Interaction between bradykinin and voltage-sensitive sodium channels in myelinated nerve fibers

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Summary. The effects of externally and internally applied bradykinin on the excitability of single myelinated nerve fibers were studied. External bradykinin (10 μM) slightly prolongs the action potential of a single myelinated nerve fiber; hence, when the fibers are stimulated by long-lasting pulses, this raises the frequency of repetitive firing in sensory fibers and evokes repetitive activity in motor fibers. Under voltage-clamp conditions, sodium channel inactivation is slowed, while sodium channel activation remains unaffected. Prolonged depolarization of the membrane leads to a maintained sodium current. The voltage dependence of the steady-state sodium current inactivation (h ∞) is shifted in the depolarized direction by ~10 mV. Internally applied bradykinin produces a frequency-dependent block of the sodium current. The phenomena described here imply that more than one site on the sodium channel is modified by bradykinin.
Key words. Bradykinin; node of Ranvier; sodium channel.

Examples of polypeptides causing pain and promoting excitation include both those such as scorpion and anemone toxins, and endogenous substances released from the tissue itself as a result of damaging stimulation. Polypeptides included in the first group modify the kinetics of sodium channel gating and increase the probability that a channel opens or remains open; they promote repetitive firing or constant depolarization of nerve and muscle[1-3]. Among polypeptides falling into the second group, there is strong evidence that bradykinin stimulates all fiber classes occurring in cutaneous nerves[4], the cells of the superior cervical ganglion of the cat[5], paravascular pain receptors[6], and polymodal nociceptors of the rabbit ear[7]. Bradykinin also acts on the nervous membrane itself (direct action); this is shown by the fact that single myelinated nerve fibers treated with bradykinin respond with long firing[8]. Since no information exists about the ionic mechanisms involved in these phenomena, we performed voltage-clamp experiments to clarify the type of interaction which occurs between bradykinin molecules and sodium channels.

Methods. The experiments were carried out on single myelinated nerve fibers from the sciatic nerve of the frog *Rana esculenta*. The membrane potential and membrane currents were recorded under current- and voltage-clamp conditions, using the method of Nonner[9]. The normal resting potential was assumed to be −70 mV, corresponding to 30% of Nas inactivation. Membrane currents were calculated assuming an axoplasmic resistance of 10 MΩ. Linear leakage and capacity currents were subtracted electronically from the total current[10].

The Ringer's solution used had the following composition (mM): NaCl 111.5, KCl 2.5, $CaCl_2$ 1.8, $NaHCO_3$ 2.4, pH 7.4. When the sodium current was being monitored[1], potassium current was suppressed by replacing the end pool solution with 110 mM CsCl + 10 mM NaCl and adding tetraethylammonium (10 mM) to the external solution. Bradykinin (from Sigma) was added to the external Ringer's solution or to the isotonic (117 mM) KCl solution bathing the two cut ends of the fiber. In the latter case, the substance reached the nodal membrane by diffusion into the axoplasm over a distance of 500–1000 μm. Taking into account the relatively large size of the molecule, the concentration at the nodal membrane was certainly smaller than the concentration in the Ringer's solution even after 1 h of diffusion from the two ends of the fiber.

Results. Effects of bradykinin on action potentials of single myelinated fibers. The sensory fibers, when stimulated by

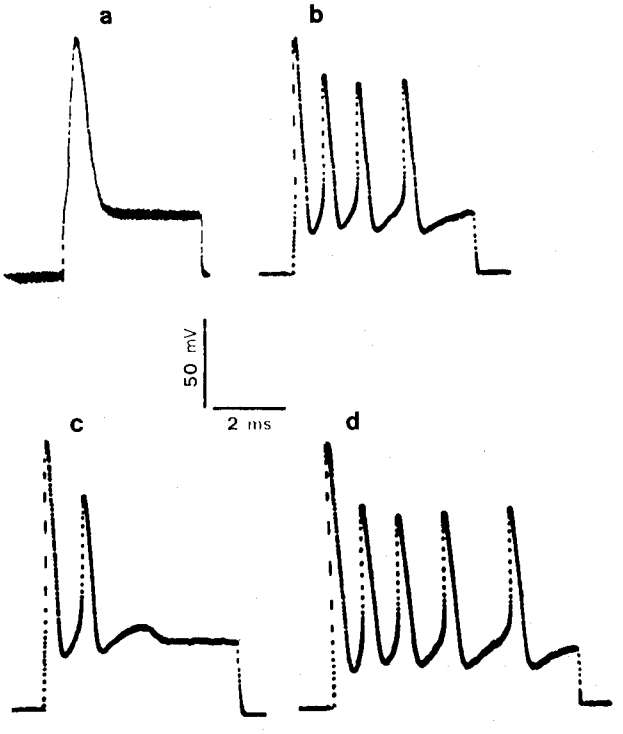

Figure 1. Effects of bradykinin on action potential recorded from single myelinated nerve fibers. Control action potentials in Ringer solution from motor (a) and sensory fibers (c). Action potentials recorded 3–5 min after the addition of 10 μM bradykinin to Ringer solution (b and d).

