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Cholecystokinin Antagonizes Morphine Induced Hypoactivity and Hyperactivity in Hamsters

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SCHNUR, P., V. P. RAIGOZA, M. R. SANCHEZ AND P. J. KULKOSKY. *Cholecystokinin antagonizes morphine induced hypoactivity and hyperactivity in hamsters.* PHARMACOL BIOCHEM BEHAV **25**(5) 1067–1070, 1986.—Three experimental replications were used to test the effects of three doses (25, 50 or 75 μg/kg) of cholecystokinin octapeptide (CCK-8) on morphine induced changes in activity. For each dose of CCK-8, running wheel activity of golden Syrian hamsters was monitored for three hours following a series of two injections. The first injection consisted of either saline or CCK-8, the second of either saline or morphine sulfate (15 mg/kg). Thus, in each replication four groups were created: Group SAL/SAL (n=8) received two saline injections, Group CCK/SAL (n=8) an injection of CCK-8 followed by an injection of saline, Group SAL/MS (n=8) an injection of saline followed by an injection of morphine and Group CCK/MS (n=8) an injection of CCK-8 followed by an injection of morphine. Results indicated that a 25 μg/kg dose of CCK-8 blocked the hypoactivity elicited by morphine 40–60 min after opiate injection, whereas a 75 μg/kg dose of CCK-8 blocked the hyperactivity elicited by morphine 80–100 min after opiate injection. These findings are consistent with previous reports that CCK-8 antagonizes the effects of opiate agonists on a variety of behaviors and is supportive of the hypothesis that endogenous CCK-8 may antagonize endogenous opioid peptides in the control of behavior.

Cholecystokinin octapeptide Hamsters Morphine Locomotor activity

CHOLECYSTOKININ, the gastrointestinal and brain octapeptide (CCK-8), exerts a variety of effects on behavior [16,33]. Moreover, accumulating evidence suggests that CCK-8 may function as an endogenous antagonist of opiate actions. First, the distribution of CCK-8 in the brain parallels that of the endogenous opiates [27]. Furthermore, CCK-8 has actions that are opposite those of the opiates: in rats, for example, food intake increases following the administration of morphine, butorphanol, beta-endorphin, and other opiate agonists [1, 13, 14, 17–19], but decreases following similar administration of CCK-8 [9,25]. In addition, CCK-8 antagonizes opiate mediated behaviors: CCK-8 has been shown to antagonize morphine and beta-endorphin induced analgesia [6,7], butorphanol induced feeding [18], as well as the catalepsy induced by beta-endorphin [10]. Also, morphine antagonizes the intestinal and analgesic effects of CCK-8 [31,32] and development of CCK antibodies potentiates morphine analgesia [8]. Finally, the CCK-8 antagonist, proglumide [4], potentiates morphine induced analgesia [29] and hypoactivity [3], and prevents or reverses morphine tolerance [11, 28, 30].

Most of the direct evidence for the role of CCK-8 as an opiate antagonist rests upon demonstrations involving opiate analgesia or feeding in murid rodents. The present study extends these findings to locomotor activity in a cricetid, the golden hamster. We investigated the effects of three doses of CCK-8 (25, 50 and 75 μg/kg) on morphine (15 mg/kg) induced changes in hamster locomotor activity. Morphine's effects on locomotor activity in the hamster are well documented: at low doses, morphine has predominantly excitatory effects, whereas at high doses, inhibitory effects predominate [21,22]. At the dose employed here, morphine's dual actions are revealed in a biphasic time effect pattern: compared with saline controls, locomotor activity is first suppressed and then elevated [22]. Recent evidence indicates that both morphine induced sedation and hyperactivity are naloxone reversible [20, 23, 24]. If CCK-8 acts as an opiate antagonist, then it too should antagonize morphine's effects on hamster locomotor activity.

METHOD

Subjects

Ninety-six experimentally naive adult (89 female, 7 male) golden Syrian hamsters with a mean weight of 104.5 g were used. Fifty-two hamsters were obtained from Harlan Sprague-Dawley (Indianapolis, IN), 32 were obtained from Sasco, Inc. (Omaha, NE), and 12 were descended from animals obtained from Sasco. Thirty-two hamsters were used in each replication of the experiment. They were housed individually in wire mesh stainless steel cages at an

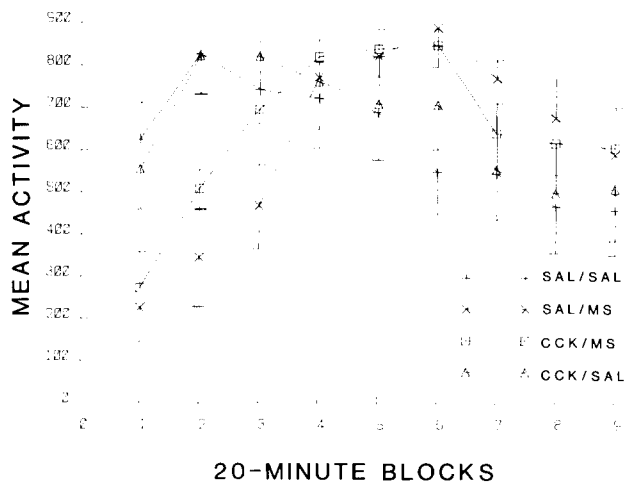

FIG. 1. Mean (±standard error) running wheel activity (revolutions) as a function of time blocks, for hamsters receiving saline and saline (SAL/SAL, n=8), saline and morphine (SAL/MS, n=8), 25 μg/kg CCK-8 and saline (CCK/SAL, n=8), or 25 μg/kg CCK-8 and morphine (CCK/MS, n=8).

