Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 7645–7649, July 1999
Colloquium Paper

This paper was presented at the National Academy of Sciences colloquium "The Neurobiology of Pain," held December 11–13, 1998, at the Arnold and Mabel Beckman Center in Irvine, CA.

Tetrodotoxin-resistant Na^+ currents and inflammatory hyperalgesia

MICHAEL S. GOLD*

Department of Oral and Craniofacial Biological Sciences, University of Maryland Dental School, 666 West Baltimore Street, Baltimore, MD 21201

ABSTRACT **Several mechanisms have been identified that may underlie inflammation-induced sensitization of high-threshold primary afferent neurons, including the modulation of voltage- and Ca^{2+}-dependent ion channels and ion channels responsible for the production of generator potentials. One such mechanism that has recently received a lot of attention is the modulation of a tetrodotoxin (TTX)-resistant voltage-gated Na^+ current. Evidence supporting a role for TTX-resistant Na^+ currents in the sensitization of primary afferent neurons and inflammatory hyperalgesia is reviewed. Such evidence is derived from studies on the distribution of TTX-resistant Na^+ currents among primary afferent neurons and other tissues of the body that suggest that these currents are expressed only in a subpopulation of primary afferent neurons that are likely to be involved in nociception. Data from studies on the biophysical properties of these currents suggest that they are ideally suited to mediate the repetitive discharge associated with prolonged membrane depolarizations. Data from studies on the effects of inflammatory mediators and antinociceptive agents on TTX-resistant Na^+ currents suggest that modulation of these currents is an underlying mechanism of primary afferent neuron sensitization. In addition, the second-messenger pathways underlying inflammatory mediator-induced modulation of these currents appear to underlie inflammatory mediator-induced hyperalgesia. Finally, recent antisense studies have also yielded data supporting a role for TTX-resistant Na^+ currents in inflammatory hyperalgesia. Although data from these studies are compelling, data presented at the Neurobiology of Pain colloquium raised a number of interesting questions regarding the role of TTX-resistant Na^+ currents in inflammatory hyperalgesia; implications of three of these questions are discussed.**

Hyperalgesia that develops in the presence of tissue injury or inflammation reflects, at least in part, an increase in the excitability of high-threshold primary afferent neurons innervating the site of injury. The increase in afferent excitability, or sensitization, develops within minutes of an inflammatory stimulus and involves a leftward shift in neuronal stimulus response function and/or an increase in spontaneous activity. The relatively rapid development of sensitization in response to inflammatory stimuli is likely to reflect the modulation of proteins within or around the afferent terminal. In contrast, a change in the expression of protein(s) appears to be involved in afferent sensitization observed in the presence of ongoing inflammation or nerve injury (see accompanying papers). At least three underlying mechanisms have been identified that may contribute to the initial phase of inflammation-induced afferent sensitization: (*i*) a change in the compliance of the tissue surrounding the afferent terminal (1); (*ii*) a change in efficacy of a transducer(s) within the afferent terminal (2–4); and (*iii*) a change in a voltage- or Ca^{2+}-dependent current within the afferent terminal (5–8). Because inflammatory mediators may sensitize sensory neurons *in vitro* to stimuli that presumably bypass the afferent transduction apparati [i.e., high extracellular potassium or current injection through a recording electrode (9, 10)], with no consistent changes either in resting membrane conductance (7) or potential (10), modulation of a voltage- or Ca^{2+}-dependent current must contribute to the sensitization of primary afferent neurons. We have focused this review on the contribution of a particular class of voltage-gated Na^+ currents (VGSCs), namely tetrodotoxin (TTX)-resistant voltage-gated Na^+ currents, to changes in afferent excitability.

Why Focus on Na^+ Channels?

