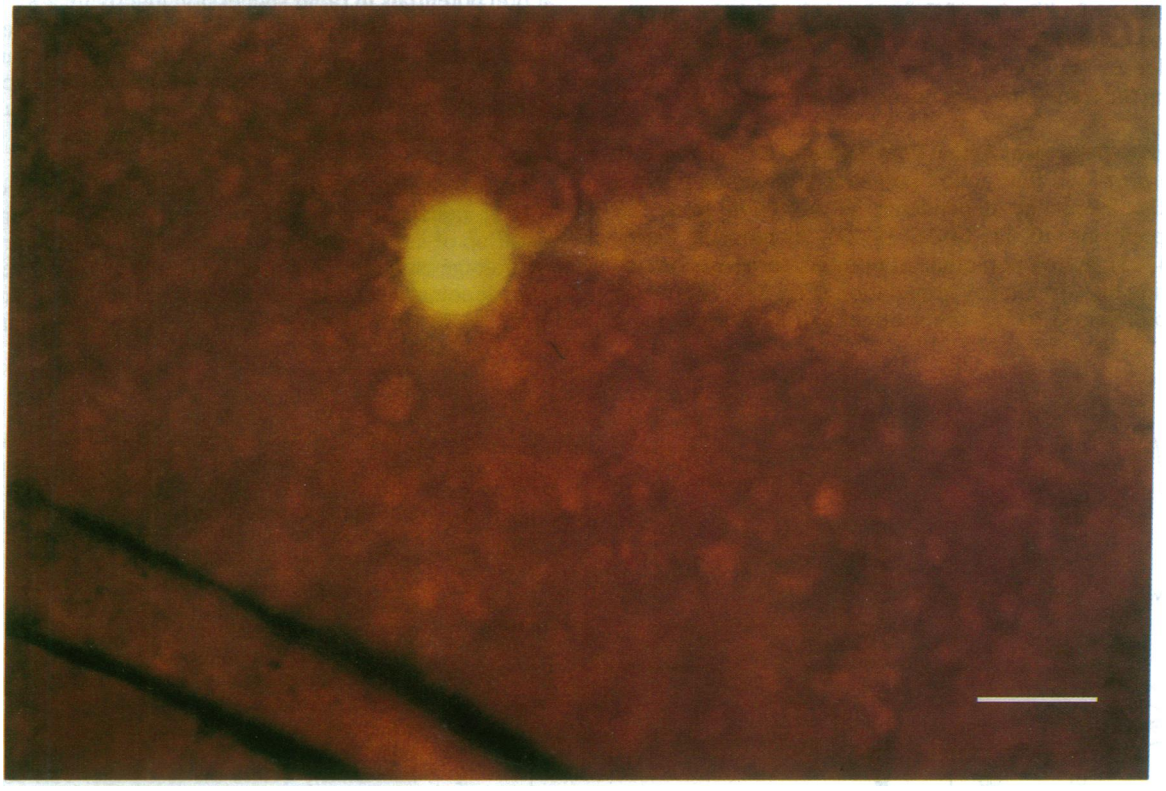

FIG. 1. Nerve terminals on the surface of a thin pituitary slice. A photomicrograph with simultaneous Nomarski and fluorescence optics shows a Lucifer yellow-filled nerve terminal. The patch electrode, filled with Lucifer yellow (0.2 mg/ml) in our normal patch-electrode filling solution, is also visible. (Bar = 20 μm.)

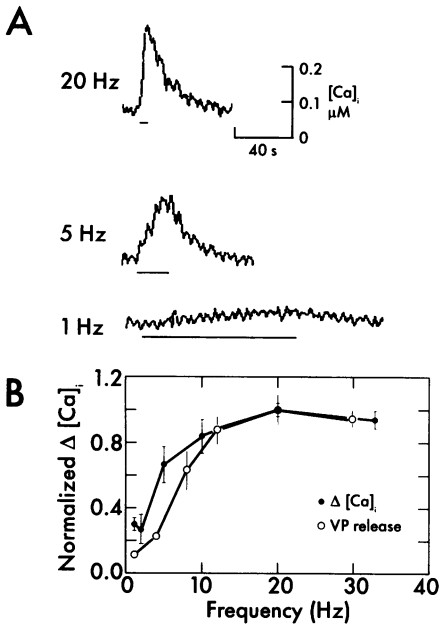

FIG. 2. (*A*) $[Ca^{2+}]_i$ in a single nerve terminal. A terminal filled with fura-2 was stimulated with 100 current pulses (3 ms, 200 pA) at the indicated frequencies. The period of stimulation is indicated by the solid line. The holding current was adjusted to produce a membrane potential of −70 mV. Under these conditions of stimulation, each current pulse produced an action potential. (*B*) Frequency dependence of $[Ca^{2+}]_i$ changes (●) determined from data such as those shown in *A*. The frequency dependence of vasopressin (VP) release (○) was replotted from ref. 13 for comparison. The data represent the arithmetic mean ± SEM of six experiments, normalized to the maximal values.