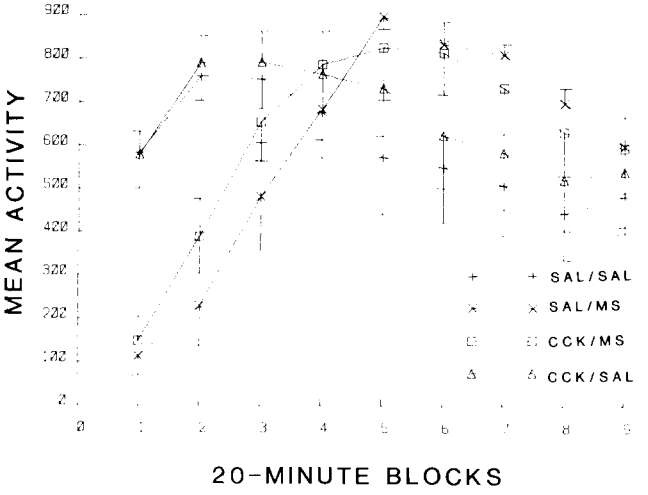

FIG. 2. Mean (±standard error) running wheel activity (revolutions) as a function of time blocks, for hamsters receiving saline and saline (SAL/SAL, n=8), saline and morphine (SAL/MS, n=8), 50 μg/kg CCK-8 and saline (CCK/SAL, n=8), or 50 μg/kg CCK-8 and morphine (CCK/MS, n=8).

ambient temperature of approximately 23°C, maintained on a 12:12 hour light-dark cycle (lights on at 7 a.m.), and given free access to tap water and shredded paper nesting material throughout the experiment. Animals received a daily food ration (Purina Rodent Lab Chow) after each experimental session sufficient to maintain 90% of their ad lib weights.

Apparatus and Materials

The apparatus consisted of sixteen identical activity wheels (Wahmann Co., Model LC-34) which were housed in a room dimly illuminated by two 15-watt bulbs. Running wheels were fitted with microswitches and interfaced (Lafayette minicomputer interface, Model No. 1180) to an Apple II Plus computer to record the number of wheel revolutions. An ambient noise level of approximately 72 dB (re: 0.0002 dynes/cm^2, A scale) was maintained.

Morphine injections consisted of 15 mg/kg doses of morphine sulfate (Lilly), expressed as the salt, dissolved in 1 ml of 0.9% w/v sodium chloride (saline). CCK-8 injections consisted of either 25, 50 or 75 μg/kg doses of sulfated cholecystokinin octapeptide (Squibb, SQ 19,844, Lot No. NN020NC) dissolved in saline. All injections were administered subcutaneously (SC) in the dorsal surface of the neck in 1 ml/kg volumes.

Procedure

Three replications of the experimental design, one at each dose, were completed. Experimental procedures in each replication spanned four successive days: on each of the first three days, animals were injected with saline and placed in the running wheels for a three hour baseline session. These sessions served to acclimate the animals to the running wheels and to the handling/injection procedures. On the fourth day, animals received two injections, spaced 10 minutes apart, before being placed in the running wheels for a three hour test session. Animals were assigned randomly to four groups: Group SAL/SAL (n=8) received two saline injections; Group CCK/SAL (n=8) an injection of CCK-8 followed by an injection of saline; Group SAL/MS (n=8) an injection of saline followed by an injection of morphine; Group CCK/MS (n=8) an injection of CCK-8 followed by an injection of morphine. This basic design was replicated three times, once for each dose of CCK-8. The number of wheel revolutions was recorded every 20 min for each animal. The level of significance for all statistical analyses was set at $p<0.05$.

RESULTS

Mean (±standard error) running wheel activity (revolutions) as a function of 20 min blocks, for hamsters injected with saline and saline (n=8), saline and morphine (n=8), CCK and saline (n=8), or CCK and morphine (n=8) is depicted in Figs. 1, 2 and 3 for CCK-8 doses of 25, 50 and 75 μg/kg, respectively.

A 2×2×3×9 [First Injection (CCK vs. saline) × Second Injection (morphine vs. saline) × CCK Dose × Time Blocks] split-plot analysis of variance revealed significant main effects of CCK dose, $F(2,84)=6.05$, and time blocks, $F(8,672)=37.60$ and significant interactions of CCK dose and time blocks, $F(16,672)=3.65$, second injection and time blocks, $F(8,672)=58.58$, and CCK, second injection and time blocks, $F(8,672)=4.84$. No other main effect or interaction was found significant.