Although a number of distinct voltage- and Ca^{2+}-dependent currents have been identified that may underlie inflammation-induced changes in afferent excitability (for review, see ref. 11), we have focused on the role of VGSCs for several reasons. First, VGSC activation is critical for the generation and propagation of neuronal action potentials. Second, there is a growing body of evidence indicating that modulation of these currents is an endogenous mechanism used to control neuronal excitability (8, 12–15). Third, evidence from injury in experimental animals (16–19) and humans (20, 21) suggests that therapeutic interventions with compounds known to block Na^+ channels may be effective for the treatment of hyperalgesia and pain.

Why TTX-Resistant Channels?

Distribution. Evidence for the selective distribution of unique VGSC(s) among sensory neurons has been obtained *in vivo* and *in vitro.* Intracellular recording from the cell bodies of sensory neurons *in vivo* indicated that the somal action potential of high-threshold receptors is resistant to tetrodotoxin (TTX) at concentrations as high as 200 μM applied to the surface of the ganglion (22). Similar results were obtained with intracellular recording from intact ganglia *in vitro*, where it was observed that TTX-resistant action potentials were present in neurons with slow-conducting axons (i.e., neurons likely to be associated with high-threshold receptors) (23). Electrophysiological studies on dissociated sensory neurons have demonstrated that while TTX-sensitive Na^+ currents are distributed throughout the population of spinal sensory neurons, TTX-resistant Na^+ currents are primarily restricted to a subpopulation of sensory neurons likely to be involved in nociception (8, 24–28). Specifically, TTX-resistant Na^+ currents are present primarily in neurons that have a small cell-body diameter (these are the neurons that tend to give rise to

Abbreviations: PGE_2, prostaglandin E_2; NGF, nerve growth factor; ODN, oligodeoxynucleotide; PKA, protein kinase A; TTX, tetrodotoxin; VGSC, voltage-gated sodium current.

*To whom reprint requests should be addressed. e-mail: msg001@dental.umaryland.edu.

PNAS is available online at www.pnas.org.

small-diameter slow-conducting axons) and that are responsive to the algogenic compound capsaicin. Of note, a TTX-insensitive Na^+ current (i.e., a current blocked by TTX at concentrations between 500 nM and 1 μM) had been described in other tissues (29–31). However, the biophysical properties of TTX-insensitive currents appears to differ from those of TTX-resistant currents (29, 31).

Identification of a gene encoding a TTX-resistant Na^+ channel confirmed the electrophysiological data indicating the existence of unique Na^+ currents in a subpopulation of primary afferent neurons. The first TTX-resistant Na^+ channel cloned, referred to as SNS (32), PN3 (33), and subsequently ScN10 (34), is only present in primary afferent neurons, in particular, a subpopulation of primary afferent neurons with small-diameter cell bodies. Heterologous expression of SNS/PN3 indicated that this clone encodes a voltage-gated Na^+ channel with biophysical properties similar to those of the TTX-resistant channels present in sensory neurons (32, 33). A second TTX-resistant Na^+ channel, referred to as NaN (35) or SNS2 (36), recently cloned from sensory neurons is also present in a subpopulation of sensory neurons with a small-diameter cell body.

