PAIN AND ANALGESIA IN REPTILES AND AMPHIBIANS

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Abstract: Pain and pain perception are difficult to evaluate in animals. Reptiles and amphibians have the appropriate anatomy and physiologic structures for nociception. Alleviation of pain and suffering is a major goal of veterinarians. Little is known about how to ameliorate pain in this group of animals. This session will focus on pain perception and what is currently known about pain relief in reptiles and amphibians.

Key words: nociception, pain, analgesia, reptiles, amphibians

In humans, pain is a multifaceted experience with the sensory component (nociception) being only one factor (Stevens, 1992). Higher limbic and cortical faculties modify the nociceptive signal to increase or decrease the pain experience. These may or may not be developed in subhuman animals. Humans that have undergone cortical ablation for treatment of severe chronic pain report an awareness of pain but that the pain no longer bothers them (Stevens, 1992), suggesting that vertebrate species that do not have developed higher cortical faculties may have very different and diminished appreciation of nociceptive sensations.

Electrophysiologic studies conducted as early as 1926 support the morphologic correlation between free nerve endings and painful sensations in non-mammalian vertebrates such as reptiles and amphibians (Adrian, 1926). Pain pathways, pain responses, and some effective analgesics have been demonstrated in amphibians and reptiles; however, little information is available to clinical veterinarians regarding appropriate drugs and dosages for potentially useful analgesics. Additionally, clinical indices for pain and relief of pain have not been documented in amphibians and reptiles. It is generally preferable and appropriate to consider procedures accepted as painful in humans to be painful in other animals (Morton and Griffiths, 1986).

Two processes may represent dual selection pressures that have guided the emergence of nociception across phylogeny. The response to pain that represents a threat of tissue damage generally induces vigorous activity and efforts to escape the stimulus. The response to pain from tissue already damaged is often inactivity (Dennis and Melzack, 1983). The former is generally easier to study in a laboratory setting while the latter more closely represents pain in a clinical setting. This underscores the philosophy that inactivity must not be interpreted as a lack of pain. Non-mammalian vertebrates demonstrate four responses to nociceptive stimulation which threaten tissue damage: a) a nonspecific flight response, b) a rapid startle reaction, c) an affective response such as vocalization, d) a coordinated reaction such as biting at the source of pain.

There is very little information available regarding pain and analgesia in reptiles. It is frequently assumed that the ranking of animals' ability to experience pain follows class lines such that Mammalia > Aves > Reptilia > Pisces (Stevens, 1992). Because of their use in the laboratory setting, pain and analgesia in amphibians has been more thoroughly investigated than in reptiles. Evidence for the ability of reptiles and amphibians to experience pain is supported by the presence of appropriate neurologic components with the capacity to elicit an action potential in response to a painful stimulus (Spray, 1976; Gans and Ulinski, 1992; Liang and Terashima, 1993; Munoz, *et al,* 1997), endogenous antinociceptive mechanisms to modulate pain (Gans and Ulinski, 1992; Stoskopf, 1994) and demonstrable modulation of pain with pharmacologic agents used in other species (Spray, 1976; Mauk, *et al,* 1981; ten Donkelarr and de Boer-van Huizen, 1987; Kanui, *et al,* 1990; Kanui and Hole, 1992).

The neuroanatomic components and pathways necessary for nociception have been demonstrated in amphibians (Dennis and Melzack, 1983; Gans and Ulinski, 1992; Liang and Terashima, 1993). Nociceptive action potentials generally have a long latency from stimulus to discharge and longer duration but lower amplitude compared to action potentials stimulated by light touch (Maruhashi, *et al,* 1952; Catton, 1958). These slow impulses are evoked by injurious stimuli such as application of weak acid to the skin, intense mechanical stimulation, and strong heat but are not seen following a gentle touch.

Neurotransmitters documented as important in pain modulation in mammals occur in reptiles and amphibians, and are believed to function in a similar manner (Davidoff, *et al,* 1973; Gans and Ulinski, 1992). As an example, gamma amino butyric acid (GABA) is responsible for primary afferent depolarization and presynaptic inhibition in both mammals and amphibians (Davidoff, *et al,* 1973) The presence of serotonin and substance P in reptiles and amphibian primary afferents also supports the similarity though their specific function has not been completely elucidated (Lorez and Kemali, 1981; Gans and Ulinski, 1992; Tan and Miletic, 1992).

Amphibians and reptiles possess well developed endogenous opioid systems. Immunohistochemical studies have demonstrated the presence of dynorphins, endorphins, and enkephalins within the central nervous system of reptiles and amphibians (Doerr-Schott, *et al,* 1981; Gans and Ulinski, 1992). These sites appear to be ubiquitous across the phylogeny (Lorez and Kemali, 1981; Stevens, 1988). Additionally, in restrained frogs, immobilization stress produces a naloxone reversible analgesia compared with control frogs (Pezalla and Dieig, 1984). This implies that there is endogenous release of opioids in frogs that are restrained.

