Opiate processes in poultry

Melinda H. Sheehan[1,2]

[1]St. Elizabeth's University, School of Public Health, Clinical Medicine Program, Bratislava, Slovakia
[2]State University of New York Old Westbury, Neuroscience Research Institute, Old Westbury, New York, USA

Submitted: 3 November 2009
Accepted: 12 November 2009

Arch Med Sci 2009; 5, 4: 626-636

Corresponding author:
Dr. Melinda H. Sheehan
Neuroscience Research Institute
SUNY College at Old Westbury
P.O. Box 210, Old Westbury, NY 11568, USA
Phone: 001-516-876-4883
Fax: 001-516-876-2727
E-mail: msheehan@sunynri.org

Abstract

The chicken is one of the most common widespread domesticated animals in the world. We rely on them as a primary source of food and consume both their meat and eggs; therefore their overall health should be of great concern and relevance. In this regard, opioid peptide and opiate alkaloid processes in mammals and invertebrates have functions that transcend their actions as anti-nociceptive agents. Many reports document their presence and action in many different physiological processes, such as neural, immune, vascular and digestive systems. Since poultry are the closest living animals to dinosaurs we examined these animals to determine if they too had an endogenous opioid system that was diffuse in function and presence. Indeed, poultry contain such an opioid system, which includes many physiological systems beside neural processes involved in pain perception. In this assessment it was also discovered that poultry opioid processes display novel idiosyncrasies that can be considered paradoxical when compared to mammalian processes. Interestingly, from the relatively sparse literature, the variations occur within the realm of pain perception and responses.

Key words: opioid peptides, morphine, nitric oxide, opiate receptors, immune, poultry, immune, central nervous system, hyperalgesia.

Introduction

The production and manufacturing of healthy poultry that is not profuse with disease and is harmless to consume is nothing less than a major accomplishment of modern agriculture. Loss of poultry population to disease represents a serious component of the cost to produce poultry. Disease is at the forefront of impediments for increased productivity [1-3]. This not only referring to increased mortality as a symptom of disease, but also referring to the increase in production costs resulting from the disease, e.g. vaccinations, veterinary care, contamination of plant sites, decreased egg production. The rate of poultry consumption, for example, in the United States has more than tripled since the 1960's. In 1960, Americans consumed 28 pounds of chicken per capita and in 2004 it was reported that figure reached 85 pounds (UDSA, ERS). With a supply and demand system as strong as this, it is extremely imperative and relevant to closely monitor the industry, including the health and welfare of the animals that create the industry. In the United States, the Hatch Act of 1887 provided the USDA the first mandate to sponsor extramural research. Interestingly, in 2007, only 0.04% of the Department of Agriculture budget was allocated to its competitive grants program for research that directly involves important domestic agricultural animals [4].

Morphine and other chemically related opiate alkaloids represent classical and reliable analgesic principles for management of severe pain associated with disease [5, 6]. Importantly, this fact alone may enable this system to serve as a sentinel of health. A cadre of cellular and physiological effects associated with the pharmacological administration of morphine and its cognates is documented by a substantial body of accumulated evidence that lie outside the realm of anti-nociception and appear to be mediated by indirect and "non-traditional" mechanisms. Opioids and opiates are neuropeptides and opiate alkaloids, respectively, which serve in many animal systems as neurotransmitters, neuromodulators and chemical messengers in systems other than the nervous system [7, 8]. Endogenous opioid peptides and opiate alkaloids modulate an array of regulatory functions such as pain perception, stress mechanisms, immune response and suppression, respiration as well as cardiovascular and diuretic functions [9-11]. Opioid peptides and their receptors are implicated in an array of behavioral and physiological functions in avian species, including reproduction, endocrinology, water balance, social behavior and pain stimulus [12-14]. This information coupled with the knowledge that various chicken tissues contain highly opioid receptors designed to bind endogenous opioids may serve as a means to understanding the overall health and wellness not only in the chicken but as a potential model for all vertebrates. Examining the mechanisms of opioid peptides in domestic poultry may provide a key to unlocking unique therapeutic opportunities through the action of opioid, for example, immunopharmacology.

Distribution of opioid binding sites in the chicken brain

Opioids are widely known to be inhibitory neurotransmitters, their receptor subtypes, denoted as mu, kappa, and delta are distributed widely throughout the central nervous system in vertebrates [12]. Immunocytochemical methods and radioimmunoassay demonstrated the existence and distribution of the opioid peptides in the avian brain over two decades ago. Autoradiographic studies have proven this system of mu, kappa, and delta subtypes intact in the domestic chicken [15]. These methods established endorphin, dynorphine and enkephalin in the forebrain, midbrain and pituitary gland [16-21]. Immunoreactive enkephalin peptides are present in the domestic fowl brain days before hatching [22]. Opioid peptides influence an array of physiological and behavioral responses in birds. Examples include embryonic motility [23], body temperature [24], pain sensitivity [25], aggressive and sexual behavior [26], vocalization [27], ingestive behaviors [24, 28] and endocrine regulation (Figure 1) [29-31].

The maximum binding capacity and affinity of [^{3}H] diprenorphine was assessed in chicken brain section homogenates [32]. Binding sites were found distributed among six regions of the brain namely the frontal cortex, lateral septum, striatum, amyglada, hippocampus and hypothalamus [32]. It has been established that the limbic system, amygdale, striatum and hippocampus in birds are involved in emotion, motivation and learning [33]. The hippocampus in birds is involved in food storage behavior and homing [33]. Distribution of opioid binding sites decrease in concentration from frontal cortex, striatum, amygdala regions and from ten

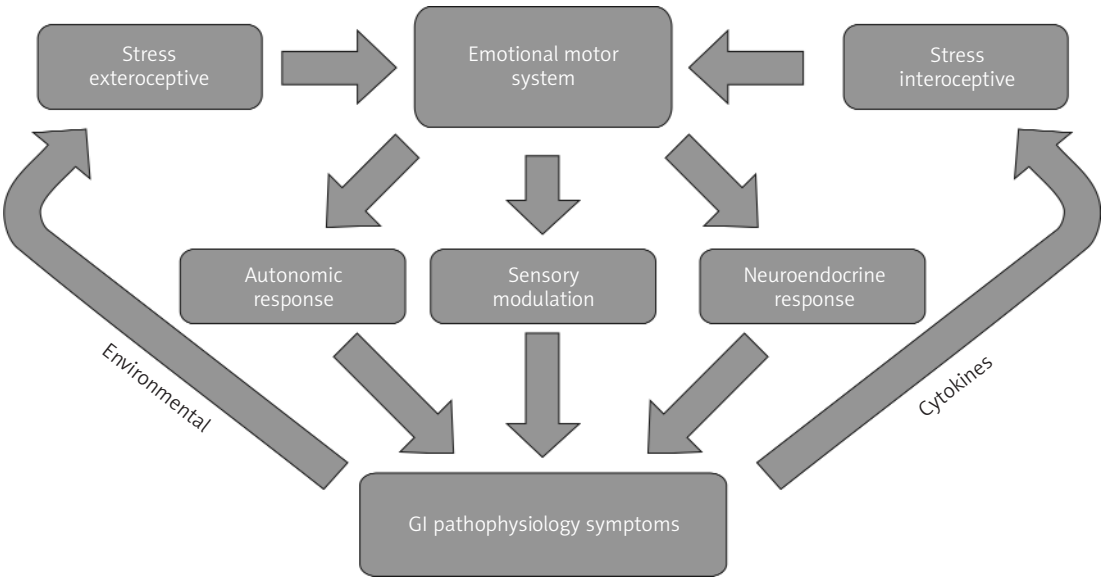

Figure 1. Multiple pathways affected/mediated/regulated by opioid/opiate receptors and their cognates in domestic chicken

day old chicks to adulthood in female chickens. Opioid binding sites in the hippocampus, hypothalamus, and lateral septum were constant for the female age groups [32]. Male chickens do not experience a reduction in opioid binding sites in the aforementioned brain regions from ten days old until adulthood that the females experienced during that same time course[32].