Biophysical Properties. At least three distinct TTX-resistant Na^+ currents have been electrophysiologically isolated in rat primary afferent neurons (37, 38). The first TTX-resistant currents to be described (25–28, 39, 40) had several unique features. First, these TTX-resistant currents have high thresholds for activation relative to TTX-sensitive currents (26, 27, 38). If the channels underlying these currents are present in the peripheral terminals of primary afferent neurons and if spike initiation involves activation of these channels, then the activation properties of these channels may explain why afferent neurons responsive to noxious stimuli have high thresholds for activation. Second, TTX-resistant currents have high thresholds for steady-state inactivation relative to TTX-sensitive currents (26, 27, 38). As a result, the majority of TTX-resistant channels are available for activation at membrane potentials as high as -40 mV (27). Consequently, it has been suggested that activation of these channels contributes to ongoing activity observed in the presence of a sustained depolarization of primary afferent neurons (38). Third, TTX-resistant currents recover from inactivation rapidly relative to TTX-sensitive currents (refs. 26 and 38, but see ref. 27). Rapid recovery from inactivation is another factor that would enable TTX-resistant currents to underlie sustained spiking in response to prolonged depolarizations (26, 41). Fourth, the inactivation rates for TTX-resistant Na^+ currents are considerably slower than those of TTX-sensitive currents (38). This is particularly true at membrane potentials close to the activation potential for TTX-resistant currents. Consequently, membrane depolarization may be facilitated after the activation of a sustained inward current carried by TTX-resistant Na^+ channels. The slow inactivation rate of TTX-resistant Na^+ currents also contributes to the broad action potential typically observed in high-threshold primary afferent neurons (41). Thus, the biophysical properties of the first and most widely studied TTX-resistant Na^+ currents are such that these currents may play a critical role in the determination of the excitability of the afferent neurons in which they are present.

The biophysical properties of recently described TTX-resistant Na^+ currents (37, 38) more closely resemble TTX-sensitive Na^+ currents than the TTX-resistant Na^+ currents described above. For example, a second and third TTX-resistant Na^+ current characterized by Rush *et al.* (38) activated and inactivated at relatively hyperpolarized membrane potentials; the third current inactivated at membrane potentials even more negative than those observed for TTX-sensitive Na^+ currents. Of note, the inactivation rate of all three TTX-resistant Na^+ currents described by Rush *et al.* was relatively slow compared with that of TTX-sensitive Na^+ currents. In contrast, a second TTX-resistant Na^+ current described by Sholz *et al.* (37) displayed both rapid kinetics for activation and inactivation *and* a voltage dependence of activation and inactivation that occurred over relatively hyperpolarized membrane potentials. The role these additional TTX-resistant Na^+ currents play in regulating the excitability of high-threshold primary afferent neurons has yet to be determined.

Effects of Inflammatory Mediators. The distribution and biophysical properties of the classically described TTX-resistant Na^+ currents suggests that these currents are involved in the control of the excitability of primary afferent neurons. Furthermore, several inflammatory mediators released in response to injury are capable of directly sensitizing subpopulations of primary afferent neurons (9, 10, 14, 42, 43). Therefore, we hypothesized that an inflammatory mediator-induced modulation of TTX-resistant Na^+ currents is a mechanism underlying the sensitization of primary afferent neurons. In support of this hypothesis, we observed that directly acting hyperalgesic inflammatory mediators such as prostaglandin E_2 (PGE_2), serotonin, and adenosine decrease the activation threshold, increase the rates of activation and inactivation, and increase the magnitude of TTX-resistant Na^+ current (8). These changes could contribute to both the decrease in threshold and increase in the number of action potentials evoked from a sensitized neuron. Further support of our hypothesis is provided by the observation that the time course of inflammatory mediator-induced modulation of TTX-resistant Na^+ currents [developing within seconds and attaining a maximum within minutes (8)] mirrors the time course of the development of hyperalgesia in response to a peripheral injection of directly acting inflammatory mediators (44). Similar observations have subsequently been reported by other investigators (14, 45).

Effects of Antinociceptive Agents. Levine and Taiwo (46) previously observed that the peripheral administration of μ-opioid receptor agonists blocked inflammatory mediator-induced hyperalgesia. Therefore, we hypothesized that if inflammatory mediator-induced modulation of TTX-resistant Na^+ currents is an underlying mechanism of inflammatory hyperalgesia, then μ-opioid receptor agonists should block inflammatory mediator-induced modulation of current. Consistent with this hypothesis, we observed that a μ-opioid receptor agonist blocked PGE_2-induced modulation of the current (47).

