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The effects of proglumide on morphine induced motility changes

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Abstract. Proglumide (0.02 mg/kg), a cholecystokinin antagonist, was administered to rats either together with or without morphine (0, 5, 15, or 45 mg/kg). Whereas proglumide in the absence of morphine showed a trend towards enhanced behavioral activation, it potentiated the hypokinesia induced by morphine. These results are consistent with the hypothesis that endogenous cholecystokinin tonically antagonizes opiate modulation of motility, irrespective of whether such modulation is produced by opiates and endogenous or exogenous origin.

Key words: Morphine – Cholecystokinin – Proglumide – Locomotion – Rat

The gastrointestinal octapeptide cholecystokinin (CCK8) has been demonstrated to exert a wide range of effects on behavior (Morley 1982). Recently, CCK8 has also been shown to affect the pain threshold in rats. Whereas CCK8 at high doses induced analgesia (Jurna and Zetler 1981), at much lower doses it did not alter the pain threshold, and was found to reduce the analgesic action of subsequently administered morphine (Faris et al. 1983).

Analgesia following electrical shocks administered to the front paws of rats, and analgesia classically conditioned upon this treatment, was demonstrated to be mediated, in part at least, by endogenous opiates (Watkins and Mayer 1982). CCK8, administered either intrathecally (IT) or intraperitoneally (IP), reduced the analgesia following these manipulations. CCK8 failed to affect analgesia induced by electrical shocks to the hind paws of rats (Faris et al. 1983), which is apparently not mediated by endogenous opiates (Watkins and Mayer 1982).

Proglumide, a competitive antagonis of CCK8 (Hahne et al. 1981), has been tested for its effects on opiate action. Microinjections of IT proglumide potentiated analgesia induced by morphine, which was injected either IT or IP, or into the periaqueductal central gray. Proglumide also enhanced analgesia induced by opioid electrical shocks to the frontpaws of rats, and classically conditioned analgesia (Kinscheck et al. 1983). In this study proglumide failed to affect the baseline pain-sensitivity of untreated rats, and analgesia produced by non-opioid hindpaw shocks. Finally, tolerance to the analgesic action of morphine was reduced or even entirely abolished by proglumide (Tang et al. 1983; Kinscheck et al. 1983). On the basis of these observations it was suggested that CCK8 in the CNS antagonizes the analgesic action of opiates (Faris et al. 1982).

CCK8 has also been found to affect motility, and it induces sedation and reduces motility (Zetler 1981) in mice. On the other hand, intracerebral injection of this compound in rats reduced the catalepsy observed following subsequent administration of β-endorphin (Itoh and Katsuura 1981). These investigators did not test the action of CCK8 in the absence of β-endorphin. The latter results suggest that CCK8 may act as an endogenous antagonist of opiate action on motility in the rat, resembling its action on opiate analgesia. If that is the case, the CCK8 antagonist proglumide should enhance opiate-induced effects on motility. The present experiment was designed to test this hypothesis.

Materials and methods

Subjects. Fifty-seven male Wistar rats (280–380 g), bred at Tel-Aviv University, were housed in a room with reversed light-dark cycle (light on from 6:00 p.m. to 6:00 a.m.) and free access to food pellets and water.

Injection procedure and drugs. On the test day the animals were randomly assigned to eight groups. Animals were injected intraperitoneally (IP) with morphine hydrochloride (0, 5, 15, or 45 mg/kg, two groups at each dose level) 30 min before the onset of the experiment. Morphine at all doses was dissolved in physiological saline, and injected in a volume of 1 ml/kg. Four of these groups (one of each dose of morphine) received 10 min prior to the experiment an injection of 0.02 mg/kg of proglumide. The proglumide was dissolved in 0.4% DMSO in 0.05 M phosphate buffer solution (pH 7.4) and injected in 1 ml/kg IP. This dose of proglumide was chosen as it was shown to reliably enhance morphine analgesia in the rat (Kinscheck et al. 1983). The other four groups were injected with equal volumes of the vehicle for proglumide, 10 min before testing.

Testing procedure. Ten minutes following the last drug administration animals were subjected to motility testing. Each animal was placed in a plastic cage measuring 25 × 40 × 12 cm, the floor of which was divided into four equal rectangles. The number of rectangles the animal crossed was recorded during the 5 min of motility testing. This procedure was chosen because it had been demonstrated to reliably reflect the suppressant effects of morphine in previous studies (Urca and Frenk 1980).