The effect of time and the interaction of morphine and time reflect the expected biphasic effect of the 15 mg/kg dose of morphine on hamster running wheel activity [21,22]. The effect of CCK dose and the interaction of dose with time blocks reflects the decreased activity at the 75 μg/kg dose of CCK. The three way interaction of CCK and morphine and time suggests that CCK affects the response to morphine at some time blocks. Student-Newman-Keuls tests revealed that, at the 25 μg/kg dose of CCK, the difference between Group SAL/MS and Group CCK/MS was significant during

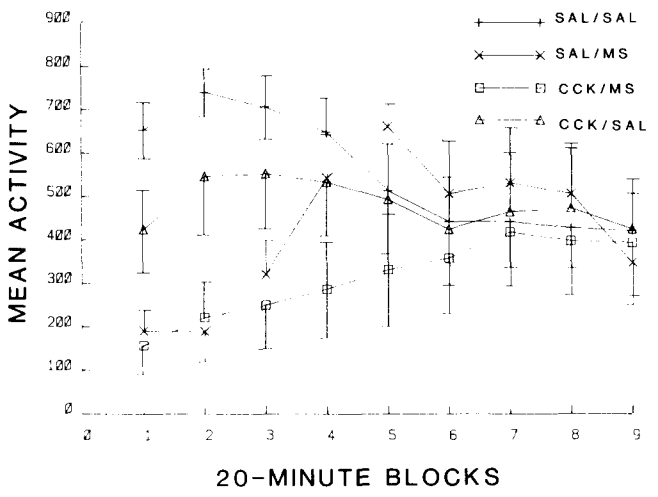

FIG. 3. Mean (±standard error) running wheel activity (revolutions) as a function of time blocks, for hamsters receiving saline and saline (SAL/SAL, n=8), saline and morphine (SAL/MS, n=8), 75 μg/kg CCK-8 and saline (CCK/SAL, n=8) or 75 μg/kg CCK-8 and morphine (CCK/MS, n=8).

the third 20 min time block (see Fig. 1). Similarly, at the 75 μg/kg dose of CCK, the difference between Group SAL/MS and Group CCK/MS was significant during the fifth 20 min time block (see Fig. 3). That is, mean running wheel activity increased 20–40 min after the morphine injection in animals receiving a prior injection of 25 μg/kg CCK, relative to hamsters receiving saline prior to morphine. Conversely, mean activity *decreased* 80–100 min after the morphine injection in animals receiving a prior injection of 75 μg/kg CCK, relative to hamsters receiving saline prior to morphine.

DISCUSSION

The present data reveal a new, though limited antagonist action of CCK-8 on morphine induced behavioral change. At the relatively high doses of 25 and 75 μg/kg SC, CCK-8 blocked the hypoactivity and hyperactivity effects of morphine on hamster running wheel behavior at 40–60 min and 80–100 min after opiate injection, respectively. In an initial pilot study, no interaction of CCK-8 and morphine on hamster running wheel activity was observed at 5.0 μg/kg CCK (unpublished observations). Comparatively high doses of CCK-8 (cf. [33]) are required to affect morphine elicited changes in activity in golden hamsters.

The effect of high doses of CCK-8 on morphine-induced hypoactivity and hyperactivity in hamsters compares with the effects of naloxone and naltrexone, which can block both morphine sedation and hyperactivity [20, 23, 24]. The present effect contrasts with the often reported analgesia and reduction in activation or motility in humans, rats and mice after administration of CCK-like peptides alone [5, 26, 32, 33], although a tendency of 75 μg/kg CCK to depress activity in hamsters was observed at 0–40 min after CCK and saline injections. Cricetids and murids display many other well-documented differences in response to opiates and peptides (e.g., [12,15]).

Results of this experiment are consistent with previous reports that CCK-8 antagonizes the effects of opiate agonists on analgesia [6,7], catalepsy [10], and feeding [1,18]. Our findings are also in accord with the reports that proglumide, a CCK blocker, or active immunization against CCK potentiates morphine-induced analgesia [8,29] and hypoactivity [3]. The coextensive distribution of CCK-8 and endogenous opiates in brain [27] allows for many central sites of opioid peptide and CCK-like peptide interaction. For example, the dorsomedial hypothalamus is required for both naloxone and CCK-8 to inhibit feeding [2]. Alternatively, the interactions of the central and peripheral actions of CCK-8 and/or opioid peptides may account for the observed antagonism.

Thus, we have found further experimental evidence to support the hypothesis that endogenous CCK-8 may antagonize endogenous opioid peptides in the control of behavior. We have extended this putative interactive control of CCK-8 and opioids to the case of locomotor behavior in hamsters. CCK-8 and the endogenous opioids may well constitute integrative peptide co-antagonists in the coordinated control of several classes of adaptive behaviors. Our evidence strengthens the notion that CCK-8 is a candidate for the hypothesized endogenous naloxone-like substance [11,13].

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