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Effects Induced by BC 264, a Selective Agonist of CCK-B Receptors, on Morphine-Dependent Rats

R. MALDONADO,[1] O. VALVERDE, M. DERRIEN, P. TEJEDOR-REAL[2] AND B. P. ROQUES

Département de Pharmacochimie Moléculaire et Structurale, U 266 INSERM, URA D1500 CNRS, Université René Descartes, UFR des Sciences Pharmaceutiques et Biologiques, Faculté de Pharmacie, 4 avenue de l'Observatoire, 75270 Paris Cedex 06, France

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MALDONADO, R., O. VALVERDE, M. DERRIEN, P. TEJEDOR-REAL AND B. P. ROQUES. *Effects induced by BC 264, a selective agonist of CCK-B receptors, on morphine-dependent rats.* PHARMACOL BIOCHEM BEHAV **48**(2) 363-369, 1994. - The aim of this study was to investigate the possible interaction between neuronal cholecystokinin (CCK) and opiate dependence. Rats were made dependent to morphine and the ability of cholecystokinin-octapeptide (CCK-8) and Tyr(SO_3H)-gNle-mGly-Trp-(NMe)Nle-Asp-Phe-NH_2 (BC 264), a selective agonist of CCK-B receptors, to induce signs of morphine withdrawal after ICV injection was tested. Behavioral responses were compared to those occuring during the naloxone-precipitated morphine withdrawal syndrome. In contrast to naloxone, CCK-8 (0.1, 1, and 10 μg, ICV) did not precipitate any sign of withdrawal. BC 264 (0.1, 1, and 10 μg, ICV) induced a strong hyperlocomotion and wet dog shakes in morphine-dependent rats, the latter effect also observed in nondependent animals. In rats receiving acute morphine, BC 264 induced an opposite effect (i.e., blockade of morphine-induced hyperactivity). Taken together, these results suggest that CCK plays only a minor role in the expression of morphine physical dependence.

Cholecystokinin BC 264 CCK-B receptors Morphine dependence Withdrawal syndrome Locomotion Naloxone

STUDIES on the mechanisms of opioid tolerance and dependence have mainly focused attention on changes at the receptor and second messenger levels (25). However, biological adaptative processes identified by these studies are reported to be not robust enough to explain the high degree of tolerance which can be reached (17) or the very low doses of naloxone required to induce a withdrawal syndrome (36). These considerations led us to consider the occurence of an antiopioid model of tolerance and dependence which postulates that neuropeptides synthesized and released in the CNS act as a part of a homeostatic mechanism to attenuate the effects of opiates (32). The brain cholecystokinin-octapeptide (CCK-8) is one of the several peptides that has been proposed to act as an antiopioid. The interaction between CCK and opioid systems has been analyzed by a variety of procedures. Thus, CCK-8-antagonized exogenous (16) and endogenous (15,26) opioids induce antinociception, whereas CCK antagonists potentiate a range of opioid-mediated antinociceptive responses (2,23). CCK antagonists are also able to prevent the development of morphine tolerance (13,14,35). The interactions between CCK and opioids are not limited to nociception, and other opioid-mediated effects, like hypothermia (20) and body shaking (19), are antagonized by CCK agonists. Besides, CCK-A and CCK-B receptor antagonists diminish and potentiate, respectively, morphine-induced behavioral reinforcement (18). However, this CCK-opioid interaction has still not been reported at the level of opiate physical dependence. Thus, the systemic injection of CCK antagonists proglumide, benzotript, devazepide, and L-365,260 did not modify the expression of morphine abstinence (13,14,28). Besides, the peripheral administration of CCK-8 failed to precipitate withdrawal syndrome after morphine chronic treatment, but the failure of CCK-8 to precipitate withdrawal could be due to its inability to cross the blood-brain barrier (30).

[1] To whom requests for reprints should be addressed.
[2] P. Tejedor-Real is on leave from Facultad de Medicina, Departamento de Neurociencias, Plaza Fragela s/n, 10003 Cádiz, Spain.