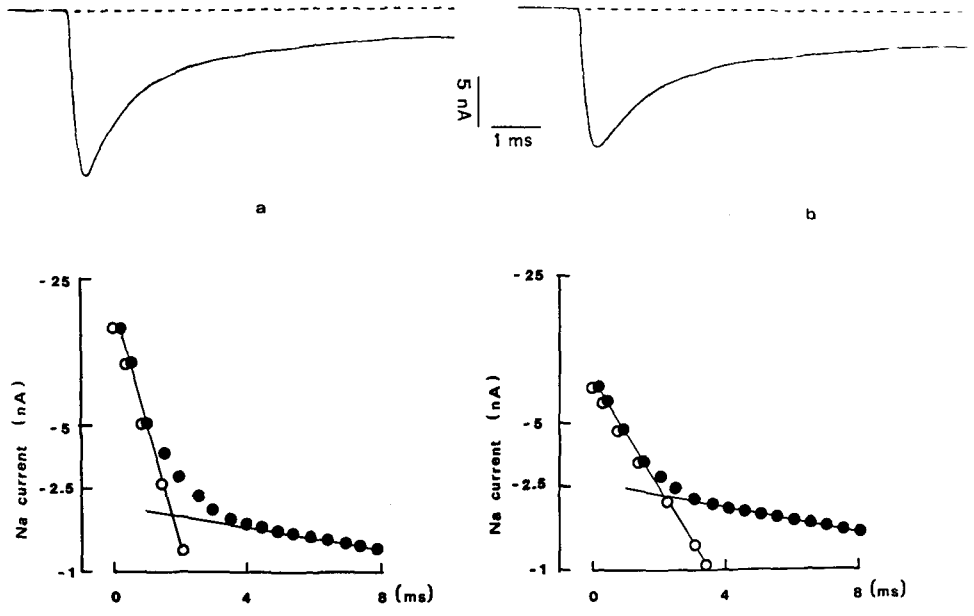

Figure 2. Effects of bradykinin on the time course of Na current. Traces of Na current (upper traces) and representations on semilogarithmic coordinates of the inactivation phases (lower panels) under control conditions (a) and in the presence of 10 μM bradykinin (b). Na current was recorded during depolarizations to 0 mV preceded by 50 ms hyperpolarizations to −120 mV. In lower panels, (●) gives the total current and (○) gives the fast phase of inactivation after subtraction of the slow phase of inactivation.

long-lasting pulses (25 ms) showed repetitive activity, which did not occur in motor fibers. Synthetic bradykinin (10 μM) raised the frequency of repetitive firing in sensory fibers and evoked repetitive activity in motor fibers (fig. 1 b, d); moreover it produced a slight increase in the duration of the action potential due to the prolongation of the repolarizing phase.

Bradykinin slows the inactivation process of sodium channels. Under voltage-clamp conditions the most immediate effect of bradykinin on the sodium current was the appearance of a persistent current during 25-ms test pulses (fig. 2 b). Control sodium currents in the untreated node had nearly decayed to the baseline level at the end of this depolarization. The magnitude of this maintained current increased progressively during continued bradykinin exposure and was not significantly dependent on the concentration. In addition, the sodium current was inactivated more slowly following bradykinin treatment. After 20 min of bradykinin treatment, the half-time for inactivation of the sodium current was at least twofold longer than that for the control current. However, as in normal fibers [6] the bradykinin-modified inactivation kinetics could still be satisfactorily described as the sum of two exponential functions (fig. 2 b). Moreover, external bradykinin produced a dose-dependent reduction of peak sodium current (fig. 3 A).

Voltage dependence of the bradykinin modified sodium channel. Sodium currents at different membrane voltages were compared in normal and bradykinin-treated fibers. The peak current-voltage relationships before and after bradykinin are shown in figure 3 A. The voltage-dependence of the peak sodium current in bradykinin-treated