Substance P is one of the many important complex molecules involved in pain perception [34, 35]. The sensory function of substance P relate to the transmission of pain information into the central nervous system. Further it also has potent effects as a vasodilator. Substance P induced vasodilatation is dependent on constitutive nitric oxide release [36]. Substance P is present in the chicken dorsal horn [37]; this same group demonstrated the presence of opioid receptors in the same neural region. The broad distribution of opioid peptides and receptors in the brain and throughout the body suggest that they serve multiple purposes, again as neurotransmitters and neuromodulators.

Prenatal exposure to opiates

The effects of opiate exposure and withdrawal on neuroendocrine function in chicks have not been extensively studied, however it is widely known and accepted that the chick responds to immune system stimulation induced by lipopolysaccharide (LPS) in a similar manner as mammals by stimulating sickness behaviors and cognitive deficits as well as an increase in body temperature [38-40]. Prenatal opiate exposure in chicks suppresses fever response and hypersensitivity in response to LPS treatment [41]. Repressed fever response to LPS has been well studied in the rodent but not as well in the avian system. Nearly equivalent results were achieved after administration of opiates prenatally to chicks, namely after exposure to interleukin (IL)-6, IL-β, and tumor necrosis factor (TNF)-α [41].

Prenatal exposure of chicks to morphine significantly impairs long term memory in the passive avoidance learning paradigm [42]. Short term memory and long term memory retention times were assessed for chicks exposed to morphine prenatally. Morphine (20 mg/kg) was injected into the airspace of the egg on days 12 to 16 of gestation, once hatched, the chicks underwent one-trial passive avoidance learning task. The task exploited the chick's innate tendency to peck at objects. While the results showed no difference in the number of spontaneous pecks between control groups and morphine treated groups during pre-training, the morphine treated groups had significantly impaired memories at the long term training time point. These experiments led to speculation that prenatal exposure to morphine may affect the process of protein synthesis and the expression of long term potentation, affecting the neural molecular cascade including NMDA-glutamate receptors or membrane protein phosphorylations [42].

Osteogenic processes are also likely regulated in part by endogenous opioids in chick embryos [43]. Embryonic osteogenesis was studied by evaluating bone growth in relation to opioid receptor blockade during different stages of embryonic development. In the Liskov *et al.* study, morphological analysis of the femoral bone after hatching showed that naloxone did significantly alter the number of osteoblasts, mitotic cells, osteocytes and the thickness of the perichondrial bone cuff [43].

Evidence for hyperalgesic effects of morphine and paradoxical pain

Opiates, typically employed to alleviate pain can often cause numerous side effects including nausea, respiratory depression, constipation addiction, and withdrawal [44-47]. Additionally, opiates can actually elicit pain, also known as opiate induced tactile hyperalgesia that may not be associated with the initial pain complaint [25, 47-54]. One explanation of this nontraditional response to opiates is that it is caused by a compensatory response to the inhibition produced by activation of the μ opioid receptor, causing hyperactivity of the system [53, 55]. Multiple opioid receptor subtypes also participate in variations of behavior and responses to stimuli, including in poultry [56-59].

Morphine initiates a signal through a G protein cascade (Figure 2) [60-65]. Morphine first interacts with the opiate receptor, which changes its conformation and interactions with a G protein within the cell causing it to expel its GDP molecule in exchange for a GTP. The G protein then breaks into 2 segments, α and β. The subunits diffuse along the cell membrane until they reach and bind their targets. Morphine also increases conduction through potassium channels, decreases conduction through calcium channels and inhibits the membrane bound enzyme adeylyl cylase, it is this trifecta that accounts for the blunting of the signal system that transmits pain (Figure 2) [62-64, 66].

Prior work from our laboratory has determined that μ_3 and μ_4 opiate receptors are selectively activated by morphine and morphine-related opiate alkaloids and are completely unresponsive to a wide variety of endogenous opioid peptides [11, 67]. Thus far this pertains to human and invertebrate tissues. These novel receptors are coupled to nitric oxide release (NO), associating morphine's actions with processes that transcend pain and include NO [53, 54, 56, 68-71]. Thus, we have defined a cellular regulatory circuit by which endogenous morphine activates its cognate μ_3 and μ_4 opiate receptors. The