Second-Messenger Pathways. In a final series of experiments designed to test the hypothesis that inflammatory mediator-induced modulation of TTX-resistant Na^+ currents is an underlying mechanism of inflammatory hyperalgesia, we attempted to determine the role of protein kinase A (PKA) in PGE_2-induced modulation of the current. Previous studies performed *in vivo* suggested that direct acting inflammatory mediators, including PGE_2, serotonin, and adenosine, produced hyperalgesia (48–52) and afferent sensitization (53) via the activation of a cAMP/PKA second-messenger cascade. The effects of these mediators were mimicked by compounds that increase the intracellular concentration of cAMP, prolonged by agents that blocked the breakdown of cAMP, and blocked by agents that inhibit adenylate cyclase and/or PKA.

Consistent with our hypothesis, England *et al.* (14) reported that PGE_2-induced modulation of the TTX-resistant Na^+ currents involved activation of a cAMP/PKA second-messenger pathway. However, these experiments were performed on primary afferent neurons from neonatal rats, and there are several lines of evidence suggesting primary afferent neurons from neonates may be qualitatively and quantitatively different than neurons from adults (39, 54–56). Furthermore, after failing to detect an effect with a membrane-permeable analog of cAMP on TTX-resistant Na^+ currents in primary afferent neurons from adult rats, Cardenas *et al.* (45) were

forced to conclude that modulation of the current must involve activation of another second-messenger pathway. We have recently reported (57) that although an increase in the intracellular concentration of cAMP may result in the modulation of TTX-resistant Na^+ current, the dose–response relationship for such manipulations is bell-shaped. This observation may explain, at least in part, differences between the observations of England *et al.* and those of Cardenas *et al*. It should be noted that a recent study involving heterologous expression and site directed mutagenesis of the cloned TTX-resistant Na^+ channel, SNS/PN3, indicates that the channel is phosphorylated after activation of PKA (58). Furthermore, PKA-induced phosphorylation of the channel results in changes in gating properties similar to those induced by inflammatory mediators (58), suggesting that inflammatory mediator-induced modulation of TTX-resistant currents reflects a direct phosphorylation of the underlying channel(s). Importantly, and more to the point, our recent results (57) strongly support the suggestion that PGE_2-induced modulation of TTX-resistant Na^+ currents in primary afferent neurons involves PKA activation.

The Function of TTX-Resistant Na^+ Currents in Peripheral Terminals. Results obtained through the study of primary afferent neuron cell bodies *in vitro* has provided compelling evidence in support of the hypothesis that modulation of TTX-resistant Na^+ currents is an underlying mechanism of inflammatory hyperalgesia. However, it is critical to determine whether these currents contribute to inflammatory hyperalgesia *in vivo*. TTX-resistant Na^+ currents are clearly present in the DRG cell body *in vivo* (22). Furthermore, results from at least two studies suggest that these currents are present in the central terminals of primary afferent neurons (59, 60). There also is evidence that TTX-resistant Na^+ currents are present in peripheral axons (61), but given that axonal conduction is blocked with TTX (22, 23), the function of TTX-resistant Na^+ current in the axon has yet to be determined. Importantly, Brock *et al.* (62) have recently obtained evidence suggesting that TTX-resistant Na^+ channels play a role in action potential generation in the peripheral terminals of corneal afferent neurons. Consistent with observations made while recording from cell bodies (22, 23), recording from the peripheral terminals revealed that electrical stimulation of the nerve trunk evoked action potentials that were blocked by TTX whereas spontaneous or naturally evoked (with pressure or capsaicin) action potential persisted in the presence of TTX (62).

