Effects of potassium channel-blocking agents on spontaneous discharges from neuromas in rats

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Thirty-five Sprague-Dawley rats with saphenous neuromas underwent acute microfilament recording in the proximal nerve. The effect of the potassium channel-blocking agents, tetraethylammonium bromide (TEA) and 4-aminopyridine, on spontaneous activity in A fibers terminating in the neuroma was observed. The effects of gallamine were also tested. Of the two channel-blocking agents, TEA reliably increased spontaneous firing in active fibers and initiated spontaneous activity in some fibers with no spontaneous baseline discharge. 4-Aminopyridine had no effect on baseline activity of either spontaneously active or quiescent fibers; however, it inhibited spontaneous activity induced by prior TEA treatment. Gallamine application produced effects similar to TEA in that spontaneous activity was dramatically increased. These results imply that a tonic potassium conductance is present in regenerating fibers in the neuroma and that this conductance moderates the tendency toward hyperexcitability and spontaneous firing. Spontaneous activity in nociceptive afferent fibers may represent the mechanism of chronic pain and paresthesias that often accompany peripheral nerve injury. These results suggest that agents which either increase potassium conductance or selectively inhibit the sodium current in regenerating axons might be effective in the treatment of these chronic pain syndromes.

Key Words • neuroma • peripheral nerve • pain • gallamine • potassium channel blocker

Damage to the peripheral nervous system can result in a sensorimotor deficit, and often in chronic dysesthesias and pain. Currently, loss of neurological function is thought to be a consequence of the failure of normal action potential propagation due to demyelinating, toxic, metabolic, or traumatic nerve injury. However, considerable evidence now indicates that the chronically painful states that develop after nerve injury may arise from abnormal neural hyperactivity rather than from inactivity. For example, experimental studies have shown that demyelinated axons and the regenerating neurites that are found in end-bulb neuromas exhibit properties of spontaneous action potential generation, action potential reflection, mechanosensitivity, and chemosensitivity to alpha-agonists. 3-6,10,14,18,21,24,27,28,30 Furthermore, after peripheral nerve injury, cells of the contiguous dorsal root ganglion, which are ordinarily silent in the absence of afferent fiber activation, exhibit spontaneous discharges. 3,4,10,16,25 Abnormal firing from these sites has been demonstrated to involve large myelinated (A-alpha) as well as small myelinated (A-delta) fibers and unmyelinated (C fiber) axons. The A-delta and C fibers are known to mediate nociception, and thus spontaneous impulse generation in these axons may conceivably be relevant to the genesis of chronic pain syndromes that often accompany nerve injury.

More direct evidence of an association between abnormal spontaneous action potential generation in damaged nerve and a behavioral pain syndrome has been provided by Wall, et al. 26-29 Although the etiology of this apparent behavior in response to pain is unclear, 23 these investigations have shown that, in the albino rat, the time course of the abnormal ongoing discharge from an experimentally produced neuroma parallels the observation of putative evidence of dysesthesias in the denervated segment (autotomy). Moreover, agents that reduce the amount of spontaneous axon discharge, such as guanethidine 29 or phenytoin, 13 also appear to reduce the behavioral manifestations of chronic pain in these animals. Further evidence to support the association of ectopic action potential generation and painful sensations has been produced by Ochoa and Torebjörk, 19 who have shown that spontaneous discharges in sensory fibers accompany postischemic paresthesias in awake human subjects.
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The ionic basis of this axonal hyperexcitability is not understood at present. However, it has recently been shown that the membrane that underlies perinodal myelin has significant potassium conductance, a feature shared by the axolemma of regenerating sprouts. Although potassium conductance is not thought to play a role in normal mammalian axonal conduction, the development of a potassium conductance in hyperexcitable axolemma might act to reduce ectopic impulse generation. Conversely, blockade of potassium conductance would be expected to increase neuroma excitability. The purpose of our present experiments was to test this hypothesis, and to determine the role of potassium conductance in the generation of spontaneous firing in neuromas.

Materials and Methods

The methods used in these experiments have been presented in detail elsewhere. Forty-five animals were anesthetized with pentobarbital (50 mg/kg intraperitoneally). The right saphenous nerve was surgically exposed in the medial thigh, and then was ligated and divided. The other five animals underwent acute recording experiments only, and served as controls.

One week following surgery, the animals in the experimental group were again anesthetized, tracheotomy and femoral venous cannulation were performed, and the saphenous nerve was reexposed. Temperature was controlled at 38°C ± 0.5°C by radiant heat, and the nerve was covered with mineral oil. The nerve was divided as far proximally in the thigh as possible, and a bipolar stimulating electrode was placed on the nerve over the neuroma. Microfilament recordings were carried out on the proximal end of the isolated nerve segment. Animals breathed spontaneously during recording until gallamine was administered, at which point mechanical ventilation was instituted and supplemental oxygen was given to maintain a PaCO2 of 35 to 40 torr and a PaO2 of over 100 torr.

Action potential data were fed from a Grass P511 amplifier to Tektronix oscilloscopes, Models 5113 and 565. The Schmidt trigger of the “B” side of the Model 565 oscilloscope was set to trigger on the action potentials of interest, and the B gate output was fed to a Nicolet computer† to generate a firing-rate plot.

