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Codeine Analgesic and Morphine Hyperalgesic Effects on Thermal Nociception in Domestic Fowl

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HUGHES, R. A. *Codeine analgesic and morphine hyperalgesic effects on thermal nociception in domestic fowl.* PHARMACOL BIOCHEM BEHAV **35**(3) 567–570, 1990.—The effects of codeine phosphate and morphine sulfate (2.5, 15.0, and 30 mg/ml/kg; IM) on latency of a jump response elicited by a noxious (61°C) thermal stimulus were studied in White Leghorn cockerels at 15–16 days posthatch. Codeine induced a significant dose-dependent increase in jump response latency (analgesic effect), whereas morphine at each dose induced a significant decrease in jump response latency (hyperalgesic effect). Naloxone (5 mg/ml/kg) reversed the hyperalgesic effect of morphine (30 mg/ml/kg) and potentiated codeine analgesic effects. It is unlikely that codeine analgesic effects in domestic fowl reflect demethylation of codeine to morphine. These opposite codeine and morphine effects may reflect the interaction of these opiates at different populations of opioid receptors or at different substrates.

Narcotic drugs Analgesic drugs Narcotic antagonists Morphine Codeine Opiate Opioid Naloxone Analgesia Hypoalgesia Hyperalgesia Pain Thermal nociception Chickens Domestic fowl

THE antinociceptive potency of the opioid codeine is, in most species, approximately ⅓ to 1/12 that of the opioid morphine (10–12, 15, 17). This potency relationship is consistent with the hypothesis that codeine exerts its antinociceptive effects through in vivo demethylation of a portion of codeine to morphine (1, 5, 6, 11, 12).

The typical antinociceptive potency relationship between codeine and morphine may not apply to young domestic fowl. In the young of this species, the analgesic ED_{50} to acute mechanical stimulation (toe pinch) was 26 mg/kg for codeine and a near LD_{50} dose of 230 mg/kg for morphine (23). This striking reversal of the more typical potency relationship for codeine and morphine is clearly incompatible with the general hypothesis that codeine analgesic effects reflect the biotransformation of some codeine to morphine.

The results of more recent research demonstrate morphine analgesic effects in domestic fowl with morphine amounts of from 5–30 mg/kg (2,4). These studies did not compare codeine and morphine effects, but the morphine doses that produced analgesic effects are substantially lower than the 230 mg/kg ED_{50} reported in earlier research (23). These conflicting results suggest that the reversed codeine-morphine potency relationship found in the earlier research may reflect a unique outcome of a particular set of procedural details and/or subject characteristics rather than a result that more generally characterizes the antinociceptive potency relationship between codeine and morphine in domestic fowl. The purpose of the present study was to examine the reliability and generality of the reversed potency relationship between codeine and morphine in domestic fowl (23).

EXPERIMENT 1

This experiment was designed to compare the antinociceptive potency of codeine and morphine on thermal nociception in young White Leghorn cockerels.

METHOD

Subjects

White Leghorn cockerels were obtained at one day posthatch (Welp, Inc., Bancroft, IA). The animals were housed in brooders (Brower model 1680-2) at a population density of 50–55 animals per brooder with free access to Wayne chick starter and tap water. Overhead fluorescent room lights were on from 0700 to 1900 hr.

Apparatus

The apparatus consisted of a $63 \times 20 \times 0.3$ cm copper plate with six 1.5 cm diameter copper tubes filled with lead shot. The tubes were spaced 0.9 cm center to center and attached to the mid portion of the copper plate. The copper plate was supported by a $63 \times 19 \times 7$ wooden base with a 23×7 cm opening in the front which permitted placement of a single element hot plate (750 W, Hamilton Beach model 812). Shims were placed under the hot plate legs to provide firm contact between the heating element and the copper plate. The thermostatic control of the hot plate was disconnected and temperature was regulated by a 7.5 amp variac (Standard Electric Co., model 300BU). Temperature was monitored (Keithley 870 digital thermometer) from a thermistor em-

TABLE 1

MEAN (SEM) JUMP LATENCY IN SECONDS OF ANIMALS PRETREATED WITH CODEINE, MORPHINE, OR SALINE (CONTROL)

	Dose (mg/kg)			
Group	0.0	2.5	15.0	30.0
Codeine	—	30.0 (4.9)	45.9 (12.0)	85.2† (4.8)
Morphine	—	17.8* (6.1)	13.5* (9.5)	12.7* (7.3)
Saline	40.8 (7.3)	—	—	—