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Table 1. Effects of morphine and proglumide treatment on the number of crossings (mean, SEM) in a 5-min period

Treatment group	*n*	Mean	SEM	
0 Morphine/vehicle	7	10.0	0.67	$P < 0.10$[a]
5 Morphine/vehicle	7	13.7	1.85	$P < 0.01$[a]
15 Morphine/vehicle	8	10.1	2.32	$P < 0.005$[a]
45 Morphine/vehicle	7	0.0	0.00	$P < 0.005$[b]
0 Morphine/proglumide	7	13.0	1.28	$P < 0.10$[b]
5 Morphine/proglumide	7	7.7	0.87	$P < 0.10$[b]
15 Morphine/proglumide	7	3.8	2.08	$P < 0.005$[b]
45 Morphine/proglumide	7	0.1	0.13	$P < 0.005$[b]

[a] Compared to the same dose of morphine followed by proglumide

[b] Compared to group 0 morphine/vehicle

All animals were tested on a single day during the dark portion of the light/dark cycle. The order of testing of the animals of all groups was randomized. Differences between groups were analyzed statistically using a two-way analysis of variance, with the first factor being dose of morphine and the second factor being proglumide or saline pretreatment. Comparisons of the means of individual groups were performed using the *t*-statistic with the error term of the ANOVA (Winer 1971, pp 445–449).

Results

The analysis of variance applied to the motility scores yielded a significant main effect of the proglumide or saline pretreatment ($F = 4.08$, $df = 1/48$, $P < 0.05$) and of the morphine doses ($F = 21.13$, $df = 3/48$, $P < 0.01$). In addition, there was a significant interaction between the main effects ($F = 4.11$, $df = 3/48$, $P < 0.05$).

Morphine, when injected with the proglumide vehicle, induced a biphasic effect on the locomotion of the animals tested (Table 1). Thus, 5 mg/kg morphine produced a trend towards increased locomotion when compared to the group injected with saline and vehicle ($P < 0.10$), whereas 15 mg/kg morphine failed to affect motility. The highest dose of morphine injected (45 mg/kg) significantly suppressed motility when compared to the control group receiving morphine vehicle alone ($P < 0.01$). Proglumide, in the absence of morphine, produced a trend towards enhanced motility when compared to the group injected with saline and proglumide vehicle ($P < 0.10$), but it significantly increased the inhibitory effects of morphine on motility. Thus, when proglumide was added to 5 mg/kg or 15 mg/kg morphine, motility was significantly lower when compared to the same doses of morphine in the absence of proglumide.

Discussion

The present results are consistent with the findings that morphine produces a biphasic effect on locomotion, with low doses inducing motility and higher doses producing hypokinesia followed by behavioral excitation (Havemann and Kuschinsky 1982). Whereas a dose of 5 mg/kg of morphine induced a nonsignificant trend towards increased motility in animals, a dose of 15 mg/kg did not alter motility, and 45 mg/kg reduced it.

When proglumide was injected following 5 mg/kg morphine, the excitatory effects of this dose of morphine were abolished. Furthermore, when proglumide was added to 15 mg/kg morphine, the combination of these drugs resulted in a sharp reduction in motility. Thus, in the present experiment proglumide consistently enhanced the suppressant effects of morphine on motility, nearly tripling the efficacy of morphine.

These results cannot be explained by assuming an additive effect of the akinesia produced by morphine, and that by proglumide. Indeed, proglumide administered to animals in the absence of morphine showed a trend towards increased motility, rather than towards hypokinesia.

The observation that proglumide, when administered in the absence of morphine, increased rather than decreased motility, supports the hypothesis that this effect results from an interaction of proglumide with endogenous opioids. Spontaneous motility in the rat is decreased by naltrexone (e.g., Katz 1979), and increased by intracerebral injections of endogenous opioids (Stinus et al. 1980). These findings suggest that endogenous opioids may tonically enhance motility in the rat. The proglumide administered in the present study would, by blocking antagonistic action of endogenous CCK, enhance the effects of endogenous opioids, resulting in similar effects to those obtained by the administration of low doses of morphine: a trend towards increased motility.

Our observation that proglumide enhances opiate hypokinesia, taken together with the finding that CCK8 decreases it (Itoh and Katsuura 1981) supports the hypothesis (Faris et al. 1983; Kinscheck et al. 1983) that endogenous CCK8 exerts a tonic antagonistic action on the behavioral effects of certain opiate systems. The nature of the CCK/opiate interaction and the site of action remain to be elucidated.

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