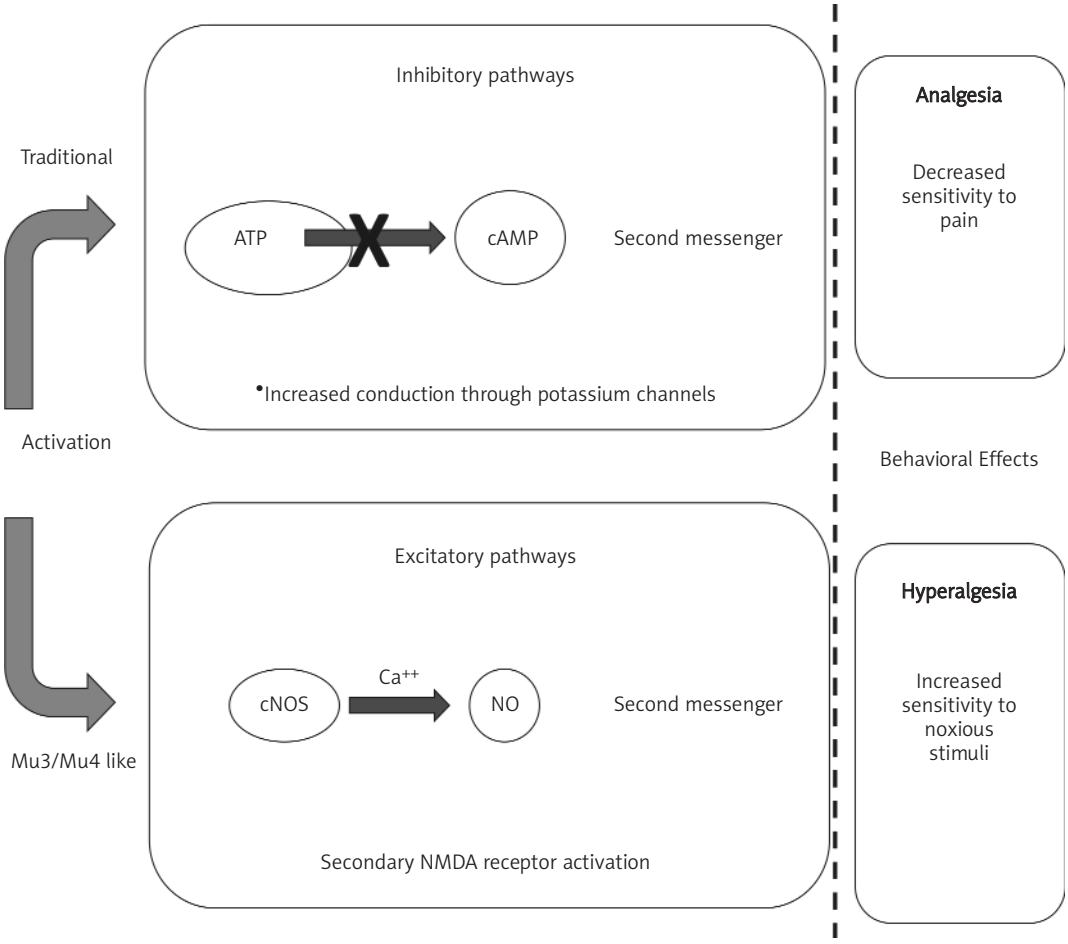

Figure 2. Inhibitory and excitatory pathways are activated by traditional and Mu3/Mu4 -like opiate receptors, respectively. Activation of the traditional receptor results in analgesia after the inhibition of adenylate cyclase stops the production of second messenger cAMP from ATP. WE surmise, based on mammalian studies, hyperalgesic effects are achieved through activation of the specialized mu3/mu4 like receptor, which cause Ca^{++} dependent cNOS to synthesize the second messenger nitric oxide, as well as activation of NMDA receptors which results in non selective cation transport through cell membranes, making Ca^{++} available for cNOS

evolutionary and biological significance of the μ_3 opiate receptor is further enhanced by our recent finding that it is present on human stem cells as is its variant, the μ_4 opiate receptor, in the absence of the traditional μ opioid receptor [67]. These specialized opiate receptors are not coupled to adenylyl cyclase activation [53], therefore excitation rather than traditional inhibition ensues cellularly (Figure 2).

There are a multiple of tests available to assess the antinociceptive action of various drugs. Stimulus type varies widely among tests, including mechanical (pinch or pressure), chemical (formalin, acetic acid), thermal (tail-flick, hot plate) stimulations. Morphine, the prototypical opiate analgesic has antinociceptive on each of the aforementioned tests [49, 57]. Non typical morphine effects have been reported pertaining to poultry undergoing thermal and chemical stimulation. Rather than the classical hypoalgesic response to morphine, White Leghorn and California White cockerels responded contradictory to this manner [25, 48-50]. The animals appeared to be experiencing sedation after morphine exposure, however when exposed to the noxious stimuli, they were more quickly to react than the control chickens that were administered saline in the stead of morphine, naloxone was able was able to block the hyperalgesic effects of morphine [48, 49]. Morphine induced hyperalgesia has also been demonstrated in rodents however it only occurs after chronic morphine exposure, and is preceded temporally by morphine's typical analgesic effects [72, 73].

The readily evident hyperalgesic effects of morphine in the chicken are not only uncommon, but paradoxical as well. Hyperalgesia in the domestic fowl was also observed in the developmental stages of the animal. A jump latency test was used to determine the effects of morphine on young chicks, at ages three, five, seven and

fourteen days. This group determined, consistent with earlier research that while chicks ages, three, five and seven days were unaffected by morphine in terms of the jump latency test, they were affected typically in terms of respiration and rates decreased. Also, the results demonstrated an age related jump latency increase [50]. The effect morphine has on these domestic fowl is both counterintuitive and paradoxical. Not only does the unexpected effect occur on the first exposure to morphine, but the animals experiencing morphine in this atypical way also display the typical temporal effects. One explanation of this nontraditional response to opiates is that it is caused by a compensatory response to the inhibition produced by activation of the μ opioid receptor, causing hyperactivity of the system [55].

Stress induced analgesia

Stress induced analgesia is an adaptive pain suppression coping behavior (mechanism) [74-77]. Endogenous analgesia occurs among chickens experiencing severe tonic pain [74]. Pain can be mimicked experimentally. Birds often suffer from gouty arthritis; this pain can be mimicked experimentally by intraarticular injection of sodium urate (SU) [74, 78, 79]. In one similar experiment, chickens were injected with SU in one ankle joint and frequency of leg lifts were recorded over time as a measure of pain. Notably, after a period of 45 to 19 min, the birds appeared hypoaesthetic, spending most of their time sitting and dosing, closing their eyes with drooping head and tail feathers [74]. The aforementioned behaviors are all common reactions to analgesic drugs, indicating an endogenous analgesic consequence. The same group also conducted a similar experiment in which they compared the results of their previous experiments where the experimental birds were kept in simple battery cages, to experimental birds kept in novel cages [74]. The birds in the novel cages received the same SU injections and observations were made at the same intervals. In the case of this experiment a novel cage contained a variety of stimuli and was considered enriched when compared to a 450 × 360 × 550mm battery cage. Lameness in the novel cages was significantly less than in the familiar battery cages. The absence of pain related behavior does not necessarily indicate absence of pain; results suggest that, like humans, the avian's motivational state can contribute to the coping of pain by altering the perception of pain [49, 51, 74, 75]. This ability of birds to demonstrate hypoalgesia may explain why commercial caponization doesn't produce behavioral evidence of pain [74], and industry is reluctant to stop this otherwise brutal procedure.

Opioid usage for relief of articular pain in domestic fowl

Spontaneous incidences of arthropathies occur frequently among the heavy breeds of domestic poultry [80-82]. Traditionally, opiates like morphine are injected or ingested systemically rather than locally administered, however studies in rats have shown that local administration of morphine or Met-enkephalin was markedly more effective than the local anesthetic lidocaine [83]. Trials among avian species make use of the microcrystalline sodium urate induced arthritis pain model to measure the analgesic effects of various opioid agonists [84]. Opioid analgesics, morphine sulfate, fentanyl citrate, and bupreorphine hydrochloride were all injected intraarticularly to subjects after injection induced arthritis symptoms were seen. None of the opioid agonists had considerable effect on pain related behavior and therefore it was concluded that opioids with a high affinity for the mu receptor had little effect when injected intraarticularly [84] since it was shown that kappa-opioid antagonists were very effective when injected intraarticularly [85]. In speculation, it is likely that all opioid receptor subtypes are not present in the joints. Although morphine binds primarily to mu opioid receptors, experiments confirm opioids such as morphine exert their effects through multiple opioid receptor subtypes with a lesser affinity. The effects of morphine or other opioids may be altered if they bind to kappa receptors before mu [59]. Selectivity of opioid receptor ligands for their cognate receptor will affect the physiological manifestations of the opiate [86, 87].

