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Time-Dependent Codeine Hypoalgesia and Hyperalgesia in Domestic Fowl

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SUFKA, K. J. AND R. A. HUGHES. *Time-dependent codeine hypoalgesia and hyperalgesia in domestic fowl.* PHARMACOL BIOCHEM BEHAV **41**(2) 349–353, 1992.—Recent research demonstrated that codeine produced hypoalgesia and morphine produced hyperalgesia against a noxious thermal stimulus in young domestic fowl. The bidirectional effects of these opiate agonists on nociception are inconsistent with the notion that codeine's algesic effects result through in vivo demethylation of codeine to yield morphine. In Experiment 1, the temporal pattern (15,30,60 and 120 min) of codeine (30 mg/kg) effects on thermal nociception and respiration were examined in 15-day-old cockerels. Codeine produced a time-dependent biphasic response: hypoalgesia at 15 min and hyperalgesia at 60 and 120 min. Respiration was depressed by codeine at all test intervals. To assess for opioid specificity, Experiment 2 examined the action of naloxone (5 mg/kg) on the temporal pattern (15 and 60 min) of codeine effects (30 mg/kg) on thermal nociception and respiration. Bidirectional codeine algesic effects were observed at the 15- and 60-min test intervals. Naloxone increased the codeine jump latency scores at the 15-min interval and decreased codeine jump latency scores at the 60-min interval. These results suggest that codeine engages opposed nonopioid-mediated hypoalgesic and opioid-mediated hyperalgesic nociceptive systems in this animal model. Codeine depressed respiration at both the 15- and 60-min test intervals and this respiratory depression was reversed by naloxone. These findings support the notion that codeine respiratory effects are mediated by opioid system activity.

Domestic fowl Chickens Thermal nociception Pain Respiration Codeine Naloxone Opioid Opiate Hypoalgesia Hyperalgesia

OPIATE agonists typically produce hypoalgesia in a variety of species (1,4,11,15). Recent research, however, reported a morphine-induced hyperalgesic response to a noxious thermal stimulus in young domestic fowl (6,12,14). This paradoxical opiate effect is strain dependent (6), naloxone reversible (6), exhibits dose and temporal characteristics inverse of typical morphine hypoalgesia (12), and is mediated primarily by μ- and, to a lesser extent, κ-receptors (14). The morphine hyperalgesic effect is not unique to thermal nociception because it is also evident in domestic fowl when they are subjected to a test of chemical nociception (7).

Research in our laboratory examined the potency relationship between the opiate agonist morphine and its methylated version, codeine, on thermal nociception in domestic fowl (5). In other species, the antinociceptive potency of codeine is approximately one-third to one-twelfth that of morphine (8,10). This differential potency is consistent with the notion that the antinociceptive action of codeine results through opioid receptor binding after in vivo demethylation to morphine (2, 3). In our potency relationship study with fowl (5), morphine produced a hyperalgesic response and codeine produced a hypoalgesic response. Moreover, the opiate antagonist naloxone attenuated the hyperalgesic action of morphine but potentiated the hypoalgesic action of codeine. This naloxone-potentiated codeine hypoalgesic effect is inconsistent with the expectation that codeine algesic effects would be like morphine effects and thus would elicit naloxone-reversible hyperalgesia in this animal model. Codeine hypoalgesic effects, however, occurred at a relatively short injection-to-test interval (10 min). As such, the failure to observe codeine hyperalgesia may be due to insufficient demethylation of codeine to morphine. Thus, longer injection-to-test intervals may be necessary to obtain hyperalgesia as greater amounts of demethylated codeine (i.e., morphine) act at opioid receptors. If codeine hyperalgesia is observed, then an important issue is whether this effect is reliant upon opioid receptor activity. Therefore, the present research examined the temporal characteristics of codeine on thermal nociception and the effect of naloxone against these codeine effects.

EXPERIMENT 1

This experiment was designed to examine the temporal characteristics of codeine on thermal nociception in young cockerels. Tests at intervals that permit greater codeine demethylation may yield hyperalgesia as this metabolite acts on opioid receptor systems. If, however, codeine is not subject to

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such biotransformation and acts directly at a receptor system to produce hypoalgesia, then its temporal characteristics should resemble a U-shaped function with peak codeine algesic effects at about 30 min and a return to baseline after several hours (12). In addition to assessing codeine effects on thermal nociception, respiration measures were included in the present study because respiration is sensitive to opiate manipulations (7,14).