Although these recent results support the suggestion that TTX-resistant currents are present and functional in the peripheral terminals of primary afferent neurons, they do not address the question of whether modulation of these currents contributes to inflammatory hyperalgesia. Given the lack of specific pharmacological agents with which to manipulate TTX-resistant Na^+ currents, it is not possible to address this issue with traditional pharmacological approaches. However, through the use of antisense oligodeoxynucleotides (ODNs) to selectively knock down expression of protein encoded by targeted mRNA (63), it has become possible to study the function of specific proteins. Furthermore, we (64), and others (65) had previously demonstrated that the intrathecal administration of ODNs could be used to knock down expression of proteins present in the peripheral terminals of primary afferent neurons. Therefore, we generated antisense ODNs to a unique region of the cloned TTX-resistant Na^+ channel, PN3/SNS, and assessed the effects of intrathecal ODN administration on PGE_2-induced hyperalgesia (66). Our results indicated that antisense, but not control, ODN sequences produced a small but significant increase in baseline threshold to mechanical nociceptive stimuli, suggesting that activity in a TTX-resistant current contributes to the determination of mechanical threshold. More importantly, antisense, but not control ODN sequences, resulted in a significant reduction in PGE_2-induced hyperalgesia. This observation is consistent with the electrophysiological data indicating that functional TTX-resistant Na^+ channels are present in the peripheral terminals of primary afferent neurons. Furthermore, it supports the hypothesis that modulation of a TTX-resistant Na^+ current is an underlying mechanism of inflammatory hyperalgesia.

Questions Concerning the Role of TTX-Resistant Na^+ Currents in Inflammatory Hyperalgesia. There are a number of questions that remain to be answered concerning the role of TTX-resistant Na^+ currents in inflammatory pain. At least three of these deserve comment in light of data presented at the Neurobiology of Pain colloquium. First, what is the function of the second TTX-resistant Na^+ current (NAN/SNS2) cloned from spinal sensory neurons? Expression of the channel appears restricted to primary afferent neurons with the smallest cell-body diameter in a subpopulation of neurons expressing PN3/SNS (36). The biophysical properties of the NAN/SNS2 expressed in HEK292 cells appear to more closely resemble TTX-sensitive Na^+ currents (i.e., with faster activation and inactivation kinetics), although these properties may not be reflective of the properties of the channel expressed in native tissue. Because there is no homology between PN3/SNS and NaN/SNS2 in the region we targeted with our antisense ODN, it is unlikely that our results with antisense ODNs reflect knock-down of both channels. Consequently, the residual PGE_2-induced hyperalgesia we observed after PN3/SNS antisense ODN administration may reflect an effect of PGE_2 on NaN/SNS2. Porreca *et al.* (67) have recently obtained data suggesting that NaN/SNS2 is not involved in either the establishment of nociceptive thresholds in control animals or in the maintenance of hyperalgesia and allodynia in a neuropathy model. However, these investigators did not investigate the role of this channel in inflammatory hyperalgesia. Thus, a role of NaN/SNS2 has yet to be determined.

The contribution of TTX-resistant Na^+ currents to nociceptive thresholds in uninjured tissue is a second question concerning the role of TTX-resistant Na^+ currents in inflammatory pain. Results from our antisense study suggest that these currents do contribute, to a limited extent, to the determination of mechanical nociceptive threshold (66). That the contribution of these currents to the determination of nociceptive threshold is small is supported by observations made by Porreca *et al.* (67). These investigators were able to clearly demonstrate a decrease in PN3/SNS protein in the cell bodies of primary afferent neurons and an attenuation of both inflammatory and neuropathic hyperalgesia, by using an antisense strategy similar to the one we used. However, antisense ODNs had no effect on baseline mechanical or thermal nociceptive thresholds. The small effect of antisense ODN treatment on baseline nociceptive threshold is striking in light of the observation that in the nociceptor cell body, TTX-resistant Na^+ current is the Na^+ current primarily responsible for action potential generation (14). The apparent difference between the role of TTX-resistant Na^+ current in the cell body and in the peripheral terminal suggests that either the current contributes little to the determination of baseline nociceptive threshold or activity in the population of TTX-resistant Na^+ current-containing afferent neurons contributes little to baseline nociceptive threshold. Although a single-unit electrophysiological study is necessary to distinguish between these possibilities, observations obtained with the neurotoxin capsaicin would suggest the latter. That is, rats treated neonatally with capsaicin to eliminate a vast majority of c-fiber afferent neurons have baseline nociceptive thresholds that are only slightly elevated. However, PGE_2-induced hyperalgesia is completely eliminated in these animals (68).