hormone secretion, together with the established requirement for Ca^{2+} in the triggering of secretion (8, 9, 31, 36), suggest that the facilitation of Ca^{2+} signals by high-frequency stimulation contributes to the frequency dependence of hormone release. We therefore investigated the mechanisms underlying the frequency dependence of facilitation of $[Ca^{2+}]_i$ signals.

Frequency-Dependent Action Potentials and K^+ Currents. It has been proposed that action potential broadening plays a role in the frequency dependence of secretion from the neurohypophysis (10, 11, 14–16, 18–22). As reported previously (14, 23), action potentials of pituitary terminals broadened during high-frequency stimulation (Fig. 3*A*). After ≈20 action potentials at 10 Hz, the width at half-height reached a maximum that was 37% larger than that of a control action potential. The frequency dependence of these changes in action potential duration (Fig. 3*B*) was also similar to that observed for $[Ca^{2+}]_i$ increases and vasopressin release (Fig. 2*B*).

Because K^+ currents are critical in determining action potential duration (37), these currents were examined with the voltage clamp technique to evaluate their role in frequency-dependent changes in action potential duration. Depolarization activated a complex pattern of outward current (Fig. 3*C*). On the basis of reversal potential, ion substitution experiments, and block by tetraethylammonium (TEA) and 4-aminopyridine, we concluded that this outward current was carried by K^+ (data not shown). Prolonged depolarization partially inactivated the K^+ current and allowed this current to be separated into rapidly and slowly inactivating components (Fig. 3*C*). These components have different kinetic and pharmacological properties and presumably are generated by different populations of K^+ channels (M.B.J. and A.K., unpublished results).

To determine how these K^+ currents change during a facilitating stimulus, trains of 30 brief (3-ms duration) pulses were applied at various frequencies. This conditioning regimen selectively reduced the rapidly inactivating component of K^+ current (Fig. 3*C*). The inactivation induced by these conditioning pulse trains was a sensitive function of pulse frequency (Fig. 3*D*), producing a relationship essentially identical to the frequency dependence of action potential duration (Fig. 3*B*). The similarity between these two plots suggests that action potential broadening by high-frequency stimulation is caused by K^+-current inactivation.

Tests of the Action Potential Broadening Model of Facilitation. The similar frequency dependence of hormone release, $[Ca^{2+}]_i$ enhancement, action potential broadening, and K^+-current inactivation supports a model for frequency-dependent facilitation in which the depolarizations of many action potentials in rapid succession inactivate a K^+ current. As the K^+ current is inactivated, action potentials repolarize more slowly, leading to increased Ca^{2+} entry, enhancement of $[Ca^{2+}]_i$, and a pursuant increase in hormone release. We next performed two types of experiments to test the hypothesis that action potential broadening is the basis for frequency-dependent enhancement of $[Ca^{2+}]_i$.

If action potential broadening contributes to the increase in $[Ca^{2+}]_i$ signals, then the $[Ca^{2+}]_i$ change should depend on the duration of a depolarizing stimulus. This was first examined by voltage clamping the terminals and applying brief, spike-like, depolarizing pulses of varying duration. Depolarizations were made to +10 mV to produce maximum signals (see