The aim of this study was to evaluate the role of the central CCK system, and particularly the different CCK receptors, on the expression of the opiate dependence. Rats were made dependent to morphine, and the ability of CCK-8 and Tyr-(SO_3H)-gNle-mGly-Trp-(NMe)Nle-Asp-Phe-NH_2 (BC 264), a selective agonist of CCK-B receptors (6), to induce behavioral signs of morphine withdrawal after ICV injection was tested. Behavioral responses were compared to those occuring during the withdrawal syndrome precipitated by ICV injection of naloxone. The effects of CCK-8 and BC 264 were also studied in rats receiving acute morphine to further characterize the significance of the behavioral effects induced by the CCK agonists.

MATERIALS AND METHODS

Animals and Surgery

Male Wistar rats (Depré, France) ranging in weight from 220 to 240 g at the beginning of the experiment were used. The animals were anaesthetized with chloral hydrate (250 mg/kg, IP), and unilateral stainless-steel cannula guides (25 gauge) were stereotaxically implanted 1 mm above the final injection site. The cannulae were secured to the skull with stainless-steel screws and dental cement. The cannula guides were kept clear with wire stylets. The guides were implanted with the bregma taken as the origin for coordinates: anterior, −0.8 mm; lateral, +1.6 mm; and ventral −3.7 mm from the skull (29). After surgery the animals were housed in cages with free access to water and food.

Injection Schedule and Drugs

ICV administration was performed with an injection apparatus consisting of 30.5-gauge stainless-steel needles attached to a 10-μl microsyringe (Hamilton, Reno, NV) by polyethylene tubing. In each animal the localization in the ventricle was checked by the ability of saline to flow rapidly into the ventricle when the extremity of the tubing of the injector was placed higher than the rat, as a consequence of negative pressure in the ventricle.

Naloxone, BC 264, CCK-8, and saline were administered ICV by an infusion pump (Precidor, Infors AG, Bottminger, Switzerland) in a constant volume of 2 μl at a rate of 0.0416 μl/s. The needle was left in situ for 30 s to allow diffusion of the drug away from the cannula guide. A blind rating procedure was used to measure the various dependent variables. Thus, injections were made by a different individual than the observer, who did not know the treatment administered to the animals.

BC 264 and CCK-8 were synthesized in the laboratory, as previously described (6). Morphine-HCl and naloxone-HCl were obtained from Sigma Chemical Co. (St. Louis). All of the drugs were dissolved in saline (NaCl 0.9%).

First Experiment

One week after surgery the rats were divided into eight groups (n = 8) corresponding to morphine treatment (four groups) and saline control (four groups). Saline and morphine were injected IP daily at 0900 and 1800 in a volume of 1 ml/kg. The morphine dose was progressively increased from 7 mg/kg to 30 mg/kg over a period of three days, and this dose was maintained during five more days. The first and second number inside the parentheses represent the dose of morphine (mg/kg) injected at 0900 and 1800, respectively, on consecutive days: 1st day (7, 10), 2nd day (15, 20), 3rd day (25, 30), 4th–7th day (30, 30), 8th day (30, only at 0900).

Ninety minutes after the last morphine injection, rats were placed in test cages for a 30-min habituation period before ICV injections. Test chambers consisted of round boxes (30 cm diameter × 35 cm height) with white floors and moderate lighting. One minute after ICV injection of naloxone, BC 264, CCK-8, or saline, animals were observed for a period of 10 min to evaluate representative signs of opiate withdrawal. This procedure was repeated at 24-h intervals until a total of three injections were made. The injection doses of naloxone (0.05, 0.5, and 5 μg), BC 264 (0.1, 1, and 10 μg), and CCK-8 (0.1, 1, and 10 μg) were randomly assigned across animals and days (Latin square). Vehicle injections were performed 24 h before the first compound administration and 24 h after the last injection to detect the appearance of any conditioned withdrawal.