Third, there is the question as to why the administration of antisense ODNs directed against SNS/PN3 had no effect on carrageenan-induced hyperalgesia as observed by Porreca *et al.*

(67). The peripheral administration of carrageenan is used as a model of acute inflammation associated with hyperalgesia that develops within tens of minutes. In their carefully controlled study, Porreca *et al.* were able to demonstrate profound effects with antisense ODN treatment on hyperalgesia resulting from the peripheral administration of Freund's adjuvant. Like carrageenan, the peripheral administration of Freund's adjuvant is used as a model of inflammation, except the hyperalgesia associated with this model develops more slowly, over many hours. Our results suggest that modulation of SNS/PN3 or a highly homologous species, contributes to inflammatory mediator-induced hyperalgesia (66). Furthermore, carrageenan-induced hyperalgesia appears to involve the production and release of hyperalgesic inflammatory mediators (i.e., see ref. 69). Consequently, we would predict that carrageenan-induced hyperalgesia should be attenuated after SNS/PN3 antisense ODN administration. Identification of the reason(s) for the discrepancy between our predicted results and the observations of Porreca *et al.* appears to require further experimentation.

Conclusions

Pain is clearly a complex process involving considerably more than the modulation of a single class of ion channels resulting in changes in the excitability of a subpopulation of neurons. That the most effective analgesics available tend to have a wide spectrum of action at a number of sites throughout the nervous system is largely reflective of this fact. Nevertheless, the study of a single class of ion channels, TTX-resistant Na^+ channels, has increased our understanding of the neurobiology of pain. Furthermore, because of the restricted distribution of TTX-resistant Na^+ currents and the observation that a decrease in the expression of these currents has little impact on low-threshold mechanical transduction, targeting these currents may lead to the development of a therapeutic modality for the treatment of hyperalgesia with fewer side effects than currently available modalities.

I would like to thank Normal Capra for helpful comments regarding the manuscript. Some of the work described in this article was supported by grants from the National Institute of Health (Grant 1RO1NS3692901A1).