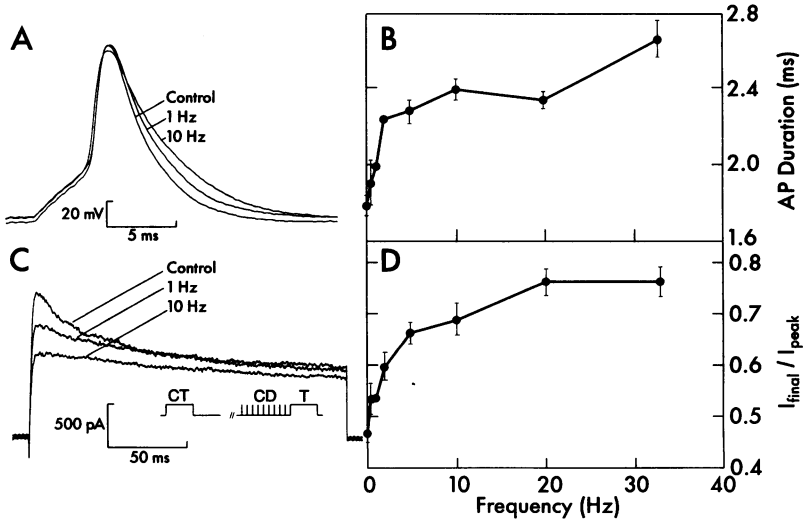

FIG. 3. Broadening of action potentials by high-frequency stimulation. (*A*) A control action potential (elicited following at least 5 s of inactivity) is displayed together with the last action potential of trains of 30 at both 1 and 10 Hz. (*B*) Dependence of action potential (AP) duration (width at half-height) on stimulus frequency. The data represent the arithmetic mean ± SEM for three cells. (*C*) Voltage clamp measurements of K^+ currents elicited by pulses from −80 mV to 50 mV. The small brief inward current at the very beginning of the pulses is a tetrodotoxin-sensitive Na^+ current. The largest K^+ current was observed with no prior conditioning. Conditioning stimuli consisted of trains of 30 pulses (3-ms duration) to 50 mV applied immediately before the test pulse. This conditioning reduced the peak current. CT, control; CD, conditioning; T, test. (*D*) Reduction in the ratio of the final K^+ current (I_{final}) to the peak K^+ current (I_{peak}) versus frequency of stimulation. The data represent the arithmetic mean ± SEM for three cells.

below). Although a quantitative comparison between the duration of action potentials and rectangular pulses is difficult, because of differences in waveform, we selected pulse durations that overlapped with the range of durations measured for action potentials. Increasing pulse durations from 2 to 6 ms caused the $[Ca^{2+}]_i$ change to increase by more than a factor of 4, indicating that the $[Ca^{2+}]_i$ change is a sensitive function of the duration of a brief depolarization (Fig. 4).

For a given pulse duration, the plot of change in $[Ca^{2+}]_i$ versus voltage was bell-shaped in appearance, reminiscent of the voltage dependence of Ca^{2+} currents in these terminals (7, 8). Depolarizations to +10 mV produced a maximal change in $[Ca^{2+}]_i$, whereas depolarizations to −40 mV or +60 mV produced extremely small changes (data not shown). The suppression of $[Ca^{2+}]_i$ changes by pulses to +60 mV is strong evidence for an adequate voltage clamp of the terminals from which these signals were measured (e.g., ref. 38). Thus, axons or other compartments that are connected to the terminal, and which might not be at the same potential as the terminal, do not contribute significantly to the $[Ca^{2+}]_i$ signals.

Another way to ask whether action potential broadening can enhance $[Ca^{2+}]_i$ changes is to measure $[Ca^{2+}]_i$ signals following pharmacological broadening of action potentials. For this purpose, we used the K^+-channel blocker TEA, which is known to increase action potential duration (10) and enhance hormone release (13, 22) in the neurohypophysis. TEA (2 mM) reduced peak K^+ current by 39% (n = 4) and broadened action potentials by 79% (n = 3). Ca^{2+} changes were increased by a factor of 6 (n = 2). Thus, both voltage clamp and pharmacological experiments indicate that action potential broadening is capable of augmenting $[Ca^{2+}]_i$ signals.

If broadening of action potentials is necessary for facilitation of $[Ca^{2+}]_i$ changes by high frequencies, then keeping the duration constant should suppress facilitation. This prediction was tested by using the voltage clamp to hold the duration of this depolarization constant while varying the stimulus frequency. As predicted, maintaining a constant duration reduced the frequency dependence of the $[Ca^{2+}]_i$ change. At 20 Hz the facilitation of $[Ca^{2+}]_i$ under voltage clamp was less than 50% of that under current clamp (Fig. 5*B*). This shows that action potential broadening contributes to the frequency dependence of $[Ca^{2+}]_i$ changes. However, increasing frequency still produced some increase in $[Ca^{2+}]_i$ signals at all pulse durations tested (from 2 to 10 ms; Fig. 5*B*). Thus, an additional, spike duration-independent mechanism is also involved in the facilitation of $[Ca^{2+}]_i$ changes by high-frequency stimulation.

