



Pain sensitivity of fishes and analgesia induced by opioid and nonopioid agents

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Abstract.

Experiments were performed on carp *Cyprinus carpio*, rainbow trout *Oncorhynchus mykiss*, cod *Gadus morhua* and sturgeon *Acipenser ruthenus*. An originally designed optico-mechanical system was used to record the response to painful electrical stimulation before and after administration of analgetic agents. Drugs used were mu agonists tramadol, dermorphine and beta-casomorphine, kappa agonist U-50488, delta agonist DADLE, and nonopioid agents sydnophenum, analginum, novocainum. Drugs were administrated by different ways - peritoneally, subcutaneously, intranasally.

Administration of drugs produced dose-dependent and lasting for at least 1 h increase of NT in 1.5-3 times. In rainbow trout, intranasal administration of dermorphine 0.20-0.75 mg/kg caused a concentration-dependent decrease in the pain sensitivity by 12-55%. The analgesic effect was usually observed within 10 min after administration and it lasted for at least 1 h (up to 2-3 h in some fish). In cod, intranasal administration of beta-casomorphine 2.5-12.5 mg/kg and peritoneal one 10-30 mg/kg decreased the pain sensitivity by 15-37% and 14-35%, respectively. In carp, nociceptive thresholds significantly increased following the intramuscular injection of agonists mu, delta, and kappa opioid receptors, tramadol 10-100 nmol/g, DADLE 10-50 nmol/g, and U-50488 30-80 nmol/g, respectively. Antinociceptive effects of opioid agents were blocked or significantly reduced by pretreatment with naloxone. In cod, injected peritoneally sydnophenum 15-100mg/kg decreased the pain sensitivity by 15-89%. Intraperitoneal injection of 50% solution of analginum 0.5-2.5 ml/100g and subcutaneous one 0.25-1 ml/100g decrease the pain sensitivity by 16-21% and 29-45%, respectively. Local subcutaneous injections of 2% solution of novocainum blocked the nociceptive reactions. Stress significantly reduced nociceptive responses.

Key Words: analgesia, fish, nonopioids, opioids, pain.

Introduction

Growth of the role of fish farm industry in the food yield of people requests the knowledge of biology and physiology of subjects of aquaculture. In fishes, the system of pain sensitivity and behavior induced by aversion stimuli are almost never investigated. The information available is fragmentary and scarce. The sensation of pain as a factor for protection has been formed during natural selection from the fundamental property of animals - to identify unfavorable external stimuli and respond to them. This property is inherent, to a certain degree, in representatives of all phylogenetic (Kavaliers, 1988). Pain as a type of sensory modality is interpreted as several complex physiological processes: information, sensation, emotion, or neurophysiological phenomenon; therefore, neither the nature of pain, nor the range of sensations involved in this concept has a definite determination, even for people. Especially pain is difficult for study and analysis in nonmammalian and voiceless species. However, the main anatomical, physiological, and biochemical components of nociception in fish are similar to those in mammals. Pain receptors in fish, i.e. nociceptors, as with other vertebrates, represent free nerve endings of spinal (on the trunk) and trigeminal (on the head) nerve fibers, are localized over the entire body surface including fins (Chervova et al., 1992; Chervova et al.,

1994; Chervova, 1997). Recently, the basic part of antinociceptive system (μ , δ and κ opioid receptors) was found also in the brain of some species of fish. Although the monitoring and estimations of analgesic efficacy of drugs in fishes is of important value, no criteria have been established for the study of their nociception (pain sensation) and analgesia.

Materials and Methods

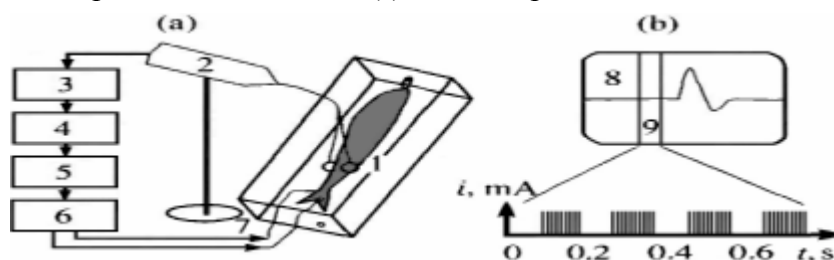
Experiments were carried out on carp *Cyprinus carpio* weighting 50-100 g, rainbow trout *Oncorhynchus mykiss* (200-400 g), cod *Gadus morhua* (100-300 g) and sturgeon *Acipenser ruthenus* (60-70 g).

