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Effects of Selective Opiate Antagonists on Morphine-Induced Hyperalgesia in Domestic Fowl[1]

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SUFKA, K. J., R. A. HUGHES AND J. GIORDANO. *Effects of selective opiate antagonists on morphine-induced hyperalgesia in domestic fowl.* PHARMACOL BIOCHEM BEHAV **38**(1) 49–54, 1991.—Although morphine typically produces analgesia in a variety of species, recent research has identified a biological model in which morphine produces a naloxone-reversible, paradoxical hyperalgesic response to a noxious thermal stimulus in young domestic fowl. The present study examined opioid receptor-mediation of this atypical opiate effect. Patterns of morphine hyperalgesia (1.25 to 5.0 mg/kg IM) were examined on a standard hot-plate test following administration (10 μg/5 μl ICV) of the mu antagonist beta-funaltrexamine, the delta antagonist naltrindole, or the kappa antagonist nor-binaltorphimine in 15-day-old White Leghorn cockerels. Respiration measures were also recorded because they are indicative of opiate effects. Morphine produced a dose-dependent decrease in mean jump latencies (i.e., hyperalgesic effect). Mu receptor antagonism attenuated this morphine-induced hyperalgesic effect. Kappa receptor antagonism attenuated morphine-induced hyperalgesia only at the highest morphine dose (i.e., 5.0 mg/kg) and delta receptor antagonism failed to attenuate morphine-induced hyperalgesia. These results suggest that morphine-induced hyperalgesia, like morphine-induced analgesia, is mediated primarily by mu receptor activation.

Domestic fowl Nociception Opioid receptors Morphine Beta-funaltrexamine Nor-binaltorphimine Naltrindole Pain Opiates

ENDOGENOUS opioid peptides and opiate drugs interact with heterogeneous populations of opioid receptors (i.e., mu, kappa, and delta) in the central nervous system (CNS) and peripheral nervous system (PNS) to mediate several behaviors (e.g., feeding, analgesia, aggression, etc.) and physiological effects [e.g., temperature, respiration, immune function, neuroendocrine status, etc.; for reviews see (20, 23, 29)]. Opiate agonists (e.g., morphine) typically produce analgesia in a variety of species (2, 7, 15, 21, 37). These analgesic effects result from the activation of an opioid-mediated antinociceptive mechanism located in the CNS (3, 4, 33, 36).

Although most animals display an analgesic response to morphine, recent research has identified a genetically variant, biological model in which morphine produced a strain-dependent hyperalgesic response (13, 14, 27). In these studies, various doses of morphine (2.5 to 30.0 mg/kg) decreased response latencies (i.e., produced hyperalgesia) to a noxious thermal stimulus in young domestic fowl. This atypical morphine effect was naloxone-reversible (13) and exhibited dose and temporal parameters (27) that were the inverse equivalent of typical opiate (analgesic) effects. These observations suggests that opioid receptors may mediate this morphine hyperalgesic effect.

Morphine is relatively specific for mu receptors (20), however, there are data that demonstrated the agonist activity of morphine at delta and kappa sites (11). It has also been suggested that mu and delta sites may be functionally interrelated (5,16). The question arises as to whether the atypical action of morphine in domestic fowl results from the activation of a single opioid receptor system (mu, delta, or kappa) or an interaction of multiple opioid receptor systems. The present research, therefore, sought to determine the contribution of various opioid receptor types in the expression of this paradoxical opiate effect in domestic fowl. In separate experiments, patterns of morphine hyperalgesic effects were examined following administration of the selective mu antagonist beta-funaltrexamine [beta-FNA; (28,32)], the selective delta antagonist naltrindole [NTI; (26)], or the selective kappa antagonist nor-binaltorphimine [nor-BNI; (24,25)].