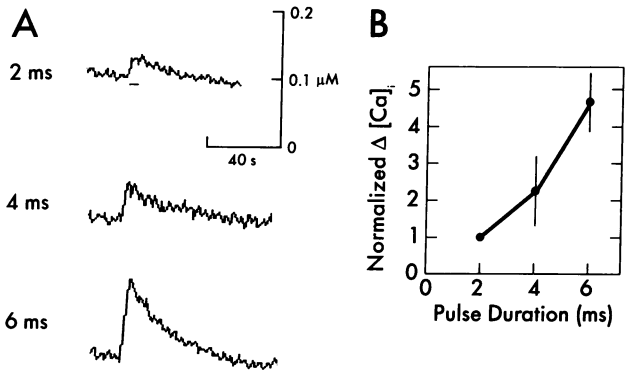

FIG. 4. $[Ca^{2+}]_i$ changes induced by constant-duration voltage pulses. A fura-2-filled nerve terminal was voltage clamped at −80 mV and stimulated with 100 voltage pulses to 10 mV of the durations indicated. (*A*) $[Ca^{2+}]_i$ responses of a single terminal to 20-Hz stimulation at the three different durations. (*B*) Relationship between pulse duration and $[Ca^{2+}]_i$ changes. For each frequency at least two durations were tested, and values were normalized to the maximal $[Ca^{2+}]_i$ change. Each point is the arithmetic mean of three to seven measurements. Data for stimulation at frequencies ranging from 1 to 20 Hz were very similar and were therefore averaged. These experiments were done in the presence of 1 μM tetrodotoxin to suppress Na^+ current and facilitate voltage control.

DISCUSSION

To our knowledge, this study reports the first recordings of $[Ca^{2+}]_i$ signals from individual vertebrate nerve endings. These $[Ca^{2+}]_i$ signals increase following electrical stimulation, as found in all cells exhibiting action potential-evoked secretion. Thus, thin slices of the posterior pituitary are a useful preparation for the study of excitation–secretion coupling in mammalian nerve terminals.

We have focused our attention on the well-documented frequency-dependent facilitation of release from pituitary nerve terminals (11, 13–17). Our experiments provide strong support for the hypothesis that broadening of action potentials contributes to this process. One line of evidence is the similar frequency dependence of action potential duration and action potential-induced $[Ca^{2+}]_i$ changes. Another line of evidence is that experimentally prolonged depolarization of the terminals enhanced $[Ca^{2+}]_i$ changes. Finally, trains of constant-duration depolarizations showed less frequency-dependent augmentation than trains of action potentials. Therefore, it is likely that broadening of action potentials during high-frequency stimulation allows more Ca^{2+} to enter with each action potential. Given that Ca^{2+} triggers secretion from these terminals (8, 9, 31), enhancement of Ca^{2+} entry should then enhance secretion.

Action potential broadening has been studied extensively in a variety of preparations. It has been shown to occur in the neurohypophysis based on experiments with microelec-

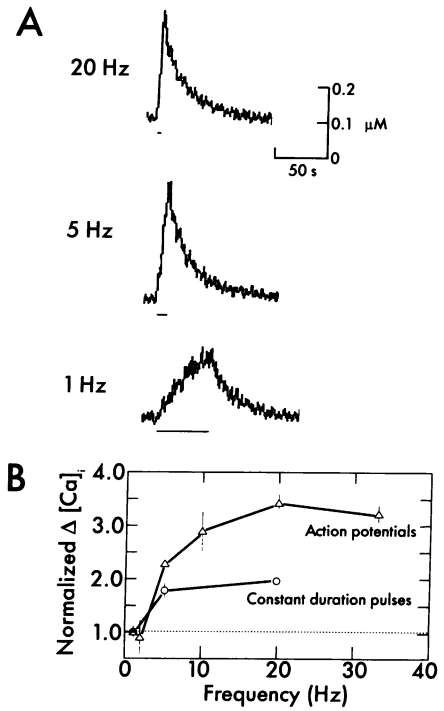

FIG. 5. Frequency dependence of $[Ca^{2+}]_i$ changes in voltage clamped nerve terminals. (*A*) $[Ca^{2+}]_i$ changes in response to 50 pulses from −80 mV to 10 mV for 6 ms, at the indicated frequency. (*B*) The frequency dependence of $[Ca^{2+}]_i$ changes evoked by constant-duration pulses (○) was plotted together with the frequency dependence of $[Ca^{2+}]_i$ changes produced by action potentials (△; from Fig. 2*B*). The results for stimulus durations of from 2 to 10 ms were similar in frequency dependence and were therefore averaged. Signals from six terminals were normalized to the value at 1 Hz and are the mean ± SEM.