For the investigation of responses of fish to painful stimuli, an originally designed optico-mechanical system was elaborated on based on the motor-locomotory reaction aimed at the removal of aversive stimulus. The fish was semirigidly fixed in a flow chamber (in the region of the mouth and pectoral fins). The gills were continuously moistened with water. The stimulating electrodes were inserted into the caudal fin blade in order to exclude the direct stimulation of muscle fibers. The recording apparatus was a movable wire “fork” embracing the caudal peduncle in the posterior third of the body. In response to painful stimulation (bursts of short pulses 0.5 ms of current 0.5-2.0 mA, with frequency 300/s), the fish moved its caudal peduncle and deviated “the fork” from the zero point. The stimulation and registration of the locomotor reaction were made by computer control, the recording system was synchronized with the painful (nociceptive) stimulus. Amplitude and duration of locomotor response was visualized on display (Fig. 1). The setup allowed to measure the nociceptive thresholds (NT) to an approximation of 10%. NT were measured at 5-min intervals for 1 h before and 1-2 h after administration of analgesic agents. Drugs used were μ agonists tramadol, dermorphine and beta-casomorphine, κ agonist U-50488, δ agonist DADLE, and nonopioid analgesic agents sydnophenum, analginum, novocainum. Sodium chloride (0,9%) served as the solvent and a control solution. Drugs were administrated by different ways - peritoneally, subcutaneously, intranasally. The data were processed using the Wilcoxon-Mann-Whitney test.

Results

It was found that the caudal, dorsal and pectoral fins, the skin surface around the eyes, and the epithelium of olfactory sacs were the most sensitive nociceptive zones; the skin on the head and trunk was less sensitive. The fishes reacted to nociceptive stimuli with a jerk of a tail. The response was the highest to the first of the applied stimuli. To

Fig. 1. (a) A scheme of an electron optomechanical 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 stimulation (s); i , the amplitude of electric stimulus (mA).



the following stimuli the responses were less intensive but, as a rule, were stable for two hours

or longer. Administration of drugs produced dose-dependent and lasting for at least 1 h increase of NT in 1.5-3 times. In cod, injected peritoneally sydnophenum 15-100 mg/kg decreased the pain sensitivity by 15-89%. Intraperitoneal injection of 50% solution of analginum 0.5-2.5 ml/100g and subcutaneous one 0.25-1 ml/100g decrease the pain sensitivity by 16-21% and 29-45%, respectively. Local subcutaneous injections of 2% solution of novocainum blocked the nociceptive reactions. Stress significantly reduced nociceptive responses in fish. In rainbow trout, intranasal administration of mu opioid agent dermorphine 0.20-0.75 mg/kg caused a concentration-dependent decrease in the pain sensitivity by 12-55%. The analgesic effect was usually observed within 10 min after administration and it lasted for at least 1 h (up to 2-3 h in some fish). In cod, intranasal administration of beta-casomorphine 2.5-12.5 mg/kg and peritoneal one 10-30 mg/kg decreased the pain sensitivity by 15-37% and 14-35%, respectively. In carp, nociceptive thresholds significantly increased following the intramuscular injection of agonists mu, delta, and kappa opioid receptors, tramadol 10-100 nmol/g (Fig. 2), DADLE 10-50 nmol/g, and U-50488 30-80 nmol/g, respectively. The result obtained indicate that individual nociceptive thresholds in intact fish ranged within 10% ($p < 0,01$) 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 responses 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. Administration of the highest concentrations of tramadol (100-50 nmol/g) elicited the decrease of the latency for first 10 min, than latency was increased. 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

Fig. 2. Changes in the nociceptive thresholds of carp to painful stimuli after administration of either tramadol solutions (1-5: 100, 80, 50, 30, and 10 nmol/g, respectively) or 0,9% NaCl (6, control). All experimental values differ significantly from control values (1-5: $P < 0,001$, $< 0,001$, $< 0,001$, $< 0,01$, $< 0,05$, respectively). Abscissa shows the time after injection of a solution; ordinate shows the degree of analgesia (A).