Two classes of signs were measured. The number of bouts of teeth chattering, mastication, rearing, wet dog shakes, and jumping were counted. Ptosis, rhinorrhea, lacrimation, salivation, and diarrhea were evaluated over 2-min periods, with one point given for the presence of each sign during each period. The number of periods showing the sign was then counted (maximum score = 5). Locomotor activity was also evaluated over 2-min periods with a value between 0 and 2 given for each period. Hypolocomotion, defined as a decrease in the ambulation of the animal in the test chamber, was assigned a score of 0, 1 refers to normal locomotion, and 2 refers to hyperlocomotion. Here the values were added for the whole 10-min period (maximum score = 10).

Second Experiment

One week after surgery the rats received an acute injection of morphine (2, 6, and 18 mg/kg, IP) or saline. Ninety minutes later, animals were placed in test cages for a 30-min habituation period before ICV injection. One minute after ICV injection of BC 264 (1 μg), CCK-8 (1μg), or saline, behavior of the animals was evaluated for a period of 10 min using the same procedure as in the first experiment.

Statistical Analysis

In the first experiment, individual group comparisons were made using a two-way analysis of variance (ANOVA) with repeated measures on two factors, dose and time. Individual dose effects were analyzed using a within-subjects Newman-Keuls comparison after significant main effects of dose by repeated-measures ANOVA. Results from the second experiment were analyzed using single-factor ANOVA, and individual mean comparisons were made using the Newman-Keuls test. The level of significance was $p < 0.05$.

RESULTS

First Experiment

Control animals chronically treated with saline. Animals chronically treated with saline did not elicit any representative behavioral sign of withdrawal after ICV administration of naloxone (0.05, 0.5, and 5 μg) or CCK-8 (0.1, 1, and 10 μg). ICV injection of BC 264 (0.1, 1, and 10 μg) induced a slight increase in locomotor activity and number of rearings at the highest dose (Table 1). A significant incidence of wet dog shake behavior (Table 1) and a slight nonsignificant presence of mastication (10 μg = 3.75 ± 1.26) were also observed

TABLE 1

BEHAVIORAL EFFECTS INDUCED BY ICV ADMINISTRATION OF NALOXONE, CCK-8, AND BC 264 IN CONTROL ANIMALS CHRONICALLY TREATED WITH SALINE

			Doses: CCK-8 & BC 264 (1), Naloxone (2)			ANOVA	
	Saline Before	Saline After	0.1 μg (1) 0.05 μg (2)	1 μg (1) 0.5 μg (2)	10 μg (1) 5 μg (2)	F	p
Locomotor activity							
Naloxone	2.87 ± 0.12	2.75 ± 0.55	3.00 ± 0.78	3.25 ± 0.77	3.25 ± 0.49	(4, 28) = 0.23	NS
CCK-8	4.10 ± 0.45	3.55 ± 0.37	2.87 ± 0.76	3.77 ± 1.10	3.77 ± 0.64	(4, 36) = 0.61	NS
BC 264	3.37 ± 0.42	3.14 ± 0.45	4.75 ± 0.49	3.87 ± 0.51	5.37 ± 0.70*	(4, 28) = 3.74	<0.02
Rearing							
Naloxone	8.57 ± 1.61	8.85 ± 2.55	10.83 ± 3.44	10.28 ± 2.94	9.28 ± 2.14	(4, 28) = 0.85	NS
CCK-8	13.55 ± 2.27	11.75 ± 2.43	8.28 ± 2.56	13.50 ± 4.59	10.62 ± 1.89	(4, 36) = 0.86	NS
BC 264	11.5 ± 3.00	11.28 ± 2.47	17.87 ± 2.90	15.87 ± 2.12	24.75 ± 4.69*	(4, 28) = 3.23	<0.05
Wet dog shakes							
Naloxone	0.12 ± 0.12	0.25 ± 0.16	0.57 ± 0.20	0.25 ± 0.16	0.37 ± 0.18	(4, 28) = 1.17	NS
CCK-8	0.50 ± 0.22	0.33 ± 0.23	0.62 ± 0.26	1.44 ± 0.70	2.11 ± 0.96	(4, 36) = 2.45	NS
BC 264	0.37 ± 0.37	0.28 ± 0.18	1.50 ± 0.80	1.50 ± 0.62	4.00 ± 1.77*	(4, 28) = 4.31	<0.01