METHOD

Subjects

Cockerels (Welp-Line 542; Welp, Inc., Bancroft, IA) were obtained 1-day posthatch and housed in pairs, under 24-h illumination, with free access to food (Wayne pullet starter) and tapwater in chambers [see (14) for complete housing apparatus description] that provided physical separation but not visual or auditory isolation. Room temperature was maintained at 32 ± 1°C for the first week and 29 ± 1°C thereafter.

Apparatus

The test apparatus consisted of a 16 × 29 × 30 cm Plexiglas™ chamber with a hinged lid. The walls of the chamber were covered with white paper except for a 5-cm opening near the chamber floor to permit observation. The floor of the test apparatus was a grid-like surface, composed of eight 1-cm diameter glass tubes spaced 2 cm center-to-center, mounted in holes drilled through the walls of the Plexiglas chamber. One end of the test chamber was occupied by a 12.5 × 15.5 × 7 cm wooden platform. The grid-like floor was heated by a Nichrome wire heating element (Eagle Glocoil Incubator) threaded through the glass tubing of the test chamber floor. Temperature was regulated by a 7.5 A variable transformer (Standard Electric Co., Model 300BU) and monitored by a digital thermometer (Fluke, Model 52) from inside one of the center glass tubes. Response latencies were measured to the nearest 0.1 s via an electronic timer (Hunter, Model 120A).

Procedure

At 15 days posthatch, chicks were removed from their home cage, weighed, given codeine sulfate (30 mg/kg dissolved in 0.9% saline) or the saline vehicle at volumes of 1 ml/kg IM, and returned to their home cage. Tests of thermal nociception were conducted either 15, 30, 60, or 120 min after codeine injections (n = 12 per group). The saline control group consisted of three animals from each of the four injection-to-test intervals. At the time of test, an animal was removed from its home cage, placed into a vented 2-quart opaque container, and transported to an adjacent room. The animal was placed on the heated grid (79.5°C) facing away from the raised platform. A timer was manually started as the chick's feet touched the grid floor and was terminated when both feet left the grid floor (i.e., escape to the raised platform or a jump response). The test session was terminated if the animal failed to respond within 70 s. After hot-plate tests, respiration was recorded by counting rhythmic chest movements for 1 min. The chick was returned to its home cage following tests.

Statistical analyses were performed using analysis of variance (ANOVA) and power-adjusted t-tests (9).

RESULTS

Results of the hot-plate tests are presented in Fig. 1. Codeine effects are evident as an initial increase and subsequent decrease in mean jump latencies. A one-way ANOVA performed on these data revealed a significant treatment effect, $F(4,55) = 5.32$, $p < 0.01$. Further analyses demonstrated that the mean jump latency for the 15-min codeine group was significantly longer (hypoalgesic effect) than the saline control group, $t(55) = 1.72$, $p < 0.05$. The mean jump latencies for the 60- and 120-min codeine groups were significantly shorter (hyperalgesic effect) than the saline control group, $t^s(55) = 2.10$ and 2.04, respectively, $p^s < 0.05$.

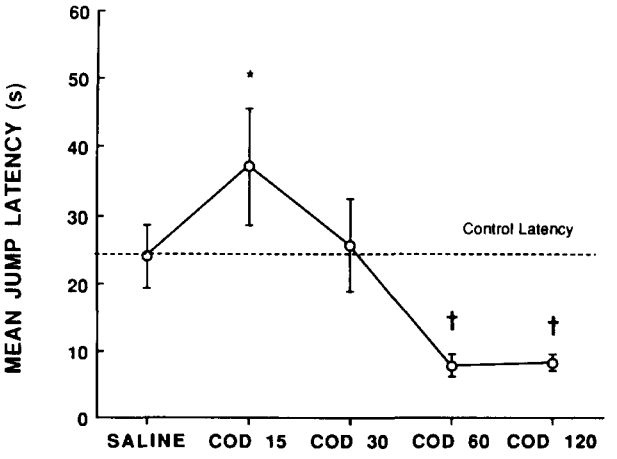

FIG. 1. Mean jump latency ± SEM as a function of injection-to-test interval (15–120 min) following IM administration of saline (1.0 ml/kg) or codeine (COD, 30 mg/kg/ml; n = 10). *Significant hypoalgesia. †Significant hyperalgesia.