1. Cooper, B. (1993) *J. Neurophysiol.* **70,** 512–521.
2. Cesare, P. & McNaughton, P. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 15435–15439.
3. Reichling, D. B. & Levine, J. D. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 7006–7011.
4. Lopshire, J. C. & Nicol, G. D. (1997) *J. Neurophysiol.* **78,** 3154–3164.
5. Nicol, G. D., Vasko, M. R. & Evans, A. R. (1997) *J. Neurophysiol.* **77,** 167–176.
6. Weinreich, D. (1995) *Pulm. Pharmacol.* **8,** 173–179.
7. Gold, M. S., Shuster, M. J. & Levine, J. D. (1996) *Neurosci. Lett.* **205,** 161–164.
8. Gold, M. S., Reichling, D. B., Shuster, M. J. & Levine, J. D. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 1108–1112.
9. Baccaglini, P. I. & Hogan, P. G. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 594–598.
10. Gold, M. S., Dastmalchi, S. & Levine, J. D. (1996) *Neuroscience* **71,** 265–275.
11. Tanner, K. D., Gold, M. S., Reichling, D. B. & Levine, J. D. (1997) in *Molecular Neurobiology of Pain,* ed. Borsook, D. (Int. Assoc. Study of Pain, Seattle), Vol. 9, pp. 79–105.
12. Cantrell, A. R., Ma, J. Y., Scheuer, T. & Catterall, W. A. (1996) *Neuron* **16,** 1019–1026.
13. Cantrell, A. R., Smith, R. D., Goldin, A. L., Scheuer, T. & Catterall, W. A. (1997) *J. Neurosci.* **17,** 7330–7338.
14. England, S., Bevan, S. & Docherty, R. J. (1996) *J. Physiol. (London)* **495,** 429–440.
15. Ono, K., Fozzard, H. A. & Hanck, D. A. (1993) *Circ. Res.* **72,** 807–815.
16. Abram, S. E. & Yaksh, T. L. (1994) *Anesthesiology* **80,** 383–391.
17. Chabal, C., Russell, L. C. & Burchiel, K. J. (1989) *Pain* **38,** 333–338.
18. Puig, S. & Sorkin, L. S. (1996) *Pain* **64,** 345–355.
19. Devor, M., Wall, P. D. & Catalan, N. (1992) *Pain* **48,** 261–268.
20. Chabal, C., Jacobson, L., Russell, L. C. & Burchiel, K. J. (1992) *Pain* **49,** 9–12.
21. Rizzo, M. A. (1997) *J. Neurol. Sci.* **152,** 103–106.
22. Ritter, A. M. & Mendell, L. M. (1992) *J. Neurophysiol.* **68,** 2033–2041.
23. Villiere, V. & McLachlan, E. M. (1996) *J. Neurophysiol.* **76,** 1924–1941.
24. Arbuckle, J. B. & Docherty, R. J. (1995) *Neurosci. Lett.* **185,** 70–73.
25. Caffrey, J. M., Eng, D. L., Black, J. A., Waxman, S. G. & Kocsis, J. D. (1992) *Brain Res.* **592,** 283–297.
26. Elliott, A. A. & Elliott, J. R. (1993) *J. Physiol. (London)* **463,** 39–56.
27. Ogata, N. & Tatebayashi, H. (1993) *J. Physiol. (London)* **466,** 9–37.
28. Roy, M. L. & Narahashi, T. (1992) *J. Neurosci.* **12,** 2104–2111.
29. Frohnwieser, B., Weigl, L. & Schreibmayer, W. (1995) *Pflugers Arch.* **430,** 751–753.
30. Donahue, L. M. (1995) *Neurochem. Res.* **20,** 713–717.
31. Thio, C. L. & Sontheimer, H. (1993) *J. Neurosci.* **13,** 4889–4897.
32. Akopian, A. N., Sivilotti, L. & Wood, J. N. (1996) *Nature (London)* **379,** 257–262.
33. Sangameswaran, L., Delgado, S. G., Fish, L. M., Koch, B. D., Jakeman, L. B., Stewart, G. R., Sze, P., Hunter, J. C., Eglen, R. M. & Herman, R. C. (1996) *J. Biol. Chem.* **271,** 5953–5956.
34. Oaklander, A. L. & Belzberg, A. J. (1997) *Brain Res. Mol. Brain Res.* **52,** 162–165.
35. Dib-Hajj, S. D., Tyrrell, L., Black, J. A. & Waxman, S. G. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 8963–8968.
36. Tate, S., Benn, S., Hick, C., Trezise, D., John, V., Mannion, R. J., Costigan, M., Plumpton, C., Grose, D., Gladwell, Z., *et al.* (1998) *Nat. Neurosci.* **1,** 653–655.
37. Scholz, A., Appel, N. & Vogel, W. (1998) *Eur. J. Neurosci.* **10** Suppl., 2547–2556.
38. Rush, A. M., Brau, M. E., Elliott, A. A. & Elliott, J. R. (1998) *J. Physiol. (London)* **511,** 771–789.
39. Schwartz, A., Palti, Y. & Meiri, H. (1990) *J. Membr. Biol.* **116,** 117–128.
40. Kostyuk, P. G., Veselovsky, N. S., Fedulova, S. A. & Tsyndrenko, A. Y. (1981) *Neuroscience* **6,** 2424–2430.
41. Schild, J. H. & Kunze, D. L. (1997) *J. Neurophysiol.* **78,** 3198–3209.
42. Levine, J. D., Basbaum, A. I. & Fields, H. L. (1993) *J. Neurosci.* **13,** 2273–2286.
43. Cui, M. & Nicol, G. D. (1995) *Neuroscience* **66,** 459–466.
44. Taiwo, Y. O., Goetzl, E. J. & Levine, J. D. (1987) *Brain Res.* **423,** 333–337.
45. Cardenas, C. G., Del Mar, L. P., Cooper, B. Y. & Scroggs, R. S. (1997) *J. Neurosci.* **17,** 7181–7189.
46. Levine, J. D. & Taiwo, Y. O. (1989) *Neuroscience* **32,** 571–575.
47. Gold, M. S. & Levine, J. D. (1996) *Neurosci. Lett.* **212,** 83–86.
48. Taiwo, Y. O., Bjerknes, L. K., Goetzl, E. J. & Levine, J. D. (1989) *Neuroscience* **32,** 577–580.
49. Taiwo, Y. O. & Levine, J. D. (1989) *Brain Res.* **492,** 400–403.
50. Taiwo, Y. O. & Levine, J. D. (1991) *Neuroscience* **44,** 131–135.
51. Taiwo, Y. O., Heller, P. H. & Levine, J. D. (1992) *Neuroscience* **48,** 479–483.
52. Taiwo, Y. O. & Levine, J. D. (1990) *Neuroscience* **38,** 752–762.
53. Wang, J. F., Khasar, S. G., Ahlgren, S. C. & Levine, J. D. (1996) *Neuroscience* **71,** 259–263.
54. Bennett, D. L., Averill, S., Clary, D. O., Priestley, J. V. & McMahon, S. B. (1996) *Eur. J. Neurosci.* **8,** 2204–2208.
55. Ogata, N. & Tatebayashi, H. (1992) *Dev. Brain Res.* **65,** 93–100.
56. Waxman, S. G., Kocsis, J. D. & Black, J. A. (1994) *J. Neurophysiol.* **72,** 466–470.
57. Gold, M. S., Levine, J. D. & Correa, A. M. (1998) *J. Neurosci.* **18,** 10345–10355.
58. Fitzgerald, E. M., Okuse, K., Wood, J. N., Dolphin, A. C. & Moss, S. J. (1998) *J. Physiol. (London),* in press.
59. Gu, J. G. & MacDermott, A. B. (1997) *Nature (London)* **389,** 749–753.