Food intake behavior

Stimulatory mechanisms for feeding in chicks are anomalous and differ from mammalian feeding stimulatory mechanisms [88]. Feeding mechanisms are important for newly hatched chicks, since they must be able to recognize and ingest food in order under their own free will to survive. It is therefore relevant to reveal the feeding behavior stimulatory mechanisms for feeding. μ-Opioid receptor antagonists, have been shown to inhibit feeding behavior in meat-type chicks, demonstrating the important roles endogenous ligands for μ receptors have in feeding regulation [89]. Results of intracerebroventricular injections of mu, kappa and delta agonists in broiler chicks suggest delta and kappa opioid receptor agonists induce hyperphagia while mu opioid receptor agonists generate a contrasting result of suppressed food consumption [89]. This further elucidates the paradoxical effects of the opioidergic involvement in the chicken while suggesting opioids do play a major role in feeding mechanisms of this precocial

animal that depends on instinct to ingest food voluntary rather than under the forceful concern of a mammalian parent.

Opioid involvement in developing social processes

Similar to many mammalian species, chicks display a well characterized stress response when separated from their group, this response includes distress vocalizations, hyperthermia and analgesia [27, 75, 90, 91]. Also, a number of studies associate the involvement of the opioid system with social attachment processes [75, 90, 92, 93]. Opioid receptor agonists attenuate separation stress, while antagonists have enhanced distress calls in chicks [90]. Only the mu opioid agonist, DAMGO was able to attenuate separation stress vocalizations, suggesting mu opioid receptor involvement and not kappa or delta [93]. However, in studies evaluating changes in this comfort response by facilitation of opiate, serotonin, and acetylcholine activity, using agonists, morphine, quipazine and pilocarpine respectively, only morphine was found to magnify the comforting effect of mirrors [90]. The effects of naloxone on contact comfort, and the acquisition and expression of imprinting were evaluated to deduce that opioid blockade reduced all these measures of social comfort. It was concluded that endogenous opioid activity contribute to the comfort which animals derive from their social environment [90]. It would be interesting in the future to compare estrogen processes in poultry given the morphine parallelisms and their ability to release constitutive nitric oxide release [94-96].

The role of opioid receptors in mediating cardioprotection

Opioid receptors appear to play important roles in protection against ischemia-reperfusion injury in several bodily systems in many vertebrates, including in the central nervous system [97], jejunum [98], kidney [99], and heart [100, 101]. Also, in whole animal models opioid receptor antagonists have effective cardioprotective effects [102]. Cardioprotection was mediated in chicken embryonic cardiomyocytes via mu opioid receptor signaling using baicalein (5, 6, 7-trihydroxy-2-phenyl-(4H)-1-benzopyran-4-one), a flavanoid compound derived from the root of Scutellaria baicalenis, Georgi, which exerts anti-inflammatory [103] and anti-oxidative [104] properties. Thus, baicalein in chicks protects cells against hypoxia-reoxygenation injury [13]. After treatment with baicalein, opioid receptor presence was determined using polymerase chain reaction. Mu-opioid receptor antagonist β-funaltrexamine was used block the mu-opioid receptor and determine presumed signal transduction pathways [13].

Avian immune system

The immune system in birds as well as all animals is a highly complex physiological system that has been the focus of many studies. A bird's ability to fight off infection, i.e., viral or bacterial, will depend on the overall condition and well being of the animal as well as its level of immunity [33, 105, 106].

Experiments were conducted on chickens to examine whether melatonin induced opioids are involved in the immunomodulatory action of melatonin. While the immunomodulatory properties of melatonin have been investigated in mammals, the underlying mechanisms are still largely misunderstood. In mammals some immuno-modulatory effects of melatonin are mediated by opioids [107, 108]. Melatonin has been shown to be involved in the regulation of both cellular and humoral immunity [107]. Melatonin not only stimulates the production of natural killer cells, monocytes and leukocytes, but also alters the balance of T helper (Th)-1 and Th-2 cells mainly towards Th-1 responses and increases the production of relevant cytokines such as interleukin (IL)-2, IL-6, IL-12 and interferon-γ [107]. In chickens results were achieved by inducing peritonitis by injecting thioglycollate, half of the chickens were pretreated with melatonin and some of these birds also underwent pretreatment with opioid antagonist naltrexone [109]. Peritoneal leukocytes were counted at different time intervals, demonstrating melatonins biphasic effect on the induced peritonitis. Initial effects of melatonin were that it blocked the development of peritonitis, by decreasing the number of peritoneal leukocytes, the authors presumed the inflammation was initially blocked by melatonin scavenging free radicals. Hours later a proinflamatory effect was seen after the increased expression of the proenkephalin gene in the peritoneal leukocytes. These effects were blocked by naltrexone, suggesting involvement of an opioid mechanism [109]. Melatonin has been shown to cause synthesis of β-endorphin and dynorphin in chicken immune cells [109]. This same group also demonstrated exogenous opiate alkaloids stimulated pro-inflammatory effects on the induced peritonitis in chickens. Majewski provided an explanation that suggests the different effects between mammals and birds in the action of melatonin on immunity could be attributed to the different immuno-regulatory activity of endogenous opioids [109].

Discussion

As one of the largest and most diverse protein families, the GPCR superfamily mediates important regulatory roles in a multiplicity of biological and pathological processes such as development and

proliferation, neuromodulation, angiogenesis, metabolic disorders, inflammation, and viral infection [62, 65]. The traditional GPCR protein has three intracellular and three extracellular loops linked to a 7 TMH domain core. The three types of opioid receptors cloned are designated mu, delta, and kappa, and they are standard 7 TMH domain GPCRs. They share a high degree of sequence homology and are most divergent at the N- and C-termini [110]. Although mechanisms of action for the three types of opioid receptors may be somewhat similar, selectivity for the different ligands depends on variations with the N- and C- terminal regions and the cellular loops. All three major subtypes of opioid receptors use cyclic AMP as their second messenger [66]. Activation of these receptors inhibits the production of adenyl cyclase, which catalyzes the formation of cyclic AMP from ATP. The inhibitory effects of the opioid receptors are mediated by inhibitory guanine triphosphate (GTP) binding regulatory protein [111]. The broad distribution of opioid peptides and receptors in the chicken brain and throughout the body suggest that they serve multiple purposes as neurotransmitters, neuromodulators and chemical messsengers.

The fact that the domestic chicken displays some paradoxical opioid actions may be an atavistic regression linking domestic chickens to their prehistoric predecessors, dinosaurs. Although domestic chickens have mu, kappa and delta opioidergic systems at play, responses to stimulation or repression of these systems does differ notably from other vertebrates. Evidence for paradoxical pain in chickens is the most notable difference among vertebrates (Figure 2). Morphine is best known for its analgesic effects, these effects are highly altered in the domestic chicken. Interestingly, stress induced analgesia seems to be more effective than morphine for treatment of arthropathic pain for domestic chickens. Further compelling evidence of the hyperalgesic response to morphine is particularly baffling since the animal does exhibit the typical side effects of morphine. Amonst prenatal chicks, endogenous opioids play a role in cognition and learning, thermoregulation, as well as osteogenic processes, suggesting the primitive role this system represents for these vertebrates.