As evident in Fig. 2, codeine produced respiratory depression at each of the four temporal intervals tested. Analysis of variance performed on the respiration data revealed a significant treatment effect, $F(4,55) = 6.30$, $p < 0.01$. Further analyses demonstrated that each of the four codeine groups (i.e., 15, 30, 60, and 120 min) had significantly lower mean respiration scores than the saline control group, $t^s(55) = 3.79$, 3.59, 4.60, and 2.65, respectively, $p^s < 0.01$.

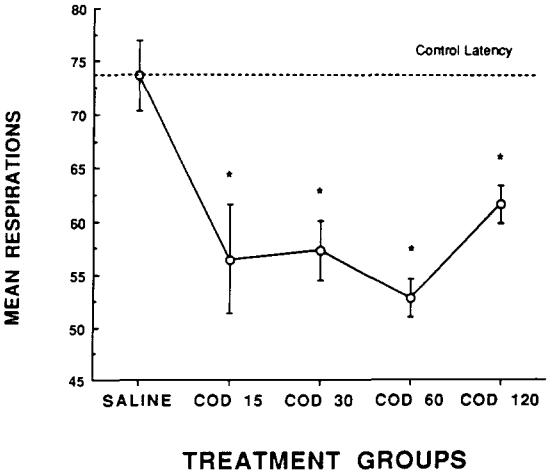

FIG. 2. Mean respirations ± SEM as a function of injection-to-test interval (15–120 min) following IM administration of saline (1.0 ml/kg) or codeine (COD, 30 mg/kg/ml; n = 10). *Significant respiratory depression.

DISCUSSION

This first experiment examined the temporal pattern of codeine effects on thermal nociception. Codeine effects were evident as a time-dependent, biphasic response. Codeine produced a hypoalgesic response at 15 min postinjection, a return to baseline at the 30-min test interval, and a hyperalgesic response at 60 and 120 min postinjection. The early codeine hypoalgesic response, observed at 15 min, is consistent with previous evidence (5); the subsequent codeine hyperalgesic response at 60 and 120 min is the first report of codeine hyperalgesia in this animal model. This time-dependent biphasic codeine algesic effect is consistent with the possibility that, in this animal model, hyperalgesia is detected at temporal intervals (>30 min) that permit sufficient in vivo codeine demethylation to yield its morphine analog. Our earlier research demonstrated a naloxone potentiation of codeine hypoalgesic effects and an attenuation of morphine hyperalgesic effects. If the present time-dependent codeine hyperalgesic effects, in fact, reflects demethylated codeine (i.e., morphine), then naloxone should potentiate codeine hypoalgesic effects and attenuate later (e.g., 60–120 min) hyperalgesic phenomena.

Codeine depressed respiration at each test interval. This respiratory effect is unlike the biphasic effect of codeine on nociception. Morphine depresses respiration in most species by activating μ-receptors and does so in fowl as well (14). Whether codeine produces respiratory depression in fowl by interaction with opioid receptors is unknown. Naloxone antagonism of codeine respiratory depression would imply opioid receptor mediation.

EXPERIMENT 2

This experiment was designed to examine the effects of the opioid receptor antagonist naloxone on time-dependent codeine hypo- and hyperalgesic effects. Naloxone effects on codeine respiratory depression were also examined.

METHOD

Subject characteristics, housing facility, hot-plate apparatus, and test procedures were as described in Experiment 1. In this study, thermal nociception was evaluated either 15 or 60 min after codeine sulfate (30 mg/kg) or 15 min after 0.9% saline injections. In all cases, naloxone hydrochloride (5 mg/kg) or its saline vehicle was administered 15 min before hot-plate tests (n = 10 per group). All injections were IM in volumes of 1.0 ml/kg. As the experimental groups do not form a complete two-factor design, statistical analyses were performed using one-way ANOVA and power-adjusted t-tests (9).

RESULTS

Results of the hot-plate tests are summarized in Fig. 3. A bidirectional codeine algesic response was observed at the 15- and 60-min test interval. Naloxone increased jump latency scores at the 15-min interval and attenuated jump latency scores at the 60-min interval. A one-way ANOVA of these data revealed a significant treatment effect, $F(5,54) = 3.99$, $p < 0.01$. No significant differences were observed between the mean jump latencies of the saline groups. As well, no significant codeine algesic effects were detected in the saline-pretreated animals. However, the mean jump latencies for chicks at the codeine 60-min interval were significantly lower than the mean jump latencies of chicks at the codeine 15-min interval, $t(54) = 2.10$, $p < 0.05$. Naloxone-pretreated chicks had significantly longer mean jump latencies than their respective saline control chicks at both the codeine 15- and 60-min intervals, $t'(54) = 2.16$ and 2.44, respectively, $p' < 0.05$.