In any pain test there are two basic components - a noxious input and a behavioral output. The input must exclusively activate the pain fibers and the behavioral output should occur as a result of a specific noxious input (Stevens, 1992). Most common is a motor response occurring shortly after the noxious stimulation. Morphine is an exogenous opioid commonly used to provide analgesia in mammals. It exerts its effect by interacting with a mu receptor whose ligand is an endogenous opioid peptide (Terenius, 1978). Initial studies using a hot plate test or electric shock did not show any analgesic effect of morphine at any doses studied in frogs. An analgesic effect was easily demonstrated with morphine in frogs (10-100 mg/kg s.c.) using an acetic acid test (Pezalla, 1983). Application of acetic acid to the skin of the hind leg in frogs induces a spinal wiping reflex which is reproducible and, now, commonly used for testing nociception in frogs. The failure of the hot plate and electric shock to detect opioid analgesia was likely due to using an inappropriate test. Frogs show great variability in their latency for jumping off hot plates and responding to

electric shock. Analgesia with morphine in amphibians has little effect on motor activity, feeding or general behavior, but can be blocked with naloxone. High doses of morphine (320-640 mg/kg) produce hyper-responsiveness to sensory stimuli similar to the reaction produced in cats given high doses of morphine (Pezalla, 1983).

In Nile crocodiles, *Crocodylus niloticus*, nociception has been studied using a hot plate test, a formalin test where formalin is injected into the hindlimb, and a capsaicin instillation test where capsaicin is instilled onto the eye (Kanui, *et al,* 1990). The hot plate test was the most reliable. This test was then used to study the analgesic effect of morphine and pethidine in crocodiles. Morphine at 0.05-1.0 mg/kg intracoelomically (i.c.o.) induced a significant increase in response latency at all doses studied with a maximum effect observed at a dose of 0.3 mg/kg (Kanui and Hole, 1992). Onset of action was 30-120 min lasting the entire 6 hr of the study. The crocodiles weighed only 619 ≠325 g. Pethidine also induced an increase in response latency at 1-8 mg/kg i.c.o. with a maximum response at 2 and 4 mg/kg (different responses - escape maneuver vs. foot lift). The onset of action was 30-180 min lasting the entire 6 hr of the study. These doses are considerably lower than expected. In mice and rats, doses more than 20 times greater are required to observe increased response times. Additionally, both drugs demonstrated a dose dependent response but also reached a plateau effect.

A tail-flick apparatus was used in a study examining the neural mechanism underlying tonic immobility in green anoles, *Anolis carolinensis* (Mauk, *et al,* 1981). Morphine at 5 mg/kg i.c.o. caused a significant increase in the latency of the response. Since the purpose of the study was to study tonic immobility, other doses were not evaluated.

In amphibians, systemic administration of the mu agonists fentanyl, levophanol, methadone, morphine, meperidine and codeine; partial mu agonists buprenorphine; and kappa agonists U50488, nalorphine, and bremazocine produced dose dependent analgesia for at least 4 hr with a gradual increase in effect during the first hour reaching a peak at 60-90 min (Stevens, *et al,* 1994). The order of analgesic potency in amphibians was fentanyl > levophanol > U50488 > methadone > bremazocine > morphine > codeine > nalorphine which parallels the potency of mu agonists found in mammals. Kappa agonist potency was not similar to that found in mammals, potentially related to the difference in concentration of kappa receptors in amphibians compared to mammals (Stevens, *et al,* 1994).

The pharmacokinetics of synthetic opioids differs greatly between amphibians and mammals. Amphibians appear to require higher doses as compared with rodents; however, the algesiometric tests used are different (hot plate vs. acetic acid) (Stevens, *et al,* 1994). Endogenous opioids play a role in hibernating in both mammals (Margules, *et al,* 1979) and amphibians (Steven and Pezalla, 1989). Cold adapted frogs returned to room temperature showed a naloxone attenuated increase in nociceptive threshold (Steven and Pezalla, 1989).

Mammals, amphibians, and reptiles have the same anatomical and functional characteristics in $\alpha 2$ adrenoceptor mediated analgesia (Gans and Ulinski, 1992; Stevens, 1992). They produce a dose dependent sedation in mammals; however, 10 mg/kg of dexmedetomidine or of clonidine in frogs provides analgesia but does not produce sedation. Dose dependent analgesia following systemic (Brenner, *et al,* 1994) and intraspinal (Stevens and Brenner, 1996) administration of dexmedetomidine, clonidine, epinephrine, and norepinephrine was demonstrated using the acetic acid test. The effects lasted 6-8 hr following systemic administration and 4 hr following

intraspinal administration. The relative potency following systemic administration is dexmedetomidine > epinephrine > norepinephrine > clonidine. Following spinal administration, all were equivalent except for clonidine which was not as potent as the others.

Ketamine produces profound somatic analgesia but little visceral analgesia in mammals. A proposed mechanism for its analgesic effects in amphibians may be through one of the opioid receptors since naloxone significantly blocks the effects of ketamine on nerves and skeletal muscle (Lee and Frank, 1991).

Pain perception in reptiles and amphibians is, therefore, likely analogous to mammals. When performing invasive and painful procedures appropriate anesthesia and analgesia should be administered. Though specific doses have not been established in clinical trials, research indicates a potential benefit to the use of opioids in reptiles and amphibians. Other analgesics such as $\alpha 2$ agonists and ketamine also have analgesic benefits in reptiles and amphibians.

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