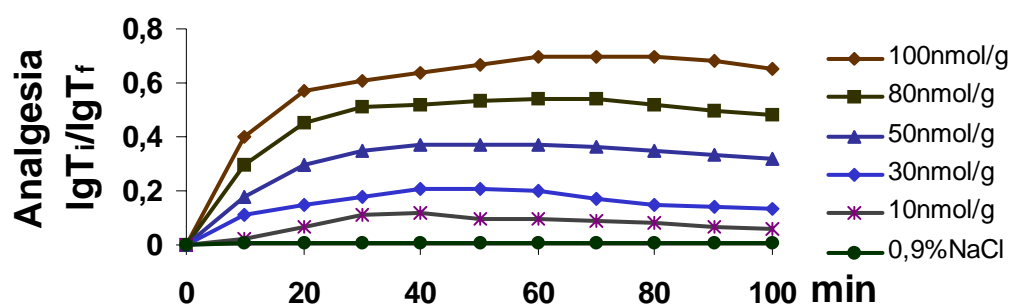
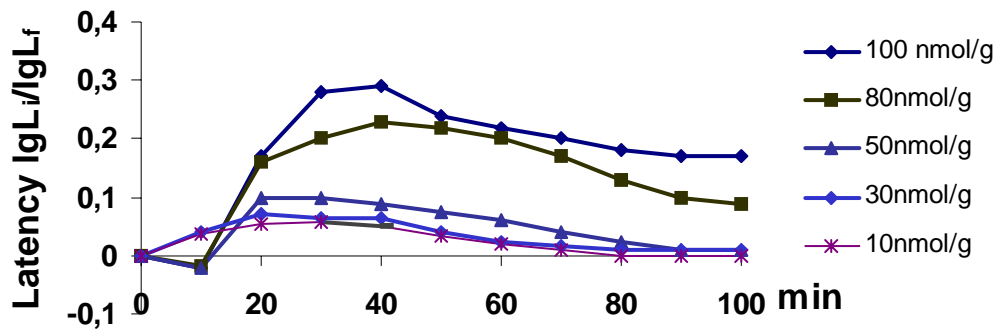


Fig. 3. Changes in the latency of the nociceptive responses of carp to painful stimuli after administration of either tramadol solutions (1-5: 100, 80, 50, 30, and 10 nmol/g, respectively).



response to stimuli ($p < 0.01$).

Preliminary experiments have shown that sturgeon *Acipenser ruthenus* possess of nociception as well as bony fishes. Its reacted to painful electrical stimuli with the same behavior – a jerk of the tail. Their nociceptive thresholds were comparable to that of carp. The pattern of response was the same after administration of the 100 nmol/g tramadol solution to carp and sturgeon. However, the period of recovery in sturgenn lasted more than in carp, for at least 3-5 days – fish demonstrated slowly swimming and preferred to lie at the bottom.

Discussion

Studies performed on *Cyprinus carpio*, *Parasalmo mykiss*, *Gadus morhua*, and *Acipenser ruthenus* indicated that the fishes possess a developed system of pain sensitivity with receptors (nociceptors) presented on the whole body. The most sensitive to noxious stimuli in these species were the blade of the caudal fin, dorsal and pectoral fins, skin around eyes, and epithelium of the olfactory sac; the skin of the head and body surface was less sensitive. NT of fish under this condition was comparable with human's one. The high density of nociceptors on fins is likely to be related to the fact, in particular, that fins are damaged in fish during their nest-building activity or aggressive interactions (Lorenz, 1984). The information from nociceptors comes to the neurons of the spinal cord or the trigeminal caudal nucleus of the brain stem; then, it is distributed in the central nervous system to provide for the formation of a rapid behavioral response. In fishes as in other vertebrates, the behavioral defensive response to a painful stimulus is manifested – movement aimed to the removal of the aversion sensation. In terrestrial animals this may be the withdrawal of a limb, the drawing in of the tail or the body, escape, jumping etc. Fish attempts to swim away starting with movement of caudal peduncle.