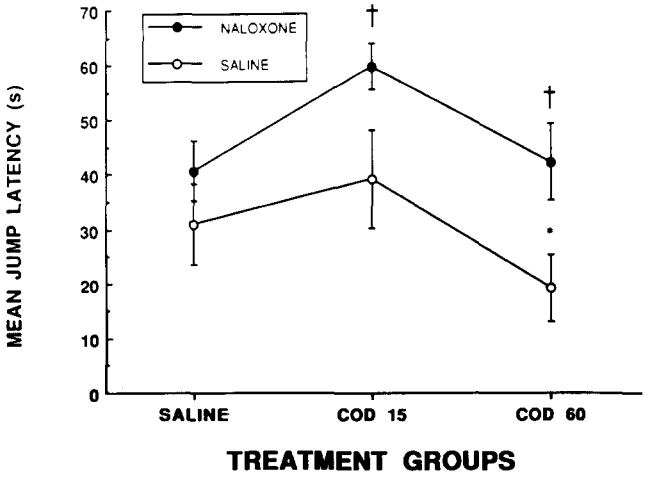

FIG. 3. Effects of naloxone (5.0 mg/kg/ml, IM) or saline (1.0 ml/kg) on temporal (15 and 60 min postinjection) patterns of codeine (COD, 30.0 mg/kg/ml) algesia on thermal nociception. Points represent mean jump latency ± SEM (n = 10). * Significant hyperalgesia compared to the COD 15-min group. †Significant naloxone antagonism.

Effects of naloxone and codeine on respiration are summarized in Fig. 4. Codeine produced respiratory depression at both the 15- and 60-min intervals and this codeine effect was attenuated by naloxone. A one-way ANOVA revealed a significant treatment effect, $F(5,54) = 9.48$, $p < 0.01$. Further analyses demonstrated that mean respiration for the codeine-treated chicks at the 15- and 60-min intervals was significantly lower than the control group, $t'(54) = 4.93$ and 4.77, respectively, $p' < 0.01$. Further analyses revealed that at the codeine 15- and 60-min intervals naloxone-treated chicks had signifi-

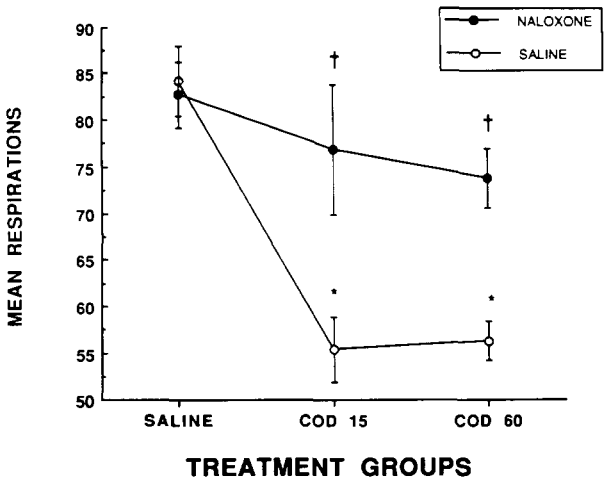

FIG. 4. Effects of naloxone (5.0 mg/kg/ml, IM) or saline (1.0 ml/kg) on temporal (15 and 60 min postinjection) patterns of codeine (COD, 30.0 mg/kg/ml) respiratory depression. Points represent mean respirations ± SEM (n = 10). *Significant respiratory depression. †Significant naloxone antagonism.

cantly higher mean respiration than their respective saline controls, $t^s(54) = 3.66$ and 2.99, respectively, $p^s < 0.01$.

DISCUSSION

Codeine produced an initial increase and a subsequent decrease in jump latency. These directional changes in response latency are indicative of hypo- and hyperalgesia, respectively, but were not statistically significant. Naloxone, nevertheless, potentiated codeine's hypoalgesic effect and attenuated the hyperalgesic effect; these results were statistically significant. Naloxone potentiation of codeine hypoalgesia is consistent with earlier results (5) and suggests that codeine hypoalgesia is mediated by nonopioid systems. However, the observation that naloxone reversed codeine hyperalgesia suggests that hyperalgesia is mediated by the action of codeine or its metabolite at opioid receptors.