*Significantly different from saline, $p<0.05$.
†Significantly different from saline, $p<0.01$.

bedded in the upper surface, in the middle of one of the center copper tubes. A 17 × 16 × 31 cm Plexiglas chamber, with a hinged lid, was fitted over the tubes. The outside chamber walls were covered with onion skin paper except for a 3.5 × 16 cm opening at the base of the front wall. All exposed copper areas, outside the chamber, were covered with 2.6 cm thick rigid foam insulation which was fastened to the apparatus base with duct tape. Response latencies were recorded to the nearest 0.1 sec with an electronic timer (Lafayette Industries, model 54030).

Procedure

Animals were assigned by a random block procedure to one of seven independent treatment groups (n = 10 per group). Treatment groups received a 1 ml/kg IM injection of 2.5, 15.0, or 30.0 mg/kg codeine phosphate or morphine sulfate or a 1 ml/kg injection of physiological saline. At 15 days posthatch, an animal was removed from a brooder, weighed, given the assigned injection and placed in a vented, opaque plastic container. Ten min after injection the animal was taken to an adjacent room and tested for thermal nociception. For this test the animal was placed on the enclosed, heated (61.0 ± 0.5°C) grid and latency to perform an upward jump response with both feet off the grid was recorded. If the animal did not perform a jump response within 90 sec it was removed and assigned a latency score of 90.

RESULTS

The results of Experiment 1 are summarized in Table 1. A comparison of mean jump latency for the drug-treatment groups with mean latency of the saline control group shows that codeine produced a dose-dependent increase in jump latency indicative of analgesia and that morphine produced an opposite and dose-independent decrease in jump latency indicative of hyperalgesia. These opposite effects were confirmed statistically. A one-way ANOVA demonstrated a significant treatment effect, $F(6,63) = 16.90$, $p<0.001$. Drug treatment groups were compared with the saline group by Dunnett's test (14). The results of these comparisons demonstrated a significant analgesic effect for codeine only at the 30 mg/kg dose ($p<0.01$) and significant hyperalgesic effects for morphine at the 2.5, 15 and 30 mg/kg doses ($ps<0.05$).

The morphine hyperalgesic effect was unexpected. Previous research suggested that morphine either would not affect nociception in young chickens (23) or would induce analgesic effects (2,4). Moreover, observations of animals during preliminary research, designed to establish dose-response parameters for the present study, indicated that codeine and morphine had sedative effects as chicks tended to sit quietly with head down and eyes closed. These observations and evidence that morphine, in chicks, reduces distress vocalizations induced by social separation (21), suggested that the codeine- and the morphine-treated animals in the present study would display analgesic rather than hyperalgesic effects. The unexpected results of Experiment 1 prompted a second experiment which was designed to replicate the codeine analgesic and morphine hyperalgesic effects and to determine if these opposite effects involved codeine and morphine interactions at opioid receptors.

EXPERIMENT 2

This study was designed to determine if the relatively specific opioid antagonist, naloxone, would reverse or attenuate the codeine analgesic and morphine hyperalgesic effects obtained in Experiment 1.

METHOD

The subjects, apparatus, and procedures were as described in Experiment 1. At sixteen days posthatch naive brooder-housed White Leghorn cockerels were assigned by a random block procedure to receive a 1 ml/kg IM injection of physiological saline or naloxone hydrochloride (5 mg/kg) followed immediately by a second IM injection of either saline, codeine phosphate (30 mg/kg) or morphine sulfate (30 mg/kg). These double injections thus formed six independent treatment groups (n = 15 per group). The injection order for each group was: saline-saline, naloxone-saline, saline-codeine, naloxone-codeine, saline-morphine, and naloxone-morphine. After drug administration, animals were placed in a holding container and tested for thermal nociception 10 min later as described in Experiment 1.

