Substance P, opioid, and catecholamine systems in the mouse central nervous system (CNS)

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In an article in this issue of PNAS, Jasmin *et al.* (1) provide new evidence that noradrenaline is a key neurotransmitter in the endogenous pain inhibitory systems in the central nervous system (CNS) of the mouse. They show that this adrenergic inhibitory system interacts with that part of the sensory nociceptive system by using the neuropeptide substance P in a mutually antagonistic manner. They conclude that substance P, when unopposed by tonic release of noradrenaline, is the major factor underlying thermal hyperalgesia. Jasmin *et al.* also present evidence that the reduced opioid efficacy seen in the absence of noradrenaline is the result of increased NK₁ receptor stimulation by endogenous substance P. Their paper (1) is a good example of the way in which critical, and well controlled, experiments on transgenic animals can help to elucidate complex problems in neurobiology. This fascinating study supports other recently published work suggesting that substance P has a key role in pain perception in the mouse by way of critical interactions with other systems, e.g., the PAR2 protease-dependent receptor (2, 3).

It has been accepted for many years that noradrenaline is a key neurotransmitter in the descending inhibitory systems by which the brainstem controls the sensitivity of the dorsal horn of the spinal cord to nociceptive sensory inputs (4). Synaptically released noradrenaline acts through α_2 adrenoceptors to reduce the sensitivity of dorsal horn relay neurons to noxious but not to nonnoxious stimuli and it also potentiates the effects of opioid drugs, such as morphine (5, 6). Investigations using agonist and antagonist drugs to examine interactions between effects mediated by opioid receptors and adrenoceptors have sometimes given confusing results, as have studies where pathways have been lesioned or chemically depleted of their transmitter content. It has thus been difficult to investigate the putative noradrenergic dysfunction believed to underlie some chronic pain conditions in humans (7). This variability fits with current ideas on

the plastic nature of pain but underlines the need for new animal models in which chronic pain states can be studied. Accordingly, to avoid the problems that plagued earlier studies, Jasmin *et al.* (1) chose to do experiments in mice in which the gene for dopamine- β -hydroxylase (DBH, the enzyme that converts dopamine to noradrenaline) had been genetically deleted (8). These mice can have their normal noradrenergic function restored by the ingenious expedient of dosing them with L-threo-3,4-dihydroxyphenylserine (DOPS), a synthetic amino acid precursor of noradrenaline that can be converted to noradrenaline by aromatic amino acid decarboxylase (AADC, which is present in both control and DBH $-/-$ mice). A further refinement is that, if the mice are dosed with carbidopa, an AADC inhibitor which does not cross the blood-brain barrier, and then given DOPS, it is possible to replace the brain noradrenaline in the DBH knockout mice, leaving peripheral noradrenaline absent (9). The DBH knockout mice have normal baseline sensory and nociceptive behaviors and are viable for long-term experiments. This latter point is important as the lack of a severe phenotype in the knockout animals renders the conclusions from experiments less open to misinterpretation.

Nociceptive testing of the DBH knockout mice revealed that they were thermally hyperalgesic compared with control animals but had normal responses to mechanical stimuli. When inflammation was produced in a paw, then the DBH knockout animals displayed mechanical hyperalgesia, but to a similar extent to control animals. Restoring noradrenaline in the CNS, by giving DOPS plus carbidopa, abolished the thermal hyperalgesia in DBH knockout mice, but this treatment had no effect on control mice.

The antinociceptive effect of DOPS/carbidopa, in the DBH knockout mice, was reversed by the α_2 -adrenoceptor blocker, SKF-86466, but not by the α_1 -adrenoceptor blocker HEAT. CNS noradrenergic neurons and their projections were preserved in DBH knockout mice, thereby providing the circuitry from which noradrenaline could be released once its synthesis was established by giving DOPS/carbidopa. As Jasmin *et al.* (1) believed that part of the antinociceptive effect of α_2 -adrenoceptor stimulation might be the result of the inhibition of substance P release from primary afferent fibers, they studied the effects of drugs that blocked the action of substance P at its target NK₁ receptors on the thermal hyperalgesia seen in DBH knockout mice. All three antagonists used (RP-67580, CP-96345, and

L-733060) reversed the hyperalgesia in the knockout mice but not in control mice, and inactive enantiomers of two of these blockers were without effect, confirming that the responses studied were indeed operated through NK₁ receptors. It was found

that the amounts of NK₁ receptor in spinal cord dorsal horn were similar in DBH knockout and control mice, but that the amount of substance P immunoreactivity in DBH knockout mice was lower than in controls, suggesting increased release/turnover leading to depletion of stores. It was also found that the DBH knockout mice were less sensitive to the antinociceptive effects of morphine than were control mice, consistent with the idea that part of the action of morphine is through stimulation of descending inhibitory circuits by using noradrenaline as a transmitter (5). This observation was confirmed by giv-

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ing DOPS/carbidopa, which increased the sensitivity of the DBH knockout mice to morphine, making it similar to that in the control animals. Surprisingly, it was found that the NK₁ receptor antagonist L-733060 had a similar effect to DOPS/carbidopa in increasing sensitivity to morphine in the DBH $-/-$ mice.