Consistent with earlier reports (7,14) and the results from Experiment 1, codeine produced respiratory depression at both the 15- and 60-min test intervals. The observation that naloxone reversed this respiratory depression supports the notion that codeine effects on respiration are mediated through opioid receptor functioning. Whether these effects result from the action of codeine or its metabolite at opioid receptors remains to be determined.

GENERAL DISCUSSION

In certain strains of domestic fowl, morphine has hyperalgesic effects neither preceeded nor followed by hypoalgesia (12). These hyperalgesic effects are attenuated, primarily, by μ-receptor antagonists (14). In this same animal model, codeine exerts hypoalgesic effects potentiated by naloxone (5). This outcome is unusual in two respects: first, codeine is thought to exert hypoalgesic effects by in vivo demethylation to morphine (2,3) and hence would be expected to exert hyperalgesic effects in fowl; second, any effects exerted by codeine on nociception via demethylation ought to reflect interactions with opioid receptors. Codeine hyperalgesic effects, therefore, should be blocked or attenuated, rather than potentiated, by the opioid receptor antagonist naloxone. In earlier research (5), codeine effects were assessed after a 10-min interval. This interval may have been too brief for sufficient codeine biotransformation to occur and elicit hyperalgesia. Thus, the present research sought to characterize the temporal pattern of codeine algesic effects. In Experiment 1, codeine produced a time-dependent biphasic hypoalgesic and hyperalgesic response. The initial codeine hypoalgesic effect is consistent with earlier results (5) and the later-appearing codeine hyperalgesic effect confirms the prediction that longer injection-to-test intervals (i.e., 60 and 120 min) would be required to reveal hyperalgesic effects due to in vivo biotransformation of codeine to morphine.

In Experiment 2, the opioid receptor antagonist naloxone potentiated the initial increase and attenuated the subsequent decrease in jump latency produced by codeine. The potentiation of the initial codeine hypoalgesic effect is consistent with the results of earlier research (5) and suggests that this codeine effect is not mediated by a direct codeine action at opioid receptors. The late-appearing codeine hyperalgesic effect (Exp. 1) and its attenuation by naloxone (Exp. 2) is consistent with the assumption that this codeine effect reflects in vivo demethylation of codeine. Collectively, these results suggest the existence of opposing algesic systems that are differentially affected, as a function of time, by codeine administration. Hypoalgesia is detected at early test intervals (<30 min) when little codeine biotransformation has occurred. Test intervals that permit sufficient codeine demethylation (>30 min) allow greater amounts of its metabolite to activate opioid receptors and produce hyperalgesia.

We attempted to provide additional evidence that codeine hyperalgesic effects result from in vivo demethylation by hepatic microsomal enzymes (8). Unpublished research from this laboratory has shown that the P-450 microsomal enzyme inhibitor SKF-525A prevented codeine hyperalgesia. However, the dose of SKF-525A that significantly increased phenobarbital-induced sleep time produced hypoalgesic effects. Studies investigating the possible role of seizure activity in the expression of codeine hypoalgesia met with similar results; the antiepileptic compound diphenylhydantoin exhibits hypoalgesic effects.

Research in this laboratory has shown that changes in testing procedures can affect nociceptive responses in fowl (13). It is possible that the changes in procedures between Experiment 1 and Experiment 2 (e.g., double injections) may have contributed to the inability to detect significant hypo- and hyperalgesia in Experiment 2. Moreover, if codeine differentially engages opposing algesic systems, time of test will be an important determinant of the nociceptive response; the nociceptive outcome will depend on the summation of activity in these opposing algesic systems.

In fowl, respiratory depression is mediated primarily by μ- and, to a lesser extent, δ-opioid receptors (14). In the present study, codeine uniformly depressed respiration at each test interval and this respiratory depression was reversed by naloxone. These findings suggest that adequate codeine to morphine biotransformation may have occurred at early test intervals to access opioid receptors to depress respiration. Alternatively, it is possible that codeine exerts direct effects on opioid receptors involved in respiration, thereby producing respiratory depression. However, the pattern of codeine and naloxone effects on nociception argues against this latter possibility and suggests that the initial codeine effect on nociception and on respiration are not mediated by the same neurochemical substrates.

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