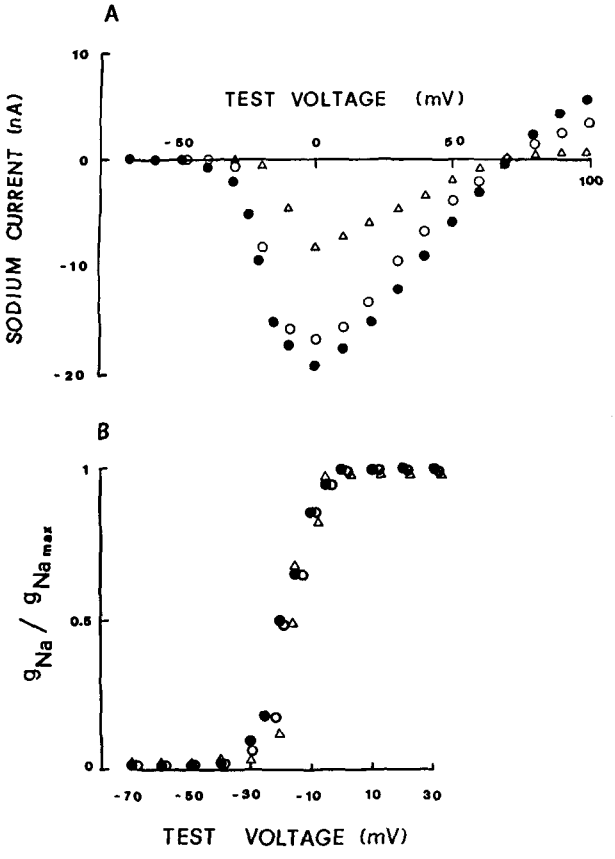

Figure 3. Effects of bradykinin on the voltage dependence of the activation of Na current. *A* Peak Na current-voltage curves and *B* peak Na conductance-voltage curve. Na current was recorded under control conditions (●), and in the presence of 10 μM (○) or 100 μM (△) bradykinin during depolarizations of various amplitudes preceded by 50-ms hyperpolarizations to −120 mV.

fibers was very similar to that of untreated fibers; the sodium channels were activated beginning at a membrane potential near − 45 mV and the reversal potentials were almost identical. In figure 3 B conductances were calculated from peak sodium current-voltage curves according to the equation:

$$g_{Na} = I/(V - V_{eq}) \quad (1)$$

where g is the conductance, I is the peak sodium current amplitude, V is the test voltage and V_{eq} is the equilibrium potential of sodium ions corresponding to the reversal potential of the peak sodium current. The rising branches of these curves almost coincide, indicating that the activation process of sodium channels was hardly affected by bradykinin.

Shift of voltage dependence of steady state channel inactivation. Bradykinin not only modified the time course of sodium channel inactivation, it also modified the voltage-dependence of the stationary sodium channel inactivation (h ∞). The parameter was measured by applying 50-ms prepulses to various membrane potentials followed directly by a standard test pulse. In normal nodes sodium channels are inactivated by increasingly more positive conditioning prepulses. This voltage dependence was modified by bradykinin, resulting in a shift of the h ∞ curve in the depolarizing direction by ~10 mV (fig. 4).

Frequency dependent block of sodium-current by internally applied bradykinin. The internodes of a single myelinated fibers were cut in a solution containing 120 mM caesium chloride and 10 µM bradykinin. Under such conditions, a small maintained current appeared during a prolonged depolarization after 20 min; this current, however, never exceeded 10% of the total current. When the membrane was stimulated at a low frequency (0.2 Hz), the block of the sodium current (I_{Na}) reached about 25% after 30 min; when the fiber was stimulated at a frequency of 10 Hz, the block of I_{Na} increased (fig. 5). Figure 5 shows superimposed I_{Na} traces recorded during 10 depolarizations to 0 mV applied at a frequency of

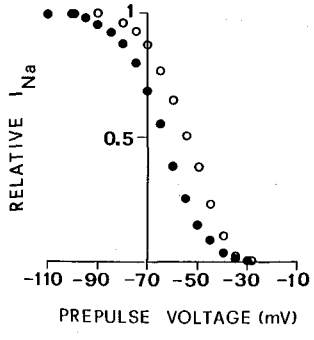

Figure 4. Effects of bradykinin on the steady-state inactivation-voltage curve of Na current. Na current was recorded under control conditions (●) and in the presence of 10 µM bradykinin (○), during depolarizations to 0 mV preceded by 50-ms pulses of various amplitudes. Peak Na current was normalized to its respective value at large hyperpolarizations and plotted against prepulse voltage.

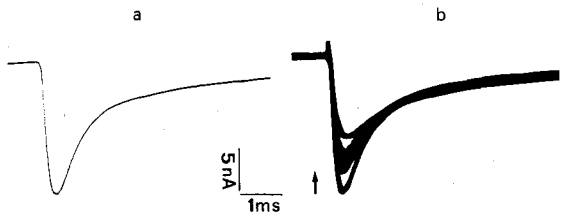

Figure 5. Frequency-dependent block of Na current by internally applied bradykinin. Superimposed traces of Na currents recorded during 10 depolarizations to 0 mV in control conditions (a) and 30 min after addition of 10 µM bradykinin (b) to the solution bathing the ends of the fiber. The arrow indicates the direction of peak Na current change.