RESULTS

A one-way ANOVA performed on the data that are summarized in Fig. 1, demonstrated a significant treatment effect, $F(5,84) = 16.36$, $p<0.001$. Subsequent comparisons by Dunnett's test demonstrated a significantly shorter mean jump response latency for the morphine group ($p<0.05$; control vs. M30) and a significantly longer mean jump response latency for the codeine group ($p<0.05$; control vs. C30). These findings replicate the codeine analgesic and morphine hyperalgesic effects of Experiment 1. Neither the mean jump latency of the naloxone-morphine group nor that of the naloxone-saline group was significantly different from the control group jump latency. Naloxone did not alter the control group jump latency. This opioid antagonist reversed the hyperalgesic effects of morphine and appeared to potentiate the analgesic effects of codeine. Statistical examination of this apparent potentiation effect demonstrated that mean jump latency of the naloxone codeine (NXC) group was significantly greater than the mean latency of the codeine control group ($p<0.05$).

DISCUSSION

In the present research, as is common in similar research with rats and mice, changes in nociception were inferred from changes in response latency elicited by a noxious stimulus (13,15). The assumption underlying this inference is that increases and decreases in some aspect of nociception (i.e., sensitivity or reactivity) are reflected in decreases (hyperalgesic effect) and increases (analgesic effect) respectively, in response latency. Evidence presented elsewhere demonstrates that the jump response, as operationally defined herein, reflects thermal nociception (8). In that research, domestic fowl displayed a mean jump latency that

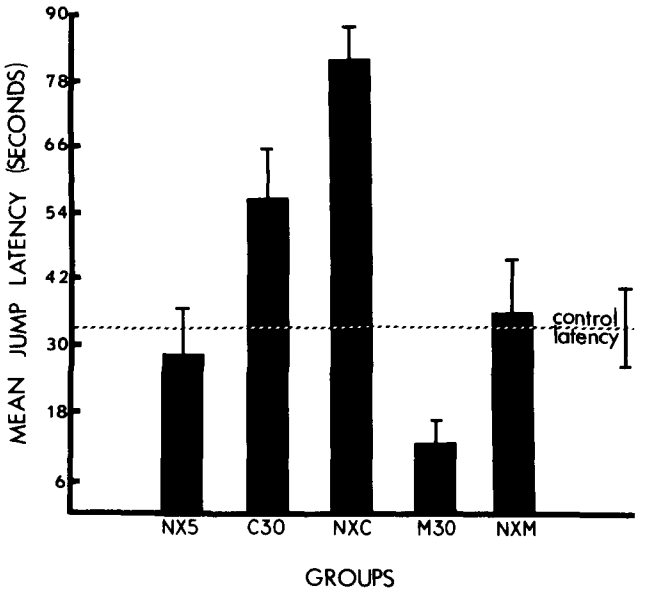

FIG. 1. Mean jump latency elicited by a noxious thermal stimulus in 15-day-old White Leghorn cockerels 10 minutes after sequential IM injection of saline-5 mg/ml/kg naloxone (NX5), saline-30 mg/ml/kg codeine phosphate (C30), saline-30 mg/ml/kg morphine sulfate (M30), same dose naloxone-codeine (NXC), naloxone-morphine (NXM), or saline-saline (control). The vertical bars represent SEM.

was inversely related to grid temperature, but did not perform the response within a 90-sec criterion when the grid was at room temperature. The present results demonstrated that codeine increased and morphine decreased jump response latencies of young White Leghorn cockerels when these animals were placed on a heated grid. Thus, in these chickens, codeine produced analgesic effects and morphine produced hyperalgesic effects on tests of thermal nociception.

The demonstration of codeine analgesic effects in domestic fowl is consistent with previous research showing similar codeine effects at approximately the same dose when animals of this species were exposed to noxious mechanical stimulation (23). The occurrence of codeine analgesic effects in domestic fowl is neither unusual nor unexpected as it is a well-documented codeine effect in a variety of other species. On the other hand, because codeine effects on nociception are thought to reflect demethylation of some codeine to morphine (1, 5, 6, 11, 12), insensitivity to morphine analgesic effects under circumstances where codeine exerts such effects (23) and the demonstration herein of opposite codeine and morphine effects on nociception, is both unusual and unexpected. These findings suggest that codeine may not exert its analgesic effects in domestic fowl through demethylation to morphine. Additional support for this possibility is provided by the results of Experiment 2.

