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PHYSIOLOGY

Opioid Modulation of Pain Threshold in Fish

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Pain is a signal and defense response developed in the course of evolution as an adaptation increasing individuals' survival in their environment. Pain sensation allows an individual to identify the irritators that may injure its body and threaten its life. In addition to the system of pain sensation (nociceptive), humans and terrestrial vertebrates have an antipain (antinociceptive) morphofunctional system, which controls the threshold of pain sensitivity to sustain it at a functional level. This system consists mostly of endogenous opioid peptides interacting with three main types of opioid receptors: μ, δ, and κ [1].

We previously showed that, like higher vertebrates, fish also have a system of pain sensation with receptive areas spread over the entire body surface [2-4]. However, little is known about nociception and antinociception in fish, which is mainly due to the lack of adequate methods for the quantitative estimation of nociceptive stimuli and the organism's response to them.

We studied the behavioral (locomotor) response of fish to a threshold level of pain and the effect of tramadol, a central analgesic, which is an agonist of (μ opioid receptors.

Forty-two carp (*Cyprinus carpio*) specimens weighing 50 to 100 g were used in our experiments. An improved technique, as compared to that used previously [2-4], allowed us to measure the thresholds for pain sensation and changes in this parameter after administering the drug. We developed a computerized opticomechanical system to record fish behavioral responses, namely, horizontal movements of the caudal peduncle in response to the stimuli (Fig. 1a).

A fish was fixed (near the mouth and pectoral fins) in a chamber with current water. Electrodes were introduced into the tissue of the caudal fin blade, which excluded the possibility of the direct stimulation of muscle fibers. A series of short-term (0.5 ms) impulses of current (amplitude, 0.5-2.0 mA; frequency, 300 s^{-1}; total duration, 0.7 s) served as stimuli. The temporal structure of the stimulus is shown in Fig. 1b.

Both stimulation and locomotor responses were measured by computer. Amplitudes and lag periods of responses were displayed on a monitor. Our method allowed the nociceptive thresholds to be determined at an accuracy of 10%; the measurements were made at 5-min intervals during 1 h before (the background) and 1.5-2 h after administration of tramadol. The drug was injected intramuscularly at closes of 10, 30, 50, 80, and 100 nmol/g body weight in a volume of 1 μl/g. Sodium chloride (0.9%) served as the solvent and a control solution. In a special experimental series (six fish), 100 nmol/g naloxone, which is a universal antagonist of opioid receptors, was introduced 1 h before the experiment. The degree of analgesia (A) was calculated as a decimal logarithm of the ratio between the nociceptive thresholds determined every five minutes after tramadol administration and the average threshold value determined prior to drug administration: $A = \lg(T_i/T_f)$. The results obtained were statistically treated using the Mann-Wilcoxon-Whitney test.

The results obtained indicate that individual nociceptive thresholds in normal fish ranged within 10%

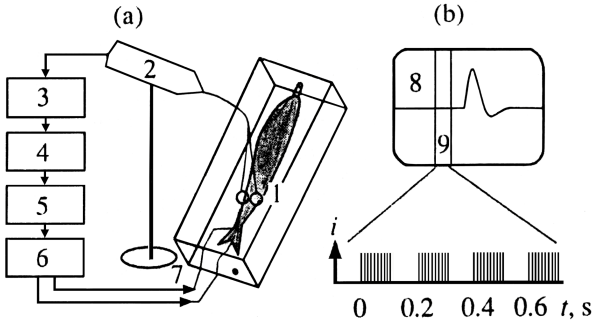

Fig. 1. (a) A scheme of an electron opticomechanical system for the measurement of nociceptive thresholds in fish, (b) An example of fish response to a painful stimulus as displayed on the monitor. Designations: 1, a chamber with current water; 2, sensor; 3, analog-to-digital converter; 4, computer; 5, stimulator; 6, amplifier; 7, electrodes; 8, the monitor screen; 9, period of irritation (s); *i*, the amplitude of irritating stimulus (mA).

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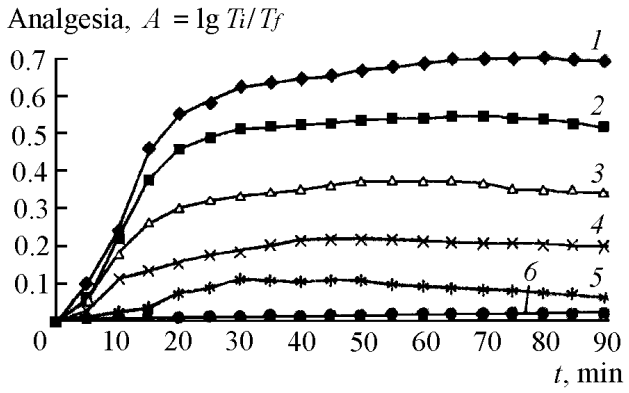

Fig. 2. Changes in the nociceptive threshold to painful stimuli after the administration of either tramadol solutions (*1 - 5*: 100, 80, 50, 30, and 10 nmol/g. respectively) or 0.9% NaCI (6: control). All experimental values differ significantly from control values (*1-5:* p<0.001, <0.001. <0.001. <0.01, and <0.05, respectively). Abscissa shows the time after injection of a solution; ordinate shows the degree of analgesia (*A*).

(p < 0.01) and remained stable for 1 - 2 h or even longer. Five to fifteen minutes after the administration of tramadol, changes in fish sensitivity to painful stimuli were observed. The analgetic effect was dose-dependent; the higher the dose, the more quickly it acted (Fig. 2). In some experiments, the overall time of analgesia was more than 2 h. The lack of response to increasing pain could not be blamed on tramadol immobilizing the fish, because the same fish placed into an aquarium showed normal swimming and behavior. Tramadol had no analgetic effect if naloxone, an antagonist of opioid receptors, was administered before (p<0.05). After the administration of a control solution, fish showed no changes in their response to stimuli (p < 0.01).

Our results indicate that, like higher vertebrates, fish also develop a prolonged analgesia in response to an agonist of the opioid μ receptors. Hence, fish have an antinociceptive system consisting of the opioid receptors similar to those in terrestrial vertebrates. The opioid receptors were first found in mammals and shown to mediate the effects of morphine and its derivatives (analgesia, addiction, etc.) They are also targets for endogenous opioid peptides: enkephalins and endorphins [1].

Opioid receptors were later found in lower vertebrates. In amphibians, μ, δ, and κ opioids were suggested to mediate antinociception via a single type of opioid receptor, unireceptor [5]. In the bony fish *Catostomus commersoni* and *Danio rerio*, cDNA for opiod receptors were isolated to completely clone the μ, δ, and partially clone κ receptors (G proteins). All fish and mammalian opioid receptors showed a high degree of amino acid homology [6, 7]. In mammals, the analgesic effects are primarily mediated through μ opioid receptors. Our results indicate that, in fish, the same receptors are responsible for increasing the pain threshold.

Thus, the opioid antinociceptive system seems to have evolved earlier than was previously believed. Even in the bony fish, this system shares structural and functional similarities with that of modern mammals.

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