Lactoferrin elicits opioid-mediated antinociception without development of tolerance: central nNOS-1 set off duty?

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Patients with the most severe pain, e.g., suffering from traumatic injury or from cancer, receive the most potent analgesics we have: opioids. This treatment, however, is still impaired by severe opioid side effects such as respiratory depression, constipation, or the development of tolerance and dependence. In this issue of the American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, Hayashida and colleagues (5) present evidence that a multifunctional protein, lactoferrin, is able to elicit antinociceptive effects via central opioidergic systems coupled to a nitric oxide (NO) synthase (NOS) system without development of tolerance. Will lactoferrin analogs replace opioids in future pharmacotherapeutic treatment of pain?

Information about tissue injury due to chemical, thermal, or mechanical attacks is picked up by nociceptor units at peripheral sensory nerve endings and is conveyed to laminae I, II, or V of the dorsal horn in the spinal cord by nonmyelinated C- or by myelinated Aδ-fibers. Ascending pathways, e.g., the spinothalamic system, under their experimental conditions, was not coupled to an opioidergic system: the antinociceptive effect, whereas inhibition of the antinociceptive system, e.g., by an NOS inhibitor, elicited an antinociceptive effect by intraperitoneal, oral, or intracerebroventricular administration of L-NAME was also demonstrated in mice after subplantar injection of formalin; the effect was either attenuated or not observed at all during the first nociceptive phase (0–5 min after formalin injection) related to a direct reaction to the stimulus, but it was observed during the second phase (15–30 min after formalin injection), during which a hyperalgesic state is believed to develop due to an inflammatory response with release of algesic mediators (15). Recently, in addition to this “nociceptive” NOS system, indication for an “antinociceptive” NOS system coupled to guanylate cyclase as well was obtained (17); in rats, in a mechanical allodynia test, intrathecal administration of an NO donor, 3-morpholinosydnonimine (SIN-1), produced an antinociceptive effect in the tail-flick test, which was inhibited by an NO scavenger or a guanylate cyclase inhibitor. Thus, in a hyperalgesic state, inhibition of the nociceptive NOS system, e.g., by an NOS inhibitor, elicited an antinociceptive effect, whereas inhibition of the antinociceptive NOS system, e.g., by an NO scavenger, led to enhanced nociception.

A variety of compounds is involved in the humoral transmission of nociceptive information or its modulatory enhancement or suppression such as serotonin, norepinephrine, GABA, substance P, cholecystokinin, neuropeptides, prostaglandins, or cytokines (20); this led to various pharmacotherapeutic attempts to block nociceptive information by such compounds or analogs thereof and, thus, to fight pain (22). One of those central messengers involved in transmission of nociceptive information is NO. Already more than a decade ago, NO was demonstrated to be involved in mechanisms underlying the development of certain types of hyperalgesia (11). Apparently, in situations of pain persisting more than a few minutes, N-methyl-D-aspartate (NMDA) receptor activation occurs in the spinal cord, leading to the increase of intracellular calcium concentration with subsequent activation of a neuronal NOS (nNOS); NO then obviously activates a soluble guanylate cyclase (GC-S), which finally leads to a hyperalgesic state. The blockade of this “nociceptive” NMDA/NOS/GC-S cascade at the NOS level by Nω-nitro-L-arginine methyl ester (L-NAME), an NOS inhibitor, or at the GC-S level by methylene blue, a GC-S inhibitor, was shown to induce dose-related antinociceptive effects, e.g., in rats suffering from hyperalgesia due to sciatic nerve ligation (12). A dose-related antinociceptive effect by intraperitoneal, oral, or intracerebroventricular administration of L-NAME was also demonstrated in mice after subplantar injection of formalin; the effect was either attenuated or not observed at all during the first nociceptive phase (0–5 min after formalin injection) related to a direct reaction to the stimulus, but it was observed during the second phase (15–30 min after formalin injection), during which a hyperalgesic state is believed to develop due to an inflammatory response with release of algesic mediators (15). Recently, in addition to this “nociceptive” NOS system, indication for an “antinociceptive” NOS system coupled to guanylate cyclase as well was obtained (17); in rats, in a mechanical allodynia test, intrathecal administration of an NO donor, 3-morpholinosydnonimine (SIN-1), produced an antinociceptive effect in the tail-flick test, which was inhibited by an NO scavenger or a guanylate cyclase inhibitor. Thus, in a hyperalgesic state, inhibition of the nociceptive NOS system, e.g., by an NOS inhibitor, elicited an antinociceptive effect, whereas inhibition of the antinociceptive NOS system, e.g., by an NO scavenger, led to enhanced nociception.