Functionally, accumulated data suggest that μ_3 and μ_4 opiate receptors may be representative members of primordial family if G-protein coupled receptors responsible for autocrine/paracrine regulation of cellular metabolic activity via local circuit Ca^{++} gating and NO feedback inhibition [110]. In combination with complementary analyses demonstrating *de novo* synthesis of chemically authentic morphine by diverse classes of animal cells [112], the establishment of a "morphinergic" regulatory pathway mediated by morphine and naturally expressed active morphine congeners such as its 6-glucuronide conjugate and their cognate μ_3 and μ_4 receptors receives significant validation. However, given the paradoxical actions of morphine in chicken this system may not be present.

The observed hyperalgesia, may actually be misinterpreted hypersensitivity to noxious stimuli as a survival mechanism of an already injured animal. The chickens paradoxical hyperalgesic response to low dose exogenous morphine may be related to this primitive survival mechanism. The paradoxical response of the chicken to morphine is most simply as follows: the animal appears drowsy while experiencing a heightened motor response to pain. An animal in such an inebriated state would not be able to defend itself if it experienced further painful stimulus, but the chicken however, reverts to a fairly obvious survival mechanism while remaining somewhat aware with motor responses functioning, if not for its own survival for group survival. The survival value for of the hyperactive response to morphine rather than inhibition is possibly due to varying primitive interactions among opioid receptors, NMDA glutamate receptor, and Ca^{++} and K^{+} channels, all found to be involved in pain perception [53].

Conclusions

Opiate receptors and their natural cognates, opiate alkaloids may have a profound effect on an animal's maturation and other biological processes. It has already been demonstrated that animal cells have the ability to synthesize chemically authentic morphine via multienzyme mediated processes restricted by numerous feedback inhibitory steps [112, 113]. There is a body of evidence demonstrating that opiate alkaloids such as morphine, morphine-3-glucuronide and morphine-6-glucuronide as well as the putative precursor molecules (thebaine, salutaridine, norcocolarine, reticuline, tetrahydropapoverine and codeine) exist in vertebrates, including their modulatory enzymes as actions associated with substance abuse [114-122]. It is not just the higher order vertebrates that express these signal molecules since they have been found in the invertebrate *Mytilus edulis* [123-126]. These primitive receptors and the morphinergic signal molecules associated with them are likely affecting many underlying biochemical processes, including endocrine and within the scope of substance abuse[127-130]. Multiple and non-mutually exclusive pathways lie behind these primitive processes. These processes have not been examined in poultry instead the literature contains, in part, paradoxes. When fully understood, these processes will likely prove to have roles towards understanding processes of benefit to the commercialized food industry.

Acknowledgments

Melinda H Sheehan, is a Ph.D candidate at St. Elizabeth's University, School of Public Health, Clinical Medicine Program, Bratislava, Slovakia and wishes to thank Dr. Richard M. Kream, recipient of the SUNY Empire Professorship for his encouragment and support. I would also wish to thank Ms Danielle Benz for her critical help in preparing the manuscript.

References

1. Lamont SJ. New advances in controlling poultry disease. Br Poult Sci 2004; 45 Suppl 1: S11-2.
2. Marangon S, Busani L. The use of vaccination in poultry production. Rev Sci Tech 2007; 26: 265-74.
3. Vieira AR, Hofacre CL, Smith JA, Cole D. Human contacts and potential pathways of disease introduction on Georgia poultry farms. Avian Dis 2009; 53: 55-62.
4. Roberts RM, Smith GW, Bazer FW, et al. Farm animal research in crisis. Science 2009; 324: 468-9.
5. Merlin MD. On the trail of ancient opium poppy. London: Associated University Press; 1984.
6. Stefano GB, Fricchione GL, Goumon Y, Esch T. Pain, immunity, opiate and opioid compounds and health. Medical Science Monitor 2005; 11: MS47-53.
7. Stefano GB, Scharrer B. Endogenous morphine and related opiates, a new class of chemical messengers. Adv Neuroimmunol 1994; 4: 57-68.
8. Hokfelt T, Broberger C, Xu ZQ, Sergeyev V, Ubink R, Diez M. Neuropeptides – an overview. Neuropharmacology 2000; 39: 1337-56.
9. Stefano GB, Scharrer B, Smith EM, et al. Opioid and opiate immunoregulatory processes. Crit Rev in Immunol 1996; 16: 109-44.
10. Stefano GB. Autoimmunovascular regulation: morphine and anandamide stimulated nitric oxide release. J Neuroimmunol 1998; 83: 70-6.
11. Cadet P, Mantione KJ, Stefano GB. Molecular identification and functional expression of mu3, a novel alternatively spliced variant of the human mu opiate receptor gene. J Immunol 2003; 170: 5118-23.
12. Akil H, Watson SJ, Young E, Lewis ME, Khachaturian H, Walker JM. Endogenous opioids: biology and function. Annu Rev Neurosci 1984; 7: 223-55.
13. Tu H, Yen HT, Cheng HW, Chiu JH. Baicalein protects chicken embryonic cardiomyocyte against hypoxia-reoxygenation injury via mu and delta but not kappa opioid receptor signaling. Eur J Pharmacol 2008; 588: 251-8.
14. Stansfield SC, Cunningham FJ. Attenuation of endogenous opioid peptide inhibition of [Gln8]luteinizing hormone-releasing hormone secretion during sexual maturation in the cockerel. Endocrinology 1988; 123: 787-94.
15. Csillag A, Bourne RC, Stewart MG. Distribution of mu, delta, and kappa opioid receptor binding sites in the brain of the one-day-old domestic chick (Gallus domesticus): an in vitro quantitative autoradiographic study. J Comp Neurol 1990; 302: 543-51.
16. Bayon AL, Koda E, Battenberg RA, Bloom FE. Regional distribution of endorphin, met5 -enkephalin and leu5 -enkephalin in the pigeon brain. Neuroscience Letters 1980; 16: 75-80.
17. de Lanerolle NC, Elde RP, Sparber SB, Frick M. Distribution of methionine-enkephalin immunoreactivity in the chick brain : an immunohistochemical study. J Comparative Neurol 1981; 199: 513-33.
18. Martin R, McGregor GP, Halbinger G, Falke N, Voigt KH. Methione5-enkephalin and opiate binding sites in the neurohypophysis of the bird, Gallus domesticus. Regulatory Peptides 1992; 38: 33-44.
19. Mikami SI, Yamada S, Okada N, Yanaihara N. Localization of substance P-, met-enkephalin- gastrin releasepeptide (GRP)- and glucagon- immunoreactive neurons in the hypothalamus and telencephalon of the Japanese quail. Biomed Res Suppl 1983; 4: 103-16.
20. Ryan SM, Arnold AP, Elde RP. Enkephalin-like immunoreactivity in vocal control regions of the zebra finch brain. Brain Res 1981; 229: 236-40.
21. White JD, Krause JA, Karten HJ, Mckelvz JF. Presence and ontogeny of enkephalin and substance P in the chick cililary ganglion. J Neurochem 1985; 45: 1319-22.
22. Blahser S. Topography, ontogeny and functional aspects of immunoreactive neuropeptide systems in the domestic fowl. Tokyo: Japan Scientific Societies Press 1983; 11-24.
23. Maderdrut JL, Reitzel N, Okado N, Oppenheim RW. Behavioral analysis of opiate-mediated inhibition in the early chick embryo. Neuroscience 1985; 16: 405-16.
24. McCormack JF, Denbow DM. Feeding, drinking and temperature responses to intracerebroventricular beta-endorphin in the domestic fowl. Peptides 1988; 9: 709-15.
25. Sufka KJ, Hughes RA, Giordano J. Effects of selective opiate antagonists on morphine-induced hyperalgesia in domestic fowl. Pharmacol Biochem Behavior 1991; 38: 49-55.
26. Furukawa Y, Kotegawa T, Tsutsui K. Effects of opioid peptides on the electrical activity of preoptic and hypothalamic neurons in the quail brain. J Experim Zoology 1995; 273: 96-103.
27. Panksepp J, Vilberg T, Bean N, Coy D, Kastin A. Reduction of distress vocalization in chicks by opiate-like peptides. Bratin Research Bulletin 1978; 3: 663-7.
28. Deviche P, Schepers G. Naloxone treatment attenuates food but not water intake in domestic fowl. Psychopharmacology 1984; 82: 122-6.
29. Stansfield SC, Cunningham FJ. Modulation by endogenous opioid peptides of the secretion of LHRH from cockerel (Gallus domesticus) mediobasal hypothalamic tissue. J Endocrinol 1987; 114: 103-10.
30. Stansfield SC, Cunningham FJ. Involvement of opiate receptor subtypes in the modulation of LHRH secretion by the cockerek (Gallus domesticus) mediobasal hypothalamus in vitro. J Endocrinol 1987; 114: 111-7.
31. Fan Y. Opioid peptides and noradrenic systems regulate cGnRH-I in avian species. 1996.
32. Parvizi N, Grossmann R. Opioid receptors in the chicken brain. 2005; 1-181.
33. Kuenzel WJ. The autonomic system of avian species. In: Whittow GC (eds.). Sturkie's avian physiology. 5 ed. San Diego: Academic Press 2000; 101-22.
34. McHugh JM, McHugh WB. Pain: neuroanatomy, chemical mediators, and clinical implications. AACN Clin Issues 2000; 11: 168-78.
35. Aghabeigi B. The pathophysiology of pain. Br Dent J 1992; 173: 91-7.
36. Bossaller C, Reither K, Hehlert-Friedrich C, et al. In vivo measurement of endothelium-dependent vasodilation with substance P in man. Herz 1992; 17: 284-90.
37. Kawate T, Sakamoto H. Immunohistochemical study of delta and mu opioid receptors on synaptic glomeruli with substance P-positive central terminals in chicken dorsal horn. Neurosci Res 2005; 53: 279-87.