In Experiment 2, the opioid receptor antagonist naloxone reversed the hyperalgesic effects of morphine, but did not reverse codeine analgesic effects. Naloxone potentiated codeine analgesic effects. Clearly, a naloxone dose sufficient to reverse high-dose morphine effects should reverse effects of a lower morphine dose resulting from demethylation of some codeine. Naloxone reversed the effects of 30 mg/kg morphine, but did not reverse the effects of 30 mg/kg codeine. This result supports the conclusion that the codeine analgesic effects reported here may not reflect morphine effects resulting from demethylation of codeine. Codeine demethylation occurs primarily in the liver (10). Although liver function is well-developed in domestic fowl of the age used in the present study (3,25), it is possible that the ten-minute interval between codeine administration and test may have been too brief for biosynthesis of codeine to behaviorally significant amounts of morphine. The analgesic codeine effects in the present research may reflect a direct action of unaltered codeine.

The reversal of morphine hyperalgesia by naloxone implies that this morphine effect is mediated, in part, by morphine interactions at opioid receptors. Naloxone has a higher affinity for mu than for kappa or delta opioid receptors and naloxone dose-response information can provide some evidence bearing on the type of opioid receptor most directly involved in an opioid effect (22,26). The use of a single naloxone dose in the present study, however, does not permit a determination of the opioid receptor subtype involved in morphine hyperalgesic effects. On the other hand, the finding that naloxone reversed morphine hyperalgesic effects, but did not reverse codeine analgesic effects suggests that these morphine and codeine effects do not reflect the actions of these two drugs at the same population of opioid receptors. Moreover, although naloxone dose-response information is clearly needed, the finding that the opioid antagonist naloxone potentiated codeine analgesia rather than blocking this effect further suggests that codeine analgesia in domestic fowl may reflect a nonopioid mode of action.

The morphine hyperalgesic effects found in the present research were not evident in previous evaluations of morphine effects on nociception in domestic fowl (2, 4, 23). In one of these studies, the opportunity to observe hyperalgesic effects was excluded by the all-or-none response measure which is not sensitive to hyperalgesic effects (23). The opportunity to detect hyperalgesic effects, however, was present in the remaining studies (2,4) and these studies, in contrast to the present finding of morphine hyperalgesic effects, demonstrated morphine analgesic effects in domestic fowl. The source of these conflicting results is not clear, but animal age, morphine dose, and noxious stimulus parameters do not appear to be likely sources of the difference as there was substantial overlap across the studies in these variables. Somatotopic differences in application of the noxious stimulus may be a relevant consideration. A noxious stimulus applied to different body locations can yield differences in opioid modulation of nociception (7,29). In the present research, morphine hyperalgesic effects occurred when the noxious stimulus was applied to the animal's feet, whereas reports of morphine analgesic effects in domestic fowl were obtained when noxious stimulation was applied to the animals' wings (2) or head (4). Thus, in domestic fowl, morphine analgesic effects may occur when noxious stimuli are applied to anterior body and hyperalgesic effects may occur when stimuli are applied to more posterior body locations. This possibility remains to be tested.

Hyperalgesic effects produced by systemic administration of an opioid agonist are unusual in intact animals. However, hyperalgesic effects have been reported to occur from activation of kappa opioid receptors at a CNS medullary locus (18,30) and also to occur from local activation of peripheral opioid receptors (28). These hyperalgesic effects are not evident in the intact animal when opioid agonists are administered systemically, presumably because hyperalgesic effects, mediated by activation of medullary and peripheral kappa receptors (18, 28, 30), summate algebraically (28) with activation of prepotent analgesic effects, mediated by mu and kappa receptors at other loci (17, 22, 26), to yield typical opioid analgesic effects. Genetic factors are known to play an important role in determining sensitivity to opioid analgesic effects and this sensitivity covaries with opioid receptor binding affinities and receptor subtype ratios (9, 17, 19, 31). Kappa receptors have been implicated in the regulation of food intake (20) and there is evidence that food intake is less extensively

affected by naloxone in fowl bred for low body weight than in fowl bred for high body weight (16). All of this evidence converges to suggest that in the White Leghorn strain of domestic fowl used in the present research, selective breeding for low body weight may have altered the population or binding characteristics of opioid receptors from a bias favoring those that subserve analgesic effects (mu and kappa) to those that subserve hyperalgesic effects (medullary and/or peripheral kappa). This possibility could account for the morphine hyperalgesic effects found in the present study.

Recent evidence from the present laboratory demonstrates that morphine analgesic and hyperalgesic effects are strain-dependent (24). Whether or not these strain-dependent opposite morphine effects reflect the activation of different populations of opioid receptors or altered binding characteristics will require a comparison of the behavioral effects and binding properties of selective agonists and antagonists on nociception in the different animal strains.

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