Additional information on nociceptive and antinociceptive NOS systems resulted from their experimental linkage to central opioidergic systems. Kawabata and colleagues (7) showed that the nociceptive NOS system, under their experimental conditions, was not coupled to an opioidergic system: the antinociceptive effect achieved by intracerebroventricular administration of L-NAME, an NOS inhibitor, was not influenced by subcutaneous administration of a nonselective opioid antagonist, naltrexone, or a δ-selective opioid antagonist, naltrindole. However, in contrast to the nociceptive NOS system, the antinociceptive NOS system turned out to be coupled to an opioidergic system: the antinociceptive effect achieved by intracerebroventricular administration of L-arginine, an NO precursor, was blocked by subcutaneous administration of naltrindole.

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Extension of the experimental design by including δ-, μ-, and κ-selective opioid receptor agonists revealed further information on the two NOS systems. Classical antinociceptive opioid effects in the second phase of the formalin test (see above) were observed on intrathecal administration of highly selective δ-, μ-, and κ-opioid receptor agonists in rats suffering from prolonged pain (10). However, these effects were found to be enhanced on intrathecal administration of an NOS inhibitor (L-NAME). Although just inhibition of the nociceptive NOS system by the NOS inhibitor might appear to represent a satisfactory interpretation of the observed antinociceptive effect, it would not exactly hit the point: the L-NAME dose used had no effect by itself in absence of the opioids. Thus it is tempting to speculate that the opioids had provoked a modulation, e.g., an activation of the nociceptive or the antinociceptive NOS system. The consequence might have been that inhibition of the activated nociceptive NOS system by the NOS inhibitor allowed for a potentiation of the opioid receptor ligand effects. This complex network of interactions is discussed in detail by Machelska and colleagues (10).

Further extension of our knowledge on the two NOS systems results from investigations as conducted by Xu and Tseng (21). In this study, morphine was administered intracerebroventricularely to mice, and the observed antinociceptive effect was measured in the radiant heat tail-flick test. As compatible with the findings of Machelska and colleagues (10), morphine-induced antinociception was found to be potentiated by intrathecally administered Nω-nitro-L-arginine (L-NNA), an NOS inhibitor, which might, besides other possibilities of interpretation, indicate the inhibition of an activated nociceptive NOS system. This result was hammered down by additional tests showing potentiation of morphine-induced antinociception by hemoglobin, an NO scavenger, or methylene blue, a guanylate cyclase inhibitor, or by attenuation of morphine-induced antinociception by L-arginine, an NO precursor, or SIN-1, an NO donor. However, in sharp contrast to the findings raised with morphine, an antinociceptive effect induced by the opioid peptide β-endorphin under the same conditions in the same study was not potentiated but attenuated by L-NNA, hemoglobin, or methylene blue. Thus, whereas morphine might be speculated to be able to provoke an activation of the antinociceptive NOS system, β-endorphin obviously had no such effect. Although further interpretations may be considered (10, 11, 21), the alterations of the antinociceptive β-endorphin effect caused by an NOS inhibitor, an NO scavenger, or a GC inhibitor clearly speak in favor of inhibition of the antinociceptive NOS system.

Screening central NOS systems for an involvement in the development of opioid tolerance or dependence revealed striking results: NOS inhibitors blocked the development of tolerance to the analgesic effects of morphine (9) or U-50,488H, a κ-opioid receptor agonist (1). Although NOS inhibitors apparently were able to inhibit development of dependence as well, their effects did not cover all characteristics of dependence or withdrawal symptoms (6). The blockade of opioid tolerance by NOS inhibitors was compatible with two NOS inhibitor effects observed in the absence of opioids or after a single administration of opioids. First, it was compatible with the blockade of the nociceptive NOS system (11, 12, 15, 17), leading to an antinociceptive effect, enhancing the antinociceptive effect of the chronically administered opioid. Second, it was compatible with the previously observed potentiation of antinociceptive effects of opioids by NOS inhibitors possibly due to the blockade of an activated nociceptive NOS system (10, 21), thus strongly counteracting the development of tolerance to the antinociceptive opioid effect of the chronically administered opioid.

It was a study of Kolesnikov and colleagues (8) that allowed the replacement of speculations by facts: employing antisenis techniques, this group knocked out 50–75% of the mRNA of two types of nNOS, nNOS-1 and nNOS-2, at the spinal or at the supraspinal level in the central nervous system of CD-1 mice. Antisense probes targeting selectively nNOS-1 blocked tolerance to morphine analgesia. Antisense probes targeting selectively nNOS-2 caused a pronounced inhibition of morphine analgesia. The authors claim that nNOS-1 diminishes the analgesic actions of morphine, while nNOS-2 enhances them. Thus the results reported by Kolesnikov and colleagues (8) allow the conclusion that the nociceptive and the antinociceptive NOS systems referred to above are functionally related to or even identical with nNOS-1 or nNOS-2, respectively.