Processes that occur in the central nervous system in response to nociceptive stimulation have not been studied in fish. However, substance P, a neuropeptide that is a mediator of nociception in mammals, was found in the peripheral nerve endings of *Gadus morhua* and *Parasalmo mykiss* (Johnsson et al., 1998), and in different regions of the brain *Salmo salar* (Vecino and Ecstrom, 1991). In the spinal cord and trigeminal ganglion of *Petromizon marinus*, neurons were found that respond by pulsed discharges to a strong squeezing of skin that leaves traces of pinching, punctures with an acute needle, and cauterization up to appearance of white Matthews and Wickelgren, 1978).

Behavioral responses that in mammals are considered to be markers of primary pain sensitivity and are controlled, as is supposed, by the limbic system are primary motor start responses, a simple nonspecific avoidance (Wall, 1992). These reactions are observed also in fish in response to pain stimuli (Rekubraskii, 1967; Jansen and Green, 1970; Sickarulidze and Kadagishvili, 1974; Ehrensing et al., 1982). In mammals, nociceptor stimulation is



accompanied by an affective response, i.e., vocalization. Special sounds that are emitted at wounding were also recorded in *Misgurnus fossilis*, these sounds were generated by the swimming bladder and were characterized by a frequency spectrum of 0 to 4000 Hz with maxima of 500, 1500, and 3000 Hz and continued for 490 ms, on average. According to these parameters, this pain "shout" differed from sounds that accompanied food seizure, feeding, or spawning behavior (Nikol'skii et al., 1968). In bony fish the conditioned reflex of avoiding the aversive stimulus is easily formed if rapid impetuous swimming is peculiar to these fish in nature. Thus, *Trachurus mediterraneus ponticus* was taught to avoid a light signal that was followed by a pain electric stimulus after 12 to 13 combinations. In slow-moving fish, "ambuscaders", as with *Neogobius melanostomus* and *Symphodus roissali*, a reflex of avoidance failed to form in response to an conditional stimulus. In response to an electric stimulus, these fish shuddered, performed a short dart aside, and came to a standstill. They responded to the subsequent pain stimuli by an increase in respiration rate (Rekubratskii, 1967).

There are facts causing doubts as to whether there is any pain sensitivity in fishes, e.g., a shark's devouring its own viscera falling out of its open belly. Probably, in hungry animals, as it was shown in mammals, the feeding motivation may be so strong that a functional blockade with a feeding excitation of the mechanisms to the central structures takes place. Increasing of the pain threshold seem to be adaptive, permitting the animals in case of need to perform the vital requirements (foraging or defensive) in spite of damaging stimuli.

Our results indicate that, like higher vertebrates, fish also develop a prolonged analgesia in response to agonists of the opioid mu 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 (Dhavan et al., 1996). Opioid receptors were later found in lower vertebrates. In the bony fish *Catostomus commersony* and *Brachydanio rerio*, cDNA for opioid receptors were isolated to clone the mu, delta, and kappa receptors (G proteins). All fish's and mammalian's opioid receptors showed a high degree of amino acid homology (Darlison et al., 1997; Barallo et al., 1998). In mammals, the analgesic effects are primarily mediated through mu opioid receptors. Our results indicate that in fish, the same receptors are responsible for increasing the pain threshold. The decrease in pain sensitivity under the action of nonopioid preparations analginum and sidnophenum as well as analgesy caused by stress, illustrates the presence in fishes of other endogenous analgesic systems in addition to the opioid system.

It is reasonably safe to suggest that fish similar to higher vertebrates have nociceptive and both opioid and nonopioid antinociceptive systems that take part in the control of fish behavior and physiological status on the level of central mechanisms. This should be considered when surgical treatment is performed on fish, in particular, on sturgeons which often suffer from sex products extraction. The use of analgesics may minimize a harm and accelerate the wound healing.

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