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Commentary

More sensory competence for nociceptive neurons in culture

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> Pain is "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" [*Pain* **6,** 249–252 (1979)]

This definition of a commission of the International Association for the Study of Pain (IASP) implies that pain is a subjective experience—hard to measure in all its dimensions. However, the definition also hints at quantifiable causes of pain: tissue damage excites particular nerve terminals, which are specifically capable of foreseeing such damage to result from ongoing "noxious" stimulation. Accounting for these characteristics the term nociceptor was coined by Sherrington (1). Later, Y. Zotterman and A. Iggo were the first to record extracellular impulses from nociceptive nerve fibers of the living animal, in the early 1950s, and all information on mechanical and thermal sensitivity and electrical properties of these primary afferents has come from similar studies (2–4). However, *in vivo* experiments are generally hampered by the complexity of the living organism, which, for example, does not allow for controlled application of chemical stimuli in the investigation of the effects of algogenic and inflammatory mediators such as bradykinin (BK). Knowledge of chemosensitivity comes from a number of *in vitro* models that have been established during the last decade (5–11).

Due to their small size (<1 μM) and since they are embedded in the tissue, nociceptive nerve terminals are not accessible to direct measurements of membrane currents. Assuming that membrane receptors and channels—like other proteins—are synthesized in the neuronal nucleus and cell body, dissociated dorsal root ganglion (DRG) neurons could be anticipated to incorporate some of these membrane receptors in their somatic membrane. If so, a subpopulation of cultured sensory neurons may serve as a cellular model of the peripheral nociceptive nerve terminal and may represent a tool to investigate the transduction mechanisms leading to excitation or sensitization of nociceptors. Both phenomena are the target of Cesare and McNaughton's interesting contribution to the current issue of the *Proceedings* (12).

In recent years, the DRG model has been extensively used to investigate membrane currents induced by chemical algogenic stimuli. Among these, capsaicin, the pungent ingredient of hot chili peppers, has been preferred because, as a selective nociceptor stimulus, it produces burning pain, heat sensitization, secondary mechanical hyperalgesia, and allodynia (13–15). It selectively excites and/or sensitizes nociceptors and, in a subpopulation of cultured sensory neurons, has been found to induce a sustained excitatory inward current that is nonspecifically carried by cations (16–19). A large subset of the same subpopulation of small-sized DRG neurons responds to acidic solutions of pH below 6.3 with a sustained non-specific cation current, which likely is the functional correlate of nociceptor excitation in single fiber studies and of pain in psychophysical experiments induced by tissue acidification (16, 20–22). Further functional similarities between DRG neurons, nociceptors, and pain sensation have been found in an action of inflammatory mediators that greatly enhances responsiveness and responses to acidic pH (23, 24). In DRG neurons from adult rats, the sustained proton-induced inward current is facilitated by a combination of inflammatory mediators, BK in the first place (25).

More support for a sensory competence of DRG cells in culture comes from the paper by Cesare and McNaughton (12), who have discovered a sensitivity to noxious heat as a possible basis of heat-induced pain. An excitatory inward current, induced by temperature increases beyond 42°C, was found in about 56% of the small neurons, presumably representing the cell bodies of unmyelinated primary afferents. This prevalence corresponds nicely to the proportion of polymodal, i.e., mechano-heat sensitive (CMH) units among the C-fibers (26). The cellular heat responses show similarities as well as interesting differences to nociceptor responses to heat. The latter have heat thresholds around 40°C intracutaneous temperature and a log-linear stimulus-response function that would fit the curve describing the increase of the heat-induced membrane current with temperature (4, 26, 27). However, in experiments using steep temperature increases similar to those used to induce heat-evoked currents, a pronounced dynamic component of the CMH fiber response is generally observed that adapts to a lower, tonic level of discharge (4, 27). The obvious lack of correlate in the current kinetics could be explained by transformation of the receptor potential at the generator region of the axon, which may be the actual site where the dynamic overshoot and adaptation of the discharge rate take place. The cultured neurons did not exhibit sensitization in response to repeated application of heat, "suggesting that the process of sensitization is not intrinsic to the neuron but is instead caused by factors released by damage to adjacent cells." This point is valid but actually superfluous, since rat nociceptors in contrast to other species are not sensitized just by repetition of noxious heat stimulation (refs. 28–30, and Fig. 1).