Values of mean ± SEM and of one-way analysis of variance (ANOVA) within subjects. Saline groups represent the controls performed before and after injection of the different compounds. Number of animals per group = 7–9. *$p < 0.05$, different from the two saline controls (Newman–Keuls test).

after the administration of 10 μg of BC 264. Saline controls performed before and after ICV administration of the different compounds were similar in all the groups (Table 1), showing the absence of any conditioned behavior. Two-way ANOVA revealed no time effect or interaction between dose and time in any case.

Dependent animals chronically treated with morphine. Central administration of naloxone (0.05, 0.5, and 5 μg) in morphine chronically treated rats precipitated a dose-dependent withdrawal syndrome characterized by a significant incidence of jumping, wet dog shakes, mastication, teeth chattering, and ptosis and a significant increase in locomotor activity and rearing behavior (Fig. 1 and Table 2). The most sensitive sign was mastication, which appears significantly after the administration of 0.05 μg of naloxone (11.25 ± 3.02, $p < 0.01$). Significant presences of teeth chattering (20.62 ± 3.31, $p < 0.01$), ptosis (3.00 ± 0.80, $p < 0.01$), locomotor hyperactivity (6.50 ± 0.46, $p < 0.05$), and wet dog shakes (3.12 ± 0.63, $p < 0.01$) were also observed after naloxone 0.5 μg. Jumping behavior (6.37 ± 3.16, $p < 0.05$) and the increase in the number of rearings (37.37 ± 4.83, $p < 0.05$) appeared only after the administration of the highest dose of naloxone (5 μg). Several signs widely reported during systemic naloxone-precipitated morphine withdrawal, such as diarrhea, salivation, lacrimation, and rhinorrea, did not appear. ICV injection of CCK-8 (0.1, 1, and 10 μg) did not induce any sign of withdrawal; only slight increases in locomotion, rearing behavior, and wet dog shakes were observed, mainly after the administration of the dose of 1 μg (Fig. 1 and Table 2). BC 264 (0.1, 1, and 10 μg) administered ICV induced a strong and dose-dependent increase in locomotion and rearing behavior in morphine-dependent rats (Fig. 1 and Table 2). This hyperactivity was also reflected in animals receiving the highest dose of BC 264 by the presence of small horizontal jumps that were quite different from the vigorous vertical jumps observed during naloxone-precipitated morphine withdrawal. A dose-dependent incidence of wet dog shakes (Fig. 1 and Table 2) and a slight nonsignificant presence of mastication (10 μg = 3.25 ± 0.79) were also observed after BC 264 administration.

Saline controls performed before and after ICV administration of the different compounds were similar in all the groups (Figs. 1 and 2), showing the absence of any conditionated effect. Two-way ANOVA revealed no time effect or interaction between dose and time in any case.

Second Experiment

Acute administration of morphine (2, 6, and 18 mg/kg, IP) induced a biphasic effect on locomotion, $F(3, 36) = 11.435$, $p < 0.0001$, and rearing behavior, $F(3, 36) = 11.066$, $p < 0.0001$. Locomotor activity was significantly increased by the doses of 2 and 6 mg/kg, and decreased after the administration of the highest dose (18 mg/kg). Rearing behavior was increased at the dose of 6 mg/kg and decreased at the dose of 18 mg/kg (Fig. 3).

ICV injection of CCK-8 (1 μg) or BC 264 (1 μg) did not modify behavior in control animals or the decrease in locomotion and rearing induced by the highest dose of morphine. However, CCK-8 abolished the increase in rearing, $F(7, 65) = 9.315$, $p < 0.0001$, and partially blocked the hyperlocomotion, $F(7, 65) = 24.831$, $p < 0.0001$, induced by lower doses of morphine. BC 264 abolished both increase in rearing, $F(7, 63) = 7.693$, $p < 0.0001$, and hyperlocomotion, $F(7, 63) = 17.019$, $p < 0.0001$, induced by morphine (Fig. 3).