The results of the current study by Hayashida et al. (5) on the antinociceptive effects of lactoferrin fit in a fascinating way the findings referred to above. Lactoferrin is an iron-binding protein first isolated in 1939 from bovine milk and later also from the milk of many other species, including the human one (19). It is synthesized by the epithelial cells of the mammary gland and is also produced by the lacrimal gland, the salivary gland, the biliary tract, and the pancreas and is therefore found in tears, saliva, bile, and pancreatic juice. It also has been demonstrated in other fluids of the mammalian organism such as synovial fluid, amniotic fluid, or seminal plasma. Lactoferrin found in plasma is predominantly neutrophil derived. A variety of functions has been ascribed to lactoferrin. It appears to be involved in particular in iron metabolism or activities of the immune system, such as liberation of bacterial peptides, antifungal, antibacterial, or antiviral effects, modulation of inflammatory reactions, or inhibition of tumor growth or metastasis.

In a preceding paper (4) the authors of the current study had demonstrated antinociceptive effects in rats during phase 1 and phase 2 in the formalin test (see above) observed on intraperitoneal or intrathecal administration of bovine lactoferrin (BLF); in addition, they had shown that these effects were mediated via μ-opioid receptors. Direct interaction of BLF with opioid receptors did not appear to play a major role in this effect, in contrast to opioid effects reported for a number of further milk-derived compounds (18); moreover, lactoferrin fragments are known to behave like opioid...
antagonists rather than like opioid agonists (18). Because lactoferrin (3) as well as its receptors (2) have been demonstrated in the central nervous system, this finding in fact appears to signal a functional significance of lactoferrin in the humoral transmission of pain information.

In view of the fact that, on the one hand, lactoferrin had been shown to induce the release of NO from macrophages (16) and, on the other hand, opioidergic systems had been found to be linked to NOS systems (7, 10, 21), it was obvious to test NOS systems for an involvement in the opioid-mediated antinociceptive effects of BLF. In fact, the authors were able to show that the NOS inhibitor L-NAME inhibited the antinociceptive BLF effects in phase 1 of the formalin test (see above) completely and in phase 2 down to a certain degree of antinociception above the control level. This indicated that BLF elicited an antinociceptive effect during either phase by activation of the antinociceptive NOS system, i.e., nNOS-2. The antinociceptive rest effect in phase 2, however, turned out to be as high as the antinociceptive effect of L-NAME observed in the absence of BLF, which indicated that the antinociceptive rest effect under BLF was not related to BLF but was rather based on the well-known (11, 12, 15) inhibition of the nociceptive NOS system by L-NAME as observed in a hyperalgesic state, now assumed to be an nNOS-1 inhibition. In addition, the fact that the antinociception remaining under L-NAME in the presence of BLF was almost identical with that one measured in the absence of BLF showed that nNOS-1 had not been activated by BLF; such an activation should have led to a higher antinociceptive effect by L-NAME in the presence of BLF than observed in the absence of BLF.

The authors further showed that the antinociceptive effect of morphine was potentiated by BLF doses, which were inactive per se; such an effect had been also demonstrated for NOS inhibitors instead of BLF before (10, 21). However, whereas the potentiation of the morphine-induced antinociceptive effect by NOS inhibitors (10, 21) might be due to the inhibition of an activated nociceptive type of NOS, i.e., nNOS-1, the potentiation of the antinociceptive morphine effect induced by BLF (which apparently stimulated the antinociceptive type of NOS, i.e., nNOS-2, without exerting a major influence on nNOS-1) is thus more likely to be related to nNOS-2 stimulation. The authors further showed that this antinociceptive effect elicited by BLF together with morphine was completely blocked by L-NAME in phase 1 of the formalin test, which would be compatible with a complete blockade of nNOS-2. This nNOS-2 blockade by L-NAME has to be assumed for phase 2 as well; however, in this phase, about one-third of the antinociceptive effect elicited by morphine and BLF was further observed under L-NAME. Interestingly, practically the same level of antinociception was achieved by L-NAME alone. This effect elicited by L-NAME alone, which is due to inhibition of the nociceptive NOS system (11, 12, 15, 17), i.e., nNOS-1 inhibition (8) was almost the same under L-NAME plus BLF and under L-NAME plus BLF plus morphine. If BLF or morphine activated nNOS-1, the antinociceptive L-NAME effects under BLF or morphine should have been stronger than observed. Thus apparently BLF did not only “avoid” nNOS-1 activation by itself, it even appeared to “prevent” nNOS-1 activation by morphine.

As the most interesting result from a clinical point of view, the authors finally showed that BLF elicited antinociception without development of tolerance. This is in line with findings raised in this study indicating a lack of BLF interaction with nNOS-1; it was nNOS-1 that had shown to be relevant for the development of tolerance (8). Moreover, BLF even appeared to block an activating effect of morphine on nNOS-1.

In summary, information resulting from this study looks promising in terms of providing novel tools to approach questions as yet resistant to final elucidation such as humoral transmission of pain information, functional significance of nitric oxide, or mechanisms of opioid actions with special weight on development of tolerance and dependence. Although lactoferrin represents an entirely novel analgesic principle and, thus, its analogs might compete with opioids for a future place in the treatment of pain, it should be emphasized that there is a very long way from an interesting result at the level of basic science to its utilization under the criteria of evidence-based medicine (18).

REFERENCES