38. Schrott LM, Sparber SB. Embryonic "binge" cocaine exposure alters neural-immune and neural-endocrine interactions in young chickens: Involvement of 5-HT2 receptors. Developmental Brain Research 2001; 130: 99-107.
39. Sell KM, Crowe SF, Kent S. Lipopolysaccharide induces memory-processing deficits in day-old chicks. Pharmacol Biochem Behavior 2001; 68: 497-502.
40. Johnson RW, Curtis SE, Dantzer R, Bahr JM, Kelley KW. Sickness Behavior in Birds caused by peripheral or central injection of endotoxin. Physiology and Behavior 1993; 53: 343-8.
41. Schrott LM, Sparber SB. Suppressed fever and hypersensitivity responses in chicks prenatally exposed to opiates. Brain Behavior and Immunity 2004; 18: 515-25.
42. Che Y, Sun H, Tan H, Peng Y, Zeng T, Ma Y. The effect of prenatal morphine exposure on memory consolidation in the chick. Neurosci Lett 2005; 380: 300-4.
43. Liskov AV, Solnyshkova TG, Frolov BA, Pavlovichev SA. Effect of naloxone hydrochloride on osteogenesis in chick embryos. Bull Exp Biol Med 2005; 139: 331-3.
44. Hong D, Flood P, Diaz G. The side effects of morphine and hydromorphone patient-controlled analgesia. Anesth Analg 2008; 107: 1384-9.
45. Stanley G, Appadu B, Mead M, Rowbotham DJ. Dose requirements, efficacy and side effects of morphine and pethidine delivered by patient-controlled analgesia after gynaecological surgery. Br J Anaesth 1996; 76: 484-6.
46. Kestin I. Morphine. Update in anaesthesia 1993; 3: 24-5.
47. Mayer DJ, Mao J, Holt J, Price DD. Cellular mechanisms of neuropathic pain, morphine tolerance, and their interactions. Proc Natl Acad Sci U S A 1999; 96: 7731-6.
48. Hughes RA. Strain-dependent morphine-induced analgesic and hyperalgesic effects on thermal nociception in domestic fowl (Gallus gallus). Behav Neurosci 1990; 104: 619-24.
49. Hughes RA, Sufka KJ. Morphine hyperalgesic effects on the formalin test in domestic fowl (Gallus gallus). Pharmacol Biochem Behavior 1991; 38: 247-51.
50. Hughes RA, Bowes M, Sufka KJ. Morphine hyperalgesic effects on developmental changes in thermal nociception and respiration in domestic fowl (Gallus gallus). Pharmacol Biochem Behavior 2009; 42: 535-9.
51. Kayser V, Besson JM, Guilbaud G. Paradoxical hyperalgesic effect of exceedingly low doses of systemic morphine in an animal model of persistent pain (Freund's adjuvant-induced arthritic rats). Brain Res 1987; 414: 155-7.
52. Paul-Murphy J, Ludders JW. Avian analgesia. Vet Clin North Am Exot Anim Pract 2001; 4: 35-45, vi.
53. Stefano GB, Esch T, Kream RM. Xenobiotic perturbation of endogenous morphine signaling: paradoxical opiate hyperalgesia. Med Sci Monit 2009; 15: RA107-10.
54. Stefano GB, Kream RM, Esch T. Revisiting tolerance from the endogenous morphine perspective. Med Sci Monit 2009; 15: RA189-98.
55. Gutstein HB. The effects of pain on opioid tolerence: how do we resolve the controversy? Pharmacol Rev 1996; 48: 403-11.
56. Fricchione G, Zhu W, Cadet P, et al. Identification of endogenous morphine and a mu3-like opiate alkaloid receptor in human brain tissue taken from a patient with intractable complex partial epilepsy. Medical Science Monitor 2008; 14: CS45-9.
57. Pasternak GW. The opiate receptors. New Jersey: Humana Press 1988.
58. Cadet P. Mu opiate receptor subtypes. Medical Science Monitor 2004; 10: MS28-32.
59. Fowler CJ, Fraser GL. Mu-, delta-, kappa- opioid receptors and their subtypes. A critical review with emphasis on radioligand binding experiments. Neurochem Int 1994; 24: 401-26.
60. Stefano GB. The mikro3 opiate receptor subtype. Pain Forum 1999; 8: 206-9.
61. Nestler EJ, Duman RS. G proteins and cyclic nucleotides in the nervous system. In: Siegel BW, Agranoff RW, Albers RW et al. (eds.). Basic Neurochemistry. New York: Raven Press 1994; 429-49.
62. Connor M, Christie MD. Opioid receptor signalling mechanisms. Clin Exp Pharmacol Physiol 1999; 26: 493-9.
63. Burford NT, Wang D, Sadee W. G-protein coupling of mu-opioid receptors (OP3): elevated basal signalling activity. Biochem J 2000; 348: 531-7.
64. Maher CE, Selley DE, Childers SR. Relationship of mu opioid receptor binding to activation of G-proteins in specific rat brain regions. Biochem Pharmacol 2000; 59: 1395-401.
65. Borgland SL, Connor M, Osborne PB, Furness JB, Christie MJ. Opioid agonists have different efficacy profiles for G protein activation, rapid desensitization, and endocytosis of mu-opioid receptors. J Biol Chem 2003; 278: 18776-84.
66. Nestler EJ, Malenka RC, Hyman SE. Molecular basis of neuropharmacology. Columbus: McGraw-Hill 2001.
67. Cadet P, Mantione KJ, Zhu W, Kream RM, Sheehan M, Stefano GB. A functionally coupled mu3-like opiate receptor/nitric oxide regulatory pathway in human multi-lineage progenitor cells. J Immunol 2007; 179: 5839-44.
68. Atmanene C, Laux A, Glattard E, et al. Characterization of human and bovine phosphatidylethanolamine-binding protein (PEBP/RKIP) interactions with morphine and morphine-glucuronides determined by noncovalent mass spectrometry. Med Sci Monit 2009; 15: BR178-BR187.
69. Pflueger A, Abramowitz D, Calvin AD. Role of oxidative stress in contrast-induced acute kidney injury in diabetes mellitus. Med Sci Monit 2009; 15: RA125-36.
70. De GC, Bianchi M, Pascale V, et al. Asymmetric dimethylarginine (ADMA): an endogenous inhibitor of nitric oxide synthase and a novel cardiovascular risk molecule. Med Sci Monit 2009; 15: RA91-101.
71. Tanii H, Higashi T, Nishimura F, Higuchi Y, Saijoh K. Effects of cruciferous allyl nitrile on phase 2 antioxidant and detoxification enzymes. Med Sci Monit 2008; 14: BR189-92.
72. Kayan S, Woods LA, Mitchell CL. Morphine-induced hyperalgesia in rats tested on the hot plate. J Pharmacol Exp Ther 1971; 177: 509-13.
73. Hendrie CA. Naloxone sensitive hyperalgesia induced by morphine and environmental stimulation. Pharmacol Biochem Behavior 1989; 32: 961-6.
74. Gentle MJ, Corr SA. Endogenous analgesia in the chicken. Neurosci Lett 1995; 201: 211-4.
75. Sufka KJ, Hughes RA, McCormick T, Borland J. Opiate effects on isolation stress in domestic fowl. Pharmacol Biochem Behavior 1994; 49: 1011-5.
76. Kambur O, Mannisto PT, Viljakka K, et al. Stress-induced analgesia and morphine responses are changed in catechol-O-methyltransferase-deficient male mice. Basic Clin Pharmacol Toxicol 2008; 103: 367-73.
77. Stefano GB, Stefano JM, Esch T. Anticipatory stress response: a significant commonality in stress, relaxation, pleasure and love responses. Med Sci Monit 2008; 14: RA17-21.
78. Coderre T, Wall P. Ankle joint urate arthritis in rats provides a useful tool for the evaluation of analgesic and antiarthritic agents. Pharmacol Biochem Behavior 1988; 29: 461-6.