No representative behavioral signs of withdrawal, other than changes in locomotion and rearing, were observed in any group of animals.

DISCUSSION

In this study, central administration of naloxone precipitated a dose-dependent withdrawal syndrome in morphine chronically treated animals. Doses of naloxone as low as 0.05 μg induced a significant presence of mastication, the most sensitive sign, but a higher dose, 0.5 μg, was required to precipitate other withdrawal signs. However, some signs reported

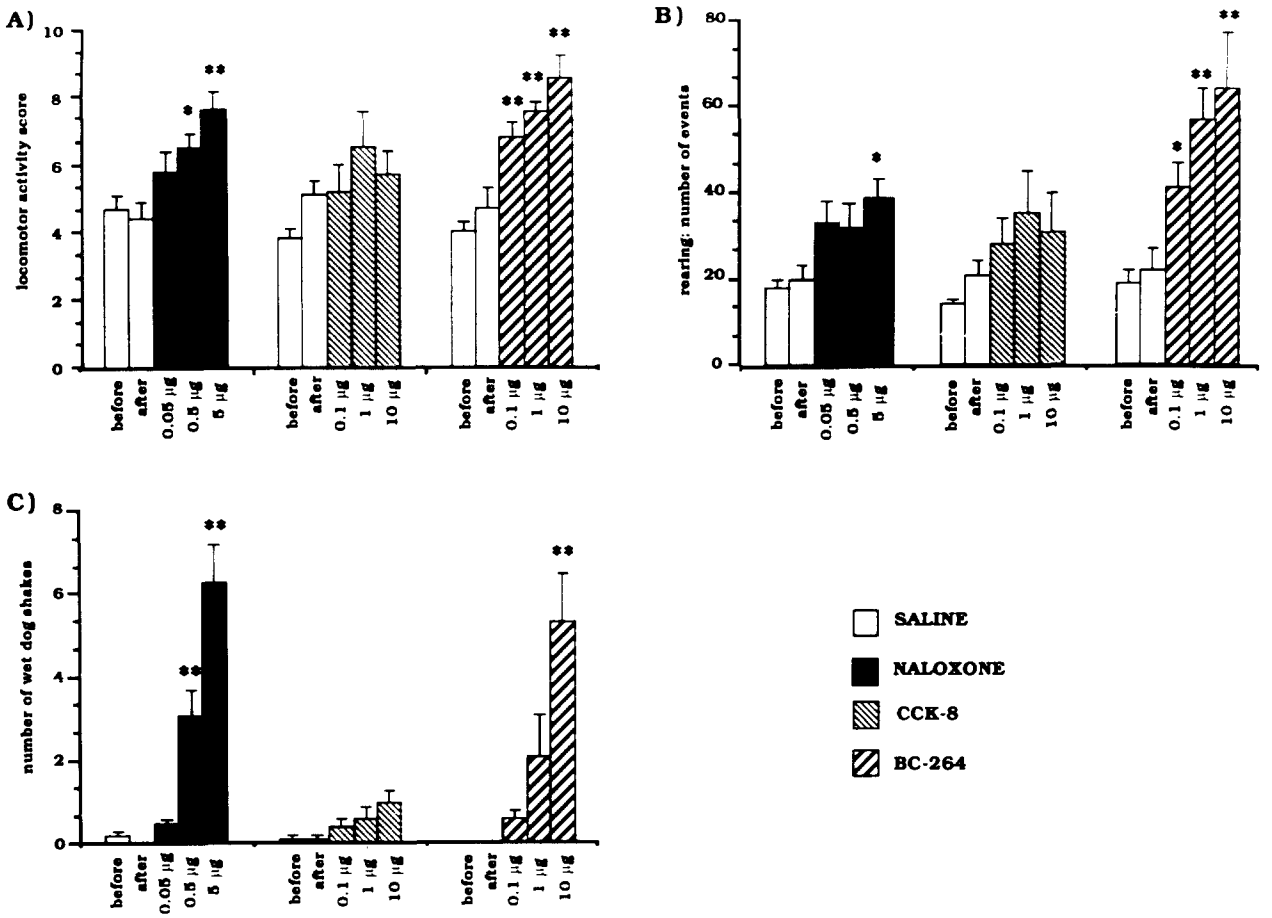

FIG. 1. Behavioral signs of withdrawal (A: locomotor activity; B: rearing; C: wet dog shakes) after ICV administration of naloxone, CCK-8, and BC 264 in morphine-dependent rats. Abscissa represents the different treatments; saline groups represent the controls performed before and after injection of the different compounds. Ordinate expresses the values of the mean ± SEM for each group. Number of animals per group = 8–11. $*p < 0.05$. $**p < 0.01$. The asterisks indicate that the group was different from the two saline controls (Newman-Keuls test).

after peripheral naloxone, such as secretory signs (salivation, rhinorrea, and salivation) and diarrhea, were not observed after central injection of the opiate antagonist, in agreement with previous studies suggesting that peripheral mechanisms are important in the triggering of these signs (24). Statistical analysis indicated that the protocol used in the present study to precipitate the withdrawal syndrome several times in the same animal shows a reliable response in the expression of the different behavioral signs of withdrawal, since no effect of time or interaction between dose and time was observed in any group of animals. Furthermore, there was no significant difference between the predrug saline injection and the postdrug saline injection, suggesting no conditioning effects due to repeated injections. The use of a similar within-subjects design for the expression of behavior signs of opiate withdrawal has been previously validated (24).