trodes (23) and voltage-sensitive dyes (14). It has also been demonstrated in the hypothalamic cell bodies from which the nerve endings of the posterior pituitary originate (19, 20). The mechanism of action potential broadening has been especially well studied in molluscan neurons (39) and can be caused by K^+-current inactivation. We tested the role of K^+-current inactivation in broadening of pituitary action potentials by using trains of brief, spike-like voltage pulses. Such trains inactivated K^+ current in a frequency-dependent manner similar to that of action potential broadening. The broadening of action potentials by treatment with 2 mM TEA, which reduces the rapidly inactivating component of K^+ current, is additional support for the involvement of this component of the K^+ current in action potential repolarization. Thus, reduction of the K^+ current by inactivation, which occurs during high-frequency stimulation (Fig. 3), would be expected to broaden action potentials.

For action potential broadening to be effective in enhancing secretion, stimulus-induced $[Ca^{2+}]_i$ increases must be a sensitive function of the duration of depolarization. We have found this to be the case for both action potentials and voltage clamp pulses. It has been proposed that a high sensitivity of Ca^{2+} flux to stimulus duration is caused by the delayed activation of voltage-gated Ca^{2+} channels (40). This delay in Ca^{2+}-current activation has been described in squid terminals (4, 38) and chromaffin cells (41, 42) and may be a general and functionally significant feature of Ca^{2+} channels that trigger secretion.

In addition to demonstrating a role for action potential broadening in the frequency dependence of secretion from the nerve endings of the neurohypophysis, this study showed that not all of the frequency dependence of Ca^{2+} signaling can be attributed to action potential broadening. When the duration of a stimulus is kept fixed, high frequencies still enhanced $[Ca^{2+}]_i$ changes to some extent (Fig. 5*B*). Because the $[Ca^{2+}]_i$ changes relax slowly, over seconds, the most likely source of this other component is temporal summation of the successive $[Ca^{2+}]_i$ increases caused by single depolarizations. Although Ca^{2+}-channel facilitation could provide an additional frequency-dependent mechanism (41, 42), facilitation of capacitance changes in secretosomes from the neurohypophysis has been reported to take place without increases in Ca^{2+} current (8). Duration-independent facilitation of Ca^{2+} signals could also involve the use-dependent generation of another, unknown second messenger.

There are many examples of use-dependent enhancement of neurotransmitter secretion. These include facilitation, posttetanic potentiation, and long-term potentiation. In no instance has an underlying presynaptic mechanism been elucidated. The two mechanisms that we have identified in pituitary nerve terminals could play roles in these other forms of synaptic plasticity. While action potential broadening is not necessary for synaptic facilitation in squid nerve terminals (43, 44), it may be involved in other forms of use-dependent plasticity. A role for action potential broadening in heterosynaptic facilitation is well documented (45, 46). Likewise, our proposal of temporal summation of $[Ca^{2+}]_i$ signals is reminiscent of the residual calcium models for synaptic facilitation (36) and posttetanic potentiation (1).