79. Seegmillar JE, Caster L, Rodney Howell R. Biochemistry of Uric acid and its relation to gout. N Engl J Med 1963; 268: 821-7.
80. Duff SR. Dyschondroplasia/osteochondrosis of the femoral trochanter in the fowl. J Comp Pathol 1985; 95: 363-71.
81. Duff SR, Thorp BH. Abnormal angulation/torsion of the pelvic appendicular skeleton in broiler fowl: morphological and radiological findings. Res Vet Sci 1985; 39: 313-9.
82. Duff SR, Hocking PM. Chronic orthopaedic disease in adult male broiler breeding fowls. Res Vet Sci 1986; 41: 340-8.
83. Ferreira SH, Nakamura M. II - Prostaglandin hyperalgesia: the peripheral analgesic activity of morphine, enkephalins and opioid antagonists. Prostaglandins 1979; 18: 191-200.
84. Gentle MJ, Hocking PM, Bernard R, Dunn LN. Evaluation of intraarticular opioid analgesia for the relief of articular pain in the domestic fowl. Pharmacol Biochem Behav 1999; 63: 339-43.
85. Rogers H, Birch PJ, Harrison SM, et al. GR94839, a kappa-opioid agonist with limited access to the central nervous system, has antinociceptive activity. Br J Pharmacol 1992; 106: 783-9.
86. Rothman RB, Bykov V, DeCosta BR, Jacobson AE, Rice KC, Brady LS. Interaction of endogenous opioid peptides and other drugs with four kappa binding sites in guinea pig brain. Peptides 1990; 11: 311-31.
87. Taub DD, Eisenstein TK, Geller EB, Adler MW, Rogers TJ. Immunomodulatory activity of mu- and kappa-selective opioid agonists. Proc Natl Acad Sci USA 1991; 88: 360-4.
88. Furose M. Central Regulation of Food Intake in the neonatal chick. Anim Sci J 2002; 73: 83-94.
89. Bungo T, Dodo KI, Kawamura K, Izumi T, Ueda H. Effects of various Mu and Delta opioid ligands on food intake in the meat-type chick. Physiol Behavior 2005; 85: 519-23.
90. Panksepp J, Bean N, Bishop P, Vilberg T, Sahley T. Opioid blockade and social comfort in chicks. Pharmacol Biochem Behavior 1980; 13: 673-83.
91. Panksepp J, Meeker R, Bean N. The neurochemical control of crying. Pharmacol Biochem Behavior 1980; 12: 437-43.
92. Panksepp J, Herman B, Conner R, Bishop P, Scott JP. The biology of social attachments: opiates alleviate separation distress. Biological Psychiatry 1978; 13: 607-18.
93. Warnick JE, McCurdy CR, Sufka KJ. Opioid receptor function in social attachment in young domestic fowl. Behav Brain Res 2005; 160: 277-85.
94. Stefano GB, Prevot V, Beauvillain JC, et al. Estradiol coupling to human monocyte nitric oxide release is dependent on intracellular calcium transients: evidence for an estrogen surface receptor. J Immunol 1999; 163: 3758-63.
95. Mantione KJ. Estrogen's actions transcend a sole reproductory function in cell signaling. Med Sci Monit 2008; 14: SC1-3.
96. Singh MN, Martin-Hirsch PL, Martin FL. The multiple applications of tamoxifen: an example pointing to SERM modulation being the aspirin of the 21st century. Med Sci Monit 2008; 14: RA144-8.
97. Takahashi H, Traystman RJ, Hashimoto K, London ED, Kirsch JR. Postischemic brain injury is affected stereospecifically by pentazocine in rats. Anesth Analgesia 1997; 85: 353-7.
98. Tubbs RJ, Porcaro WA, Lee WJ, et al. Delta opiates increase ischemic tolerance in isolated rabbit jejunum. Academic Emergency Medicine 2002; 9: 555-60.
99. Blaszcyk J, Kedziora J, Luciak M, Sibinska E, Trznadel K, Pawlicki L. Effect of Morphine and Naloxone on oxidative metabolism during experimental renal ischemia and repersusion. Experimental Nephrol 1994; 2: 364-70.
100. Shultz JE, Gross GJ. Opioids and cardioprotection. Pharmacol Therapy 2001; 89: 123-37.
101. Stefano GB, Neenan K, Cadet P, Magazine HI, Bilfinger TV. Ischemic preconditioning - An opiate constitutive nitric oxide molecular hypothesis. Med Sci Monit 2001; 7: 1357-75.
102. Fryer RM, Hsu AK, Gross GJ. ERK and p38 MAP kinase activation are components of opioid-induced delayed cardioprotection. Basic Research in Cardiology 2001; 96: 136-42.
103. Lin CC, Shieh DE. The anti-inflammatory activity of Scutellaria rivularis extracts and its active compounds, baicalin, baicalein and wogonin. Am J Chin Med 1996; 24: 31-3.
104. Gabrielska J, Oszmianski J, Zylka R, Komorowska M. Antioxidant Activity of flavones from Scutellaria baicalensis in lecithi liposomes. Z Naturforsch 1997; 52: 817-23.
105. Sharma JM. Overview of the avian immune system. Vet Immunol Immunopathol 1991; 30: 13-7.
106. Koch G. The immune system in poultry. Tijdschr Diergeneeskd 1991; 116: 728-34.
107. Srinivasan V, Spence DW, Trakht I, Pandi-Perumal SR, Cardinali DP, Maestroni GJ. Immunomodulation by Melatonin: Its significance for Seasonally occurring diseases. NeuroImmuno Modulation 2008; 15: 93-101.
108. Brotto LA, Gorzalka BB, LaMarre AK. Melatonin protects against the effects of chronic stress on sexual behaviour in male rats. Neuroreport 2001; 12: 3465-9.
109. Majewski P, Dziwinski T, Pawlak J, Waloch M, Skwarlo-Sonta K. Anti-inflammatory and opioid-mediated effects of melatonin on experimental peritonitis in chickens. Life Sciences 2005; 76: 1907-20.
110. Kream RM, Sheehan M, Cadet P, et al. Persistence of evolutionary memory: Primordial six-transmembrane helical domain mu opiate receptors selectively linked to endogenous morphine signaling. Medical Science Monitor 2007; 13: SC5-6.
111. Simon EJ. Opioid receptors and endogenous opioid peptides. Med Res Rev 1991; 11: 357-74.
112. Kream RM, Stefano GB. De novo biosynthesis of morphine in animal cells: An evidence-based model. Medical Science Monitor 2006; 12: RA207-19.
113. Kream RM, Stefano GB. Morphine synthesis in animals (Editorial). Medical Science Monitor 2006; 12: ED1-2.
114. Epple A, Nibbio B, Spector S, Brinn JE. Endogenous Codeine: Autocrine regulator of catecholamine release from chromaffin cells. Life Sci 1994; 54: 695-702.
115. Donnerer J, Oka K, Brossi A, Rice KC, Spector S. Presence and formation of codeine and morphine in the rat. Proc Natl Acad Sci U S A 1986; 83: 4566-7.
116. Zhu W, Bilfinger TV, Baggerman G, Goumon Y, Stefano GB. Presence of endogenous morphine and morphine 6 glucuronide in human heart tissue. Int J Mol Med 2001; 7: 419-22.
117. Zhu W, Ma Y, Bell A, et al. Presence of morphine in rat amygdala: Evidence for the mu3 opiate receptor subtype via nitric oxide release in limbic structures. Med Sci Monit 2004; 10: BR433-9.

118. Gu Y, Yun L, Tian Y, Hu Z. Association between COMT gene and Chinese male schizophrenic patients with violent behavior. Med Sci Monit 2009; 15: CR484-9.
119. Yokoyama H, Hirose H, Saito I. Two types of unsafe drinker judged to have metabolic syndrome: typical metabolic syndrome or alcohol-related syndrome? Med Sci Monit 2009; 15: H57-64.
120. Yokusoglu M, Sag C, Cincik M, et al. Perindopril, atenolol, and amlodipine prevent aortic ultrastructural changes in rats exposed to ethanol. Med Sci Monit 2008; 14: BR96-102.
121. Zhu W. CYP2D6: a key enzyme in morphine synthesis in animals. Med Sci Monit 2008; 14: SC15-8.
122. Castardeli E, Duarte DR, Minicucci MF, et al. Exposure time and ventricular remodeling induced by tobacco smoke exposure in rats. Med Sci Monit 2008; 14: BR62-6.
123. Stefano GB, Goumon Y, Casares F, et al. Endogenous morphine. Trends in Neurosciences 2000; 9: 436-42.
124. Stefano GB, Digenis A, Spector S, et al. Opiate-like substances in an invertebrate, an opiate receptor on invertebrate and human immunocytes, and a role in immunosuppression. Proc Natl Acad Sci USA 1993; 90: 11099-103.
125. Zhu W, Baggerman G, Goumon Y, Casares F, Brownawell B, Stefano GB. Presence of morphine and morphine-6-glucuronide in the marine mollusk Mytilus edulis ganglia determined by GC/MS and Q-TOF-MS. Starvation increases opiate alkaloid levels. Brain Res Mol Brain Res 2001; 88: 155-60.
126. Goumon Y, Casares F, Zhu W, Stefano GB. The presence of morphine in ganglionic tissues of Modiolus deminissus: A highly sensitive method of quantitation for morphine and its derivatives. Mol Brain Res 2001; 86: 184-8.
127. Nikisch G. Involvement and role of antidepressant drugs of the hypothalamic-pituitary-adrenal axis and glucocorticoid receptor function. Neuro Endocrinol Lett 2009; 30: 11-6.
128. Kopeckova M, Paclt I, Petrasek J, Pacltova D, Malikova M, Zagatova V. Some ADHD polymorphisms (in genes DAT1, DRD2, DRD3, DBH, 5-HTT) in case-control study of 100 subjects 6-10 age. Neuro Endocrinol Lett 2008; 29: 246-51.
129. Gavrilovic L, Spasojevic N, Tanic N, Dronjak S. Chronic isolation of adult rats decreases gene expression of catecholamine biosynthetic enzymes in adrenal medulla. Neuro Endocrinol Lett 2008; 29: 1015-20.
130. Zhu W, Esch T, Kream RM, Stefano GB. Converging cellular processes for substances of abuse: endogenous morphine. Neuro Endocrinol Lett 2008; 29: 63-6.