10 Hz in control conditions (a) and after internal application of bradykinin (b); in control conditions, the amplitude of I_{Na} remained constant during the 10 pulses to 0 mV; after application of bradykinin the degree of block of peak sodium current was much larger. These results indicate that the degree of the sodium current block was frequency-dependent, since the block increased with repeated stimulation.

Discussion. In this report, evidence is presented that bradykinin, when applied externally, modifies the inactivation process of sodium channels in myelinated nerve fibers, prolongs the duration of action potentials and increases repetitive activity. Under voltage-clamp conditions, two primary effects on sodium current can be resolved: a maintained sodium current persisting during long lasting depolarization, and a shift of ~10 mV in the voltage dependence of sodium inactivation. Maintained sodium currents, referred to as non-inactivating or slowly inactivating sodium currents [12, 13], are unlikely to arise from a class of slowly opening sodium channels or by reopening of the inactivated sodium channels; it is more likely that they are due to slowing of the inactivation process. One can assume that bradykinin modifies a fraction of sodium channels to give rise to a non-inactivable late sodium current.

The magnitude of the positive shift of the voltage dependence of steady-state current inactivation is ~10 mV. It is well known that by increasing the external positive surface charge of the membrane, the voltage dependence of steady-state sodium current inactivation will be shifted in the depolarizing direction [14, 15]. Bradykinin is an octapeptide with the following sequence: Arg-Pro-Pro-Gly-Phe-Ser-Pro-Arg. Arginine residues bear a positive charge on the guanidinium moiety at pH 7.0 so that charge differences on the membrane surface could be introduced. Thus, the voltage shift could be due to 'non-specific' charge differences on the membrane surface. However, the fact that neither voltage dependence of sodium current activation nor the equilibrium potential for I_{Na} is shifted, argues that the shift of the voltage-dependence of steady-state current inactivation could be due also to a modification of a site on the sodium channel.

I_{Na} was largely blocked during high frequency stimulation with bradykinin diffusing into the axoplasm from the two ends of the fiber. In many respects the effects of internally applied bradykinin resemble those of leu- and met-enkephalins[16] and those of quaternary derivatives of the local anesthetics lidocaine and trimecaine[17,18]. One interpretation of the internal bradykinin effect is that during depolarizations, the molecules would bind to a site located in the neighborhood of the sodium channel and then immobilize the sodium channel gate (related to inactivation of the sodium permeability) leaving the channel in a closed configuration. The fast sodium inactivation process can be eliminated by internal treatments with proteolytic enzymes[19], which suggests that the 'h' gate is composed of a polypeptidic chain. It is tempting to postulate that bradykinin molecules enter the sodium channels from their axoplasmic end and bind to the receptors for the 'h' gate. The results of Eaton et al.[20] make the amino acid arginine a likely component of the sodium inactivation gate; bradykinin has two arginine residues which could bind to the receptor for the 'h' gate. However, since enkephalins have no arginine residue, we postulate that enkephalins would bind to the receptor for the 'h' gate by another amino acid, the most serious candidate being phenylalanine[19]. Bradykinin has phenylalanine residue too; therefore, more than one residue may be involved in the interaction between bradykinin molecules and sodium channels; the positively charged residues could interact with a site located at or near the extracellular surface of sodium channels whereas the neutral residues could react with a site within the membrane and on the cytoplasmic surface.

The slowing of I_{Na} inactivation and the increase of late current could account for the exciting activity of bradykinin, which can form aggregates, producing polypeptidic neurotoxins acting as 'modifiers' of the sodium channel gate and promoting excitation of axons.

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Inhibition of temperature-induced spermatogenic proliferation by a brain factor in hibernating *Helix aspersa* (Mollusca)

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Summary. Ablation of the brain from hibernating *Helix aspersa* maintained at 25 °C causes a significant increase in the proliferation of male cells in the gonad, whereas the ablation of the optic tentacles has no effect. The brain, therefore, produces a factor which specifically inhibits the multiplication of spermatogonia and spermatocytes.
Key words. Spermatogenesis; temperature; brain; hibernation; *Helix aspersa.*

Hibernation is a mode of biological adaptation of animals exposed to unfavorable environmental conditions. Normally the pulmonate snail *Helix aspersa* hibernates for five months during fall-winter. When snails in their first few months of hibernation are transferred to optimal conditions which would normally permit them to reproduce[1], they fail to do so[2]. It seems that a refractory period prior to reproduction exists in hibernating snails. Our interest centers on the role of the cephalic neuroendocrine organs of snails (brain and optic tentacles) in the