Hyperalgesic effects in domestic fowl have been reported at a low dose of morphine [i.e., 2.5 mg/kg; (27)]. The effectiveness of this dose may be reliant upon mu receptor activation. Through the use of beta-funaltrexamine, an opioid receptor alkylating agent that produces irreversible mu receptor blockade and reversible

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kappa agonist effects (28,32), the involvement of mu receptors in the expression of morphine-induced hyperalgesia can be evaluated. If morphine-induced hyperalgesia is produced by selective mu receptor activation, beta-FNA administration should attenuate morphine effects. It is possible, however, that hyperalgesia in domestic fowl may reflect the activity of morphine at delta receptor sites. If morphine-induced hyperalgesia results from activation of delta sites, administration of the selective delta antagonist naltrindole (26) should attenuate morphine effects. Finally, several studies have shown kappa receptor-mediation of hyperalgesia (30,35). The involvement of kappa receptor system in morphine-induced hyperalgesia in domestic fowl has not been previously investigated. If morphine-induced hyperalgesia is subserved by a kappa receptor component, then nor-BNI should attenuate morphine effects. Together, these experiments will characterize the role of heterogeneous opioid receptors in the expression of paradoxical morphine hyperalgesia in domestic fowl. This model may provide evidence of genetic determinants that can influence opioid-mediated responses.

In addition to pain modulation, morphine has been shown to depress respiration in a number of species including domestic fowl (12, 17–19). Given the atypical effects of morphine on nociception in this species, it may be critical to include analysis of this opioid receptor mediated dependent variable. The inclusion of respiration measures will more completely evaluate functional parameters of opioid receptor activity and may serve to obviate a variety of alternative interpretations of the nociceptive data. Thus the present study also examined the effects of selective opiate receptor antagonists on morphine-induced respiratory depression in domestic fowl.

METHOD

Subjects

Chicks (Welp-Line 542 cockerels commercial stock) were obtained from Welp Incorporated (Bancroft, IA) 1-day posthatch and were housed in pairs in chambers that provided physical separation but not auditory or visual isolation. This housing arrangement, as described below, enables researchers to locate and capture animals with minimal disruption to the brood and eliminates many procedural problems (e.g., social isolation and/or social aggression confounds during injection-to-test intervals) associated with communal brooder housing.

Housing Apparatus

Four separate $186 \times 62 \times 37$ cm enclosures were constructed. Each enclosure was divided into twelve $31 \times 31 \times 37$ cm individual housing units. The outside perimeter walls were fabricated from 16-gauge aluminum sheeting and were painted gray. The floor of the housing apparatus was constructed from 2.5 cm expansion metal. The internal walls of the housing apparatus and the lids covering each individual unit were constructed of 2×2 hardware cloth. The lids were covered with white broadcloth. Illumination was provided by a 12-W fluorescent light bulb that was centered at the top and extended across the housing facility. Animals were housed under continuous illumination. Food was available from ¼ round stainless steel feeders. Water was available from J-shaped waterers mounted outside the chamber with the drinking spout protruding through a 3 cm diameter opening into each housing unit. Animals had free access to food and tap water. Room temperature was maintained at 32 ± 1°C for the first week and at 29 ± 1°C thereafter.

Test Apparatus

Jump latency in response to a thermal stimulus was used as the index of nociception. The apparatus (15) is a modified version of a hot-plate that is typically used to assess thermal nociception in rats (6). The grid-like floor of the apparatus consisted of a $63 \times 20 \times 0.3$ cm copper plate with six 1.5 cm diameter copper tubes with lead shot. The tubes were spaced 0.9 cm center to center and were attached to the middle 12.5 cm of the copper plate. The copper plate was supported by a $63 \times 19 \times 7$ cm wood base with a 23×7 cm opening in the front which enabled a single element hot plate (750 W Hamilton Beach, Model 812) to be under the center portion of the copper plate. The thermostatic control of the hot plate was disconnected and temperature was regulated by a variable transformer (Stayco Energy Products Co., Type 3PN1010). Temperature was monitored by a digital thermometer (Fluke, Model 52) from a thermistor imbedded in the upper surface of one of the center middle copper tubes. A $17 \times 16 \times 31$ cm Plexiglas chamber with a hinged lid was fitted over the tubes. The outside chamber walls were covered with onionskin paper. A 3.5×16 cm opening at the base of the front wall remained uncovered to permit observation. All the copper areas outside of the chamber were covered with 2.5 cm Styrofoam which was fastened to the base with ductape. Response latencies were recorded to the nearest 0.1 second via an electronic timer (Hunter, Model 120A).

Procedure

Injections. The groups in this study formed a 2×4 factorial design. Factors were antagonist (saline or opiate antagonist 10 μg/5 μl ICV) and morphine (0.0, 1.25, 2.5, or 5.0 mg/ml/kg; $n = 10$ per cell). Opiate antagonists were obtained from Research Biochemicals Incorporated (Natick, MA) and morphine sulfate was purchased from Sigma Chemical Company (St. Louis, MO). All drugs were dissolved in neutral pH, pyrogen-free saline. Doses for morphine were based on earlier work from this laboratory (27). The dose for the three antagonists was extrapolated from work in rodents (24,32). Chick (15 days old) pairs from each individual housing unit received either ICV saline or the opiate antagonists. Beta-FNA (and its vehicle control) was administered approximately 20 hours before morphine injections while nor-BNI and NTI (and their vehicle controls) injections were given immediately before IM morphine injections. Intracerebroventricular injections were manually performed with a 10 μl Hamilton microsyringe fitted with a 22S gauge needle (22). Solutions were directly delivered through the skull immediately anterior to the cerebellum into the vicinity of the 4th ventricle. Injection sites were verified by dye injection in a separate group of chicks. Drugs were ICV delivered in a total volume of 5 μl at a rate of 1 μl per 5 s. The needle was maintained in place for 15 seconds prior to withdrawal to prevent retrograde leakage of the injected solution. Intramuscular morphine injections were given thirty minutes before nociceptive tests. Animals were returned to their home cage during this injection-to-test interval.

Thermal nociceptive and respiration measures. Thirty minutes after morphine injections, chicks were placed into a 2-quart opaque plastic container which was covered with a vented lid. Each animal was individually transported to an adjacent experiment room where tests were administered. The chick was removed from the container and placed on an ambient temperature grid (25°C). An electronic timer was started by a manual switch as the chick's feet touched the apparatus floor and was manually terminated following a jump response or after attainment of a 70-s no-jump criterion. The nociceptive response was defined as a vertical jump with both feet leaving the grid floor. After this ambient temperature test, the chick was tested for jump latency on a 60°C hot-plate. Immediately after hot-plate tests, respirations were measured

by counting rhythmic chest movements during a 1-minute interval. All observations were performed without knowledge of the treatment conditions. Chicks were returned to their home cage following tests.

Statistics. Data were analyzed using a 2-way analysis of variance (ANOVA). Factors were antagonist (saline or opiate antagonist) and morphine (0.0, 1.25, 2.5, and 5.0 mg/kg). Simple effects analyses were used to identify sources of significant interactions. Multiple *t*-statistics (one-tailed) were used to test a priori orthogonal contrasts. These contrasts identified significant dose-related hyperalgesic effects of morphine and determined if the opiate antagonist treatment attenuated observed morphine effects.

RESULTS

Dye Spread

Verification of dye spread in a separate group of chicks was conducted with reference to the coordinates of van Tienhoven and Juhasz (31) 30 min post-ICV blue dye injection. Chicks (n = 8) were sacrificed by rapid decapitation and whole brains were immediately removed from the calvarium. A midsagittal incision through the longitudinal fissure was performed. For each chick brain, dye diffusion was observed throughout the entire ventricular system. Moreover, blue stain was observed to penetrate approximately 1.5 mm into the neural tissue surrounding the ventricular system.

The ICV method of administering drugs to the experimental animals produced no overt, long-lasting changes in the animals behavior. Some chicks (less than 10%) displayed acute motor dysfunction lasting approximately 15 seconds and/or a small amount of bleeding from the injection site. In all cases, the motoric effects of icv administration completely abated prior to nociceptive testing.

Beta-Funaltrexamine Effects

Jump latency. The effects of beta-FNA on morphine-induced hyperalgesia are presented in Fig. 1. Morphine produced a dose-dependent decrease in mean jump latencies in chicks that received ICV/saline administration. This morphine-induced hyperalgesic effect was attenuated by ICV/beta-FNA administration. A 2-way ANOVA performed on these data revealed significant treatment effects for antagonist, $F(1,72) = 11.99$, $p<0.01$, and morphine, $F(3,72) = 10.16$, $p<0.01$. The antagonist × morphine interaction was not significant, $F(3,72) = 1.85$. Subsequent analysis of the jump latency data for the ICV/saline groups demonstrated that the mean jump latency for the 2.5 and 5.0 mg/kg morphine dose groups were significantly shorter than the mean jump latency of the saline control group, ts(72) = 4.02 and 5.32, respectively, ps<0.01. Further analysis demonstrated that the mean jump latencies of the ICV/beta-FNA groups at the 2.5 and 5.0 mg/kg morphine doses were significantly longer than the mean jump latencies for comparable ICV/saline groups, ts(72) = 2.11 and 3.22, ps<0.05 and 0.01, respectively. Jump latencies from ambient temperature tests were not analyzed since animals reached the 70-second no-jump criterion on 98% of the tests at ambient grid temperature.

Respirations. As shown in Fig. 2, morphine produced a dose-dependent decrease in mean respirations in the ICV/saline groups that was attenuated by ICV/beta-FNA administration. A 2-way ANOVA performed on these data revealed significant treatment effects for antagonist, $F(1,72) = 7.13$, $p<0.01$, morphine, $F(3,72) = 19.76$, $p<0.01$, and a significant antagonist × morphine interaction, $F(3,72) = 5.30$, $p<0.01$. Analysis of simple main effects yielded significant morphine effects at each dose saline, $F(3,72) = 22.27$, $p<0.01$; beta-FNA, $F(3,72) = 2.78$, $p<0.05$, and a significant antagonist effect at only the 5.0 mg/kg morphine dose, $F(3,72) = 4.01$, $p<0.01$.

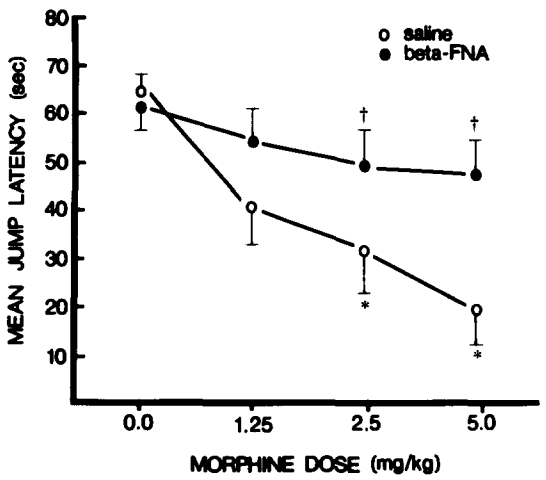

FIG. 1. Mean jump latency as a function of morphine dose for chicks treated with the selective mu antagonist beta-funaltrexamine (10 μg/5 μl ICV) or saline (vertical lines = SEM). *Indicates significant morphine hyperalgesia; †indicates significant antagonist attenuation of hyperalgesia (all ps<0.05).

Naltrindole Effects

Jump latency. The effects of NTI on morphine-induced hyperalgesia are shown in Fig. 3. Morphine produced a dose-dependent decrease in mean jump latencies in both the ICV/saline and ICV/NTI groups. This hyperalgesic effect was partially attenuated in the ICV/NTI groups at the 2.5 and 5.0 mg/kg morphine dose groups. A 2-way ANOVA, however, revealed significant treatment effects for morphine only, $F(3,72) = 13.55$, $p<0.01$. Subsequent analysis of these data for ICV/saline groups demonstrated that the mean jump latencies for the 1.25, $t(72) = 2.34$,

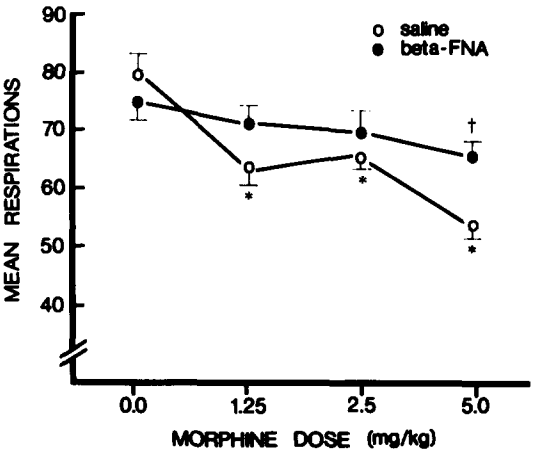

FIG. 2. Mean respirations (1 minute) as a function of morphine dose for chicks treated with the selective mu antagonist beta-funaltrexamine (10 μg/5 μl ICV) or saline (vertical lines = SEM). *Indicates significant morphine respiratory depression; †indicates significant antagonist attenuation of respiratory depression (all ps<0.05).

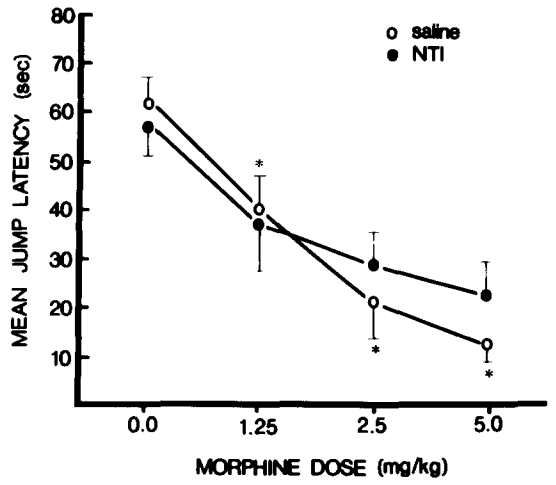

FIG. 3. Mean jump latency as a function of morphine dose for chicks treated with the selective delta antagonist naltrindole (10 μg/5 μl ICV) or saline (vertical lines = SEM). *Indicates significant morphine hyperalgesia (all $ps<0.05$).

$p<0.05$, 2.5, $t(72)=4.12$, $p<0.01$, and 5.0 mg/kg, $t(72)=4.98$, $p<0.01$, morphine dose groups were significantly shorter than the mean jump latency of the saline control group. *t*-Tests between the mean jump latencies of the ICV/saline and ICV/NTI groups at each morphine dose failed to reveal a significant attenuation of morphine-induced hyperalgesia. Jump latencies from ambient temperature tests were not analyzed since animals reached the 70 second no-jump criterion on all of the tests at ambient grid temperature.

Respirations. As presented in Fig. 4, morphine produced a dose-dependent decrease in mean respirations in both ICV/saline and ICV/NTI groups. A 2-way ANOVA of these data revealed a significant treatment effect for morphine, $F(3,72)=20.28$, $p<0.01$. The antagonist effects and morphine × antagonist interaction terms were not significant. Subsequent analysis of the respiration data for the ICV/saline groups revealed that the mean respirations of the 2.5, $t(72)=2.65$, $p<0.01$, and 5.0 mg/kg, $t(72)=4.61$, $p<0.01$, morphine dose groups were significantly

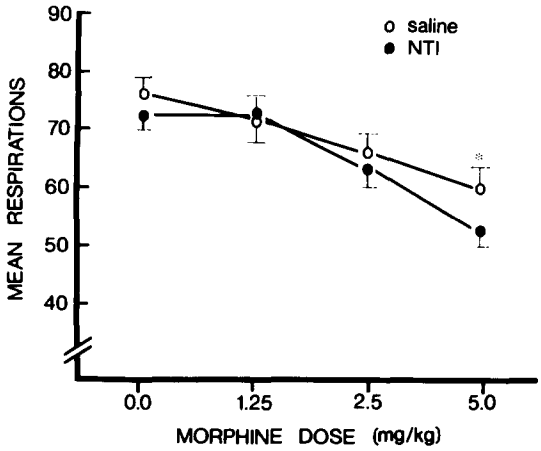

FIG. 4. Mean respirations (1 minute) as a function of morphine dose for chicks treated with the selctive delta antagonist naltrindole (10 μg/5 μl ICV) or saline (vertical lines = SEM). *Indicates significant morphine respiratory depression (all $ps<0.05$).

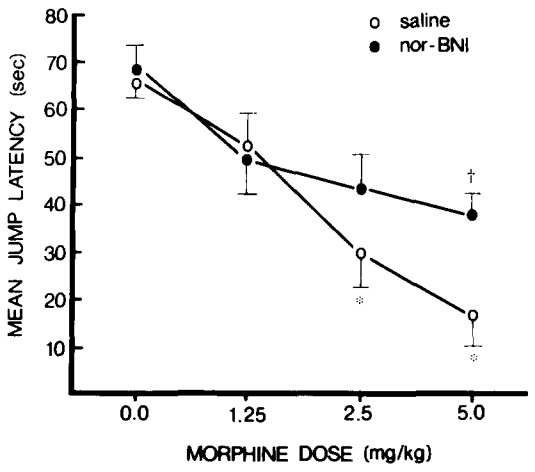

FIG. 5. Mean jump latency as a function of morphine dose for chicks treated with the selective kappa antagonist nor-binaltorphimine (10 μg/5 μl ICV) or saline (vertical lines = SEM). *Indicates significant morphine hyperalgesia; †indicates significant antagonist attenuation of hyperalgesia (all $ps<0.05$).

lower than the mean respirations of the saline control group.

Nor-Binaltorphimine Effects

Jump latency. The effects of nor-BNI on morphine-induced hyperalgesia are shown in Fig. 5. Morphine produced a dose-dependent decrease mean jump latencies in the ICV/saline groups. This hyperalgesic effect was attenuated by nor-BNI at higher doses of morphine (i.e., 2.5 and 5.0 mg/kg). A 2-way ANOVA revealed a significant effect for morphine, $F(3,72)=15.40$, $p<0.01$, a marginally significant effect for antagonist, $F(1,72)=3.54$, $p=0.06$, and a nonsignificant antagonist × morphine interaction. Subsequent analyses of these data for the ICV/saline groups demonstrated that the mean jump latencies for the 2.5, $t(72)=4.19$, $p<0.01$, and 5.0 mg/kg, $t(72)=5.20$, $p<0.01$, morphine dose groups were significantly shorter than the mean jump latency of the control group. Moreover, at the 5.0 mg/kg morphine dose, the mean jump latency of the ICV/nor-BNI group was significantly longer than the mean jump latency of the ICV/saline group, $t(72)=1.94$, $p<0.05$. Jump latencies from ambient temperature tests were not analyzed since animals reached the 70-no-jump criterion on all of the tests at ambient grid temperature.

Respirations

As shown in Fig. 6, morphine produced a dose-dependent decrease in mean respirations that was unaffected by ICV nor-BNI treatment. A 2-way ANOVA of these data revealed a significant treatment effect for morphine, $F(3,72)=24.49$, $p<0.01$. The antagonist effects and antagonist × morphine interaction terms were not significant. Subsequent analyses of the respiration data of the ICV/saline groups demonstrated that the mean respirations of the 1.25, $t(72)=3.02$, $p<0.01$, 2.5, $t(72)=3.97$, $p<0.01$, and 5.0 mg/kg, $t(72)=6.76$, $p<0.01$, morphine groups were significantly shorter than the mean respirations of the saline control group.

DISCUSSION

The purpose of the present research was to examine the involvement of opioid receptor types in the expression of paradox-

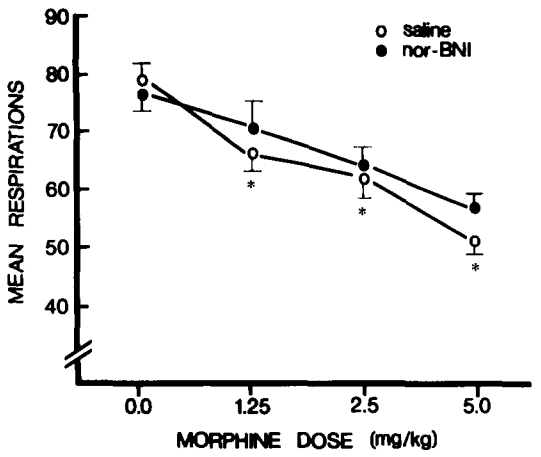

FIG. 6. Mean respirations (1 minute) as a function of morphine dose for chicks treated with the selctive kappa antagonist nor-binaltorphimine (10 μg/5 μl ICV) or saline (vertical lines = SEM). *Indicates significant morphine respiratory depression (all $ps<0.05$).

ical morphine hyperalgesia in domestic fowl. Morphine dose response effects against thermal nociception were evaluated after ICV administration of the selective mu, kappa, or delta opiate antagonists. Respiration measures were also recorded as they are indicative of opioid sensitivity. In the present study, mu, but not kappa or delta antagonism attenuated morphine-induced respiratory depression. These results with domestic fowl are consistent with earlier research that demonstrated mu receptor involvement in the regulation of respiration in other species (18,19).

In the present research, as is common in similar research with rats and mice, changes in nociception were inferred from changes in response latencies elicited by a noxious thermal stimulus. The assumption underlying this inference is that increases and decreases in some aspect of nociception are reflected in decreases and increases respectively, in response latency. Further support for this inference is provided by research that demonstrated thermal stimulus intensity is inversely related to response latency (15). Animals typically did not perform the jump response on ambient temperature tests. This failure to respond to ambient temperature suggests that chicks were responding to noxious properties of the thermal stimulus and not to confinement characteristics of the test apparatus. Furthermore, it is unlikely that morphine-induced changes in response latencies were due to motoric effects as chicks did not display overt alterations in wing or leg movements. Although it is possible that that patterns of morphine-induced hyperalgesia were the result of drug toxicity, this is improbable for several reasons. First, chicks did not present the physical characteristics representative of illness (e.g., crouching and immobility). Second, it is unlikely that gross systemic involvement of morphine-toxicity would produce hyperagitation and motoric hyperactivity. Such toxic effects would cause immobility, thereby extending response latencies. The morphine-induced decreases in response latency strongly argue against this possibility.

In the present research, morphine-induced hyperalgesia was attenuated by the mu antagonist beta-FNA at the two highest morphine doses (i.e., 2.5, and 5.0 mg/kg) tested. The kappa antagonist nor-BNI reduced this hyperalgesic response only at the highest morphine dose (i.e., 5.0 mg/kg), whereas NTI, the selective delta antagonist, failed to affect the hyperalgesic response produced by morphine. The hyperalgesic effects of morphine, therefore, may reflect the specific action of distinct opioid receptors (e.g., mu and kappa) within the CNS. Although biochemical characterization of distinct populations of opioid receptors in this animal model has not yet been conducted, the differential efficacy of the opiate antagonists in attenuating morphine-induced hyperalgesia supports such a conclusion.

Evidence suggests that multiple opioid receptor types subserve analgesia at several levels in the nociceptive neuraxis. Autoradiographic studies have identified distinct mu, delta, and kappa receptors at various CNS loci [for review see (10)]. Most notable are populations of mu receptors located within the midbrain PAG and various raphe nuclei, and mu, delta, and kappa receptors differentially distributed (i.e., in rostral and caudal regions) within the dorsal horn of the spinal cord. This regional distribution corresponds with observations that analgesia can be elicited by both mu receptor activation within the PAG (38) and kappa and delta receptor activation at spinal loci (34). Opioid receptor systems have been shown to interact with peptide and monoaminergic neuraxes that function in both local circuit and centrifugal pain modulation. The contribution of these spinal and supraspinal opioid receptor-mediated mechanisms in the paradoxical hyperalgesic effects of morphine in domestic fowl is yet unknown.

Genetic factors have been shown to affect the pharmacologic profile of opiate drugs in rodents (1). Alterations in molecular dynamics of receptor complexes, metabolic mechanisms and biochemical constituents of the central and peripheral nervous system (8) may all contribute to the differential activity of opiates in genetically variant models. It has been suggested that the observed atypical effects of morphine may reflect genetic variability in substrates contributing to the pharmacologic profile of opiate drugs in this animal model (14). These variables require further study; our laboratory is engaged in these investigations.

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