60. Jeftinija, S. (1994) *Brain Res.* **639,** 125–134.
61. Quasthoff, S., Grosskreutz, J., Schroder, J. M., Schneider, U. & Grafe, P. (1995) *Neuroscience* **69,** 955–965.
62. Brock, J. A., McLachlan, E. M. & Belmonte, C. (1998) *J. Physiol. (London)* **512,** 211–217.
63. Crooke, S. T. (1997) *Ciba Found. Symp.* **209,** 158–164.
64. Khasar, S. G., Gold, M. S., Dastmalchi, S. & Levine, J. D. (1996) *Neurosci. Lett.* **218,** 17–20.
65. Bilsky, E. J., Wang, T., Lai, J. & Porreca, F. (1996) *Neurosci. Lett.* **220,** 155–158.
66. Khasar, S. G., Gold, M. S. & Levine, J. D. (1998) *Neurosci. Lett.* **256,** 17–20.
67. Porreca, F., Lai, J., Biau, D., Wegert, S., Ossipov, M. H., Eglen, R. M., Kassotakis, L., Novakovic, S., Robert, D. K., Sangameswaran, L. & Hunter, J. C. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 7640–7644.
68. Khasar, S. G. & Levine, J. D. (1996) *Neurosci. Lett.* **205,** 141–143.
69. Dirig, D. M., Isakson, P. C. & Yaksh, T. L. (1998) *J. Pharmacol. Exp. Ther.* **285,** 1031–1038.