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1. Magleby, K. L. & Zengel, J. E. (1975) *J. Physiol. (London)* **245**, 163–182.
2. Nicoll, R. A., Kauer, J. A. & Malenka, R. C. (1988) *Neuron* **1**, 97–103.
3. Bliss, T. V. P. & Lynch, M. A. (1988) in *Long-Term Potentiation: From Biophysics to Behavior*, eds. Landsfield, P. W. & Deadwyler, S. A. (Liss, New York), pp. 3–72.
4. Llinas, R. (1984) *Curr. Top. Membr. Transp.* **22**, 519–571.
5. Augustine, G. J., Charlton, M. P. & Smith, S. J. (1987) *Annu. Rev. Neurosci.* **10**, 633–693.
6. Mason, W. T. & Dyball, R. E. J. (1986) *Brain Res.* **283**, 279–286.
7. Lemos, J. R. & Nowycky, M. C. (1989) *Neuron* **2**, 1419–1426.
8. Fidler, N. H., Nowycky, M. C. & Bookman, R. J. (1990) *Nature (London)* **344**, 449–451.
9. Cazalis, M., Dayanithi, G. & Nordmann, J. J. (1987) *J. Physiol. (London)* **390**, 55–70.
10. Obaid, A. L., Orkand, R. K., Gainer, H. & Salzberg, B. M. (1985) *J. Gen. Physiol.* **85**, 481–489.
11. Salzberg, B. M. & Obaid, A. L. (1988) *J. Exp. Biol.* **139**, 195–231.
12. Salzberg, B. M., Obaid, A. L., Senseman, D. M. & Gainer, H. (1983) *Nature (London)* **306**, 36–40.
13. Bondy, C. A., Gainer, H. & Russell, J. T. (1987) *Neuroendocrinology* **46**, 258–267.
14. Gainer, H., Wolfe, S. A., Obaid, A. L. & Salzberg, B. M. (1986) *Neuroendocrinology* **43**, 557–563.
15. Dutton, A. & Dyball, R. E. J. (1979) *J. Physiol. (London)* **290**, 433–440.
16. Dreifuss, J. J., Kalnins, I., Kelly, J. S. & Ruf, K. B. (1971) *J. Physiol. (London)* **215**, 805–817.
17. Gainer, H. (1978) in *Comparative Neuroendocrinology*, eds. Gaillard, P. J. & Boer, H. H. (Elsevier/North Holland, Amsterdam), pp. 293–304.
18. Poulain, D. A. & Wakerly, J. B. (1982) *Neuroscience* **7**, 773–808.
19. Andrew, R. D. & Dudek, F. E. (1985) *Brain Res.* **334**, 176–179.
20. Bourque, C. W. & Renaud, L. P. (1985) *J. Physiol. (London)* **363**, 429–439.
21. Nordmann, J. J. & Stuenkel, E. L. (1986) *J. Physiol. (London)* **380**, 521–539.
22. Hobbach, H. P., Hurth, S., Jost, D. & Racke, K. (1988) *J. Physiol. (London)* **397**, 539–554.
23. Bourque, C. W. (1990) *J. Physiol. (London)* **421**, 247–262.
24. Jackson, M. B., Augustine, G. J. & Konnerth, A. (1990) *Soc. Neurosci. Abstr.* **16**, 4196.
25. Edwards, F. A., Konnerth, A., Sakmann, B. & Takahashi, T. (1989) *Pflügers Arch.* **414**, 600–612.
26. Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. (1981) *Pflügers Arch.* **391**, 85–100.
27. Grynkiewicz, G., Poenie, M. & Tsien, R. Y. (1985) *J. Biol. Chem.* **260**, 3440–3450.
28. Neher, E. (1989) in *Neuromuscular Junction*, eds. Sellin, L. C., Libelius, R. & Thesleff, S. (Elsevier, Amsterdam), pp. 65–76.
29. Tsien, R. Y. (1989) *Annu. Rev. Neurosci.* **12**, 227–253.
30. Brethes, D., Dayanithi, G., Letellier, L. & Nordmann, J. J. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 1439–1443.
31. Douglas, W. W. & Poisner, A. M. (1964) *J. Physiol. (London)* **172**, 1–18.
32. Baylor, S. M. & Hollingsworth, S. (1988) *J. Physiol. (London)* **403**, 151–192.
33. Dodge, F. A. & Rahamimoff, R. (1967) *J. Physiol. (London)* **193**, 419–432.
34. Knight, D. E. & Baker, P. F. (1982) *J. Membr. Biol.* **68**, 107–140.
35. Augustine, G. J. & Charlton, M. P. (1986) *J. Physiol. (London)* **381**, 619–640.
36. Katz, B. & Miledi, R. (1967) *J. Physiol. (London)* **192**, 407–436.
37. Hodgkin, A. L. & Huxley, A. F. (1952) *J. Physiol. (London)* **117**, 500–544.
38. Augustine, G. J., Charlton, M. P. & Smith, S. J. (1985) *J. Physiol. (London)* **367**, 143–162.
39. Aldrich, R. W., Getting, P. A. & Thompson, S. H. (1979) *J. Physiol. (London)* **291**, 531–544.
40. Augustine, G. J. (1990) *J. Physiol. (London)*, in press.
41. Fenwick, E. M., Marty, A. & Neher, E. (1982) *J. Physiol. (London)* **331**, 499–535.
42. Hoshi, T., Rothlein, J. & Smith, S. J. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 5871–5875.
43. Charlton, M. P. & Bittner, G. D. (1978) *J. Gen. Physiol.* **72**, 471–486.
44. Charlton, M. P., Smith, S. J. & Zucker, R. S. (1982) *J. Physiol. (London)* **323**, 173–193.
45. Hochner, B., Klein, M., Schacher, S. & Kandel, E. R. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 8410–8414.
46. Kandel, E. R. & Schwartz, J. H. (1982) *Science* **229**, 433–443.