So what do these experiments tell us about the way in which pain is perceived and controlled? Jasmin *et al.* (1) suggest that a defect in the noradrenergic part of the descending pain inhibitory system, such as is found in the DBH knockout mice, could produce a state of chronic hyperalgesia and it is relevant to ask whether a similar situation ever exists in humans. Certainly defects in the function of DBH have been identified in human subjects (9). Additionally, Jasmin *et al.* propose that substance P and opioids have opposite effects on pain behavior with the pronociceptive effects of substance P being balanced by the antinociceptive effects of opioids. This situation should have the consequence that when a hyperalgesic state is associated with a reduced antinociceptive response to morphine, an appropriate treatment might be to give an NK₁ antagonist to block the effects of substance P and restore the balance.

Substance P has been known to be widespread in the CNS since the 1950s (10). It is more abundant in dorsal than in ventral roots and has been associated with pain because substance P was found in the smaller, unmyelinated sensory fibers. Exogenous substance P, applied to dorsal horn sensory relay neurons, had a slow and prolonged excitatory action resembling excitation seen after peripheral noxious stimuli (10). Multiple messengers often coexist in the same dorsal root ganglion neurons along with substance P. As we now accept that the activation of peripheral nociceptors is the result of an "inflammatory soup" of algogens released by tissue damage or inflammation, we must consider that the activation of nociception within the dorsal horn of the spinal cord may be the result of a

parallel release of numerous transmitters and modulators from the terminals of primary afferent fibers. Only if a single released substance has a dominant role will the specific pharmacological blockade of its effects result in analgesia. It is now well accepted that sensory neurotransmission is plastic; that synthesis of transmitters and their receptors, including that of substance P, can be up-regulated by conditions such as inflammation, which cause pain; and that synaptic connections often change after nerve injury (11). We are thus faced with a nociception system that uses numerous transmitter substances in parallel and that adjusts its sensitivity up and down in response to tissue injury. So how good is the evidence that substance P has a dominant role in nociception?

The preprotachykinin knockout mouse (which lacks both substance P and neurokinin A) has attenuated responses

to intense noxious stimuli. NK₁ receptor knockout mice show no changes in acute nociception tests but do show reduction in responses to inflam-

matory stimuli (12). NK₁ receptor antagonists can be shown to have antinociceptive effects in the presence of nerve injury or inflammation but not in acute tests such as hot plate (13). The profile of these compounds was similar to that of non-steroid anti-inflammatory drugs (NSAIDs), which are well known to be analgesic in humans. It was therefore a surprise to many when clinical trials failed to show reproducible analgesic effects with those NK₁ antagonists that so far were studied in humans (ref. 14, but see ref. 15). This anomaly might be explained by species differences in the physiology of substance P or distribution of NK₁ receptors or by differences between clinical pain and the type of noxious stimulus and response studied experimentally in small animals (12, 14). However, most of the differences in substance P and NK₁ receptor distribution between species are expressed at supraspinal sites, with remarkable similarities in distribution of both the peptide

and NK₁ receptors in the dorsal horn of the spinal cord. Also, those animal tests that revealed antinociceptive activity with NK₁ antagonists have recently been reliably predictive of analgesic activity in humans, for example, in the case of the cox-2 inhibitors (16). It is possible that there are particular pain states where the role of substance P is dominant and where these agents may be effective analgesics in man. The studies of Jasmin and colleagues suggest that adding an NK₁ antagonist to an opioid may have interesting and potentially beneficial consequences.

If one regards the noxious stimuli used in the animal behavioral studies as stressful stimuli, the published work on nociception links well with the experimental work supporting the antidepressant actions of NK₁ antagonists (17). These combined data tell us that NK₁ receptor blockade can reliably attenuate the response to a variety of stressors but may not be sufficient to result in clinical analgesia. It also adds support to the suggestion by Jasmin *et al.* (1) that there are important interactions between substance P and noradrenergic systems. This idea has already been suggested in the context of the antidepressant actions of NK₁ receptor antagonists (18), and additional evidence (19) pointing to interactions with the 5-HT system is also worth examining for an influence on nociception.

It is relevant to review our inability to accurately predict from animal experiments, including those on gene deletion mutants, the lack of clinical analgesic properties of NK₁ receptor antagonists. This mismatch has an impact on the discovery and clinical evaluation of other putative analgesics. What preclinical criteria should be used to determine whether clinical trials of a new analgesic are likely to be successful? Just how reliable will elegant transgenic animal studies such as those reported here by Jasmin *et al.* (1) turn out to be in predicting clinical outcomes in humans?

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Jasmin *et al.* propose that substance P and opioids have opposite effects on pain behavior.

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