Drugs were administered either intravenously or topically on the neuroma. Tetraethylammonium bromide (TEA), 10 mM, and 4-aminopyridine (4-AP) solutions, 0.01, 0.05, 0.1, 0.5, 1.0, 5.0, and 10.0 mM, in sterile 0.9% saline were used, as well as the commercially available solution of gallamine (Flaxedil), 20 mg/cc.

Results

Figure 1 shows a typical discharge pattern of an A-alpha fiber that responded to mechanical stimulation of the neuroma. A single microfilament containing one or, at most, two spontaneously active axons was used in each experimental animal. One fiber recording per animal was necessitated by the generalized effects of either systemic or topical application of the agents used on the whole neuroma. Isolated fiber conduction velocities ranged from 6 to 73 m/sec (A-alpha to A-delta). No unmyelinated fibers were tested during these studies.

These experiments are largely derived from an early observation that gallamine, used to afford muscle paralysis in our animals, produced a marked increase in spontaneous activity recorded from neuromas. In five of 35 rats with neuromas, no spontaneous activity could be recorded in the nerve, even with a meticulous search for active fibers. After administration of either gallamine or TEA, spontaneous activity became abundant. In the six rats with neuromas that were tested, either intravenous or topical application of 2 to 3 mg TEA reliably increased the basal rate of discharge of active fibers, and often a quiescent microfilament would develop several spontaneously discharging fibers after TEA application. After 2 to 10 mg TEA was applied, firing rates typically increased to approximately 50 to 75 Hz, and often doublet and triplet spiking was produced with firing rates in the range of 150 to 250 Hz (Fig. 2 upper). Gallamine, 4 to 20 mg, produced effects similar to TEA in 12 of 13 rats with neuromas (Fig. 2).

The hyperexcitability produced by gallamine appeared to be additive to that produced by TEA, and on a molar basis the two agents were equally potent in producing increased spontaneous activity.

In contrast to results with TEA, topical or systemic application of 4-AP (200 µl, 0.01 to 10.0 mM) alone produced no change in the firing rate of neuroma fibers. At first, this was quite disturbing to us since 4-AP and TEA are thought to block potassium conductance by similar mechanisms. Further experiments revealed that,
in all 20 rats with neuromas that were tested, 4-AP actually showed a dose-related antagonization of the excitatory effects of TEA and gallamine (Fig. 2 lower). This effect was highly reproducible, even after multiple injections of TEA and gallamine. After gallamine or TEA administration, the effect of topical 4-AP application on spontaneous firing from the neuroma was often quite profound, in that 50 to 100 μl of 5 mM 4-AP applied to the neuroma produced a virtually immediate cessation of the spontaneous discharge (Fig. 3). Interestingly, 4-AP had no effect on the high-frequency discharges that can be recorded from the neuroma during periods of induced hypoxia.4

In five rats, systemic or topical application of TEA, gallamine, and 4-AP to otherwise normal nerves after acute transection produced no change in the uniformly silent recordings from these filaments. Thus, spontaneous firing and the effects of these agents appeared to be dependent upon the presence of a neuroma.

Discussion

Blockade of axoplasmic transport in a transected nerve reduces the ectopic excitability of the axons in the end-bulb neuroma.12 Sodium channel constituents are probably produced in the cell soma and exported to the regenerating neurite, where they are incorporated in large numbers.20 Potassium channels are also likely to be involved in this process of transport and incorporation into axonal sprouts. Our results indicate that potassium conductance appears to mediate the hyperexcitability of axons in neuromas. Blockade of an outward potassium current with a continued tonic inward sodium current would permit spontaneous depolarization of the membrane, ectopic spike initiation, and increased spontaneous firing. Our findings of increased spontaneous firing in neuroma fibers but no change in normal fibers exposed to TEA are consistent with the hypothesis that a potassium current develops in neuroma fibers, and that this conductance inhibits the generation of abnormal spontaneous activity.

Our conclusions are corroborated by the work of Devor,11 with some important differences. Contrary to his study, we have found that 4-AP does not have an excitatory effect on spontaneous firing from the neuroma. In fact, 4-AP appears to antagonize the excitatory effects of TEA. This implies that, while 4-AP and TEA both inhibit potassium conductance in normal unmyelinated or demyelinated axons, their mechanisms of action may differ. Existing evidence does, in fact, indicate that their modes of action differ in several respects.1

Our results also suggest that potassium channels in neuroma fibers may have unique functional characteristics, since the effects of TEA and 4-AP are similar in unmyelinated and demyelinated axons1 but differ in neuroma fibers. Further work on the biophysical properties of potassium channels in regenerating axons will be necessary to establish this.

If spontaneous impulse generation in nociceptive afferent fibers is the physiological correlate of chronic pain or paresthesias resulting from peripheral nerve injury, then inhibition of this activity should alleviate the pain syndrome. On the basis of the present study, agents which either increase the potassium conductance in regenerating axons or selectively inhibit the sodium
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current would be predicted to decrease ectopic impulse generation and, thus, the accompanying painful sensation.

Finally, we have found that gallamine, a commonly used paralyzing agent in animal research, has potent excitatory properties in neuroma axons. This is almost certainly due to the well established TEA-like properties of gallamine.²³ Our results suggest the appropriateness of a quantitative, if not qualitative, reevaluation of previous data on spontaneous impulse generation from experimental neuromas and demyelinating injuries in animals treated with gallamine.

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