Under inflammatory conditions, BK is synthesized from ubiquitous precursors and exerts two distinct effects on CMH fibers: it directly but transiently excites about one-half of the C afferents excited by noxious heat. In the same population a sensitization to heat is induced, which recovers within few minutes after removal of BK (Fig. 1); this is due to the activation of the B2 receptor subtype, which also mediates the excitatory effect (29, 31, 32). This finding is reflected in the cellular model by the facilitation of the heat-induced current, which outlasted the BK application only briefly. Sensitization to heat is yet observed in C polymodals that do not respond to BK with discharge, and the B1 receptor subtype has proven effective in one-half of these cases (refs. 32 and 33, and Fig. 1). In contrast, in those cultured DRG neurons that did not respond to BK with an inward current, no sensitization of the heat-induced current was obtained by BK treatment. The discrepancy to the single fiber studies is probably due to the fact that excitatory effects on primary afferents only become evident if they exceed the threshold for action potential

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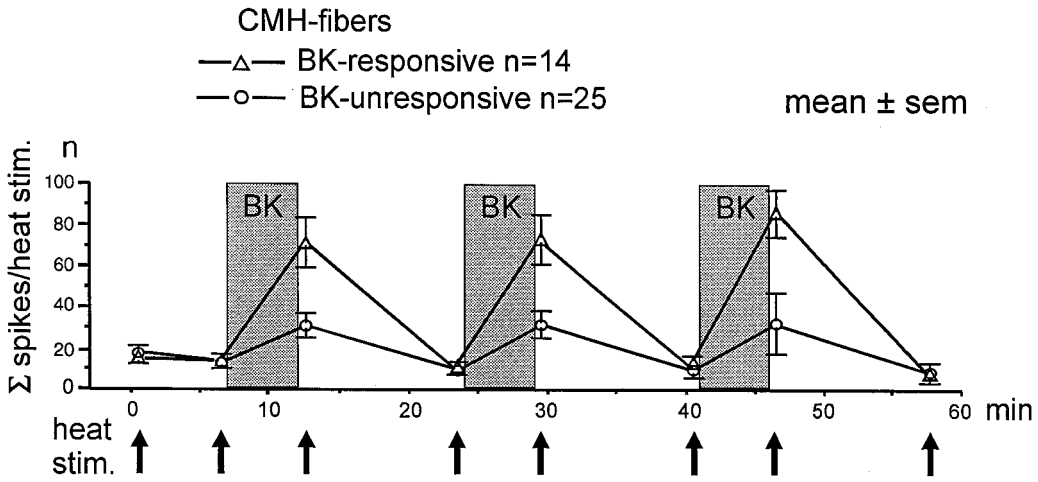

FIG. 1. Characteristics of BK-induced nociceptor sensitization to heat. Cutaneous receptive fields of mechano-heat-sensitive C-fibers (CMH) of the rat were repeatedly superfused (at the corium side, *in vitro*) with 10 μM BK, and sensitization to radiant heat stimulation (32–45°C in 20 s) resulted, which was significant within both groups of fibers and considerably different between units excited by BK and those not excited (Wilcoxon matched pairs and *U* test, respectively). (Y.-F. Liang, B. Haake, and P.W.R., unpublished data)

generation. Determination of sensory properties, on the other hand, is possible at higher resolution and reveals the BK effect even when insufficient to excite the sensory terminal (Fig. 1).

A major characteristic of excitatory BK effects in many preparations is its tachyphylaxis upon repeated application, which is not addressed in Cesare and McNaughton's paper. This will need to be explored in future studies, since it has recently been discovered in a single fiber study that tachyphylaxis does not apply to BK-induced sensitization to heat, which can be reproduced or sustained without reduction (ref. 33, and Fig. 1). This is reminding for the sustained sensitization achieved in the cellular model through direct activation of protein kinase C (PKC) or through inhibition of protein dephosphorylation by calyculin A. However, the phorbol 12-myristate 13-acetate did not evoke a direct current response nor did phosphatase inhibition prolong the BK-induced inward current (see figures 5 and 6 in ref. 12), which may mean that the sensitizing but not the excitatory effect of BK results from PKC activation. An essential difference between the two BK effects is already indicated by the above discrepancy in tachyphylaxis, which becomes puzzling in view of the B2 receptor mediating both effects. PKC has multiple effects among which it can mediate cross-talk between second messenger pathways by activating phospholipase A2 and certain isoforms of adenylyl cyclase (34). Both actions finally increase intracellular cAMP levels and these are again involved in mechanisms of nociceptor sensitization to heat (30, 35). Further work is needed to show whether there are distinct pathways toward sensitization, as Cesare and McNaughton suggest, or only one final second or third messenger onto which different mediator effects converge.

Cellular models and current measurements using the conventional whole cell configuration of the patch-clamp technique have inherent problems of their own. Wash-out of the cytosol and "space-clamp" in cases of cells growing processes meet with hardly predictable membrane characteristics induced by long-term cultivation, and it is difficult to foresee or recognize all resulting consequences. Therefore, any model should keep in touch with the "real thing," here the nociceptive nerve ending, that is heat-sensitive and chemosensitive by nature not by culture.

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