Central administration of BC 264 induced a strong and dose-dependent increase in locomotor activity and rearing behavior in morphine-dependent rats. This response was not due to an intrinsic effect of BC 264, since it was observed with doses that did not modify locomotion in saline chronically treated animals. CCK-8, at the dose of 1 μg, induced on these behavioral responses a slight and nonsignificant effect which was not observed at a higher dose. The higher effectiveness of BC 264 in inducing hyperlocomotion in morphine chronically treated animals indicates a selective participation of CCK-B receptors. Besides, the lack of effect following administration of the highest dose of CCK-8 could be due to an opposite effect of CCK-A and CCK-B receptor stimulation. BC 264 also induced a significant incidence of wet dog shakes when administered at the highest dose; however, this behavior was observed in both morphine-dependent and nondependent animals.

The behavioral responses induced by BC 264 and CCK-8 in control rats chronically treated with saline were also different. Thus, BC 264 administered at the highest dose increased locomotor activity and rearing behavior and induced the presence of wet dog shakes. Interestingly, it has been reported that peripheral administration of BC 264 in monkeys induced anxiety behavior with hypervigilance and stereotypy (27). Anxiety behavior after peripheral BC 264 has also recently been reported in mice (10). The behavioral changes observed in the present study after central administration of BC 264 are in agreement with these observations. These behavioral changes were not observed after CCK-8 injection. On the contrary, CCK-8 administered at the lowest dose showed a slight trend

TABLE 2

SUMMARY TABLE OF ONE-WAY ANALYSIS OF VARIANCE (ANOVA) WITHIN SUBJECTS PERFORMED IN MORPHINE-DEPENDENT ANIMALS AFTER ICV ADMINISTRATION OF NALOXONE (0.05, 0.5, AND 5 μg), CCK-8 (0.1, 1, and 10 μg), AND BC 264 (0.1, 1, AND 10 μg)

	ANOVA	
	F	*p*
Locomotor activity		
Naloxone	(4, 32) = 7.42	<0.0002
CCK-8	(4, 32) = 2.79	<0.05
BC 264	(4, 44) = 21.94	<0.0001
Wet dog shakes		
Naloxone	(4, 32) = 35.93	<0.0001
CCK-8	(4, 32) = 2.81	<0.05
BC 264	(4, 44) = 15.803	<0.0001
Teeth chattering		
Naloxone	(4, 32) = 93.94	<0.0001
CCK-8	(4, 32) = 2.43	NS
BC 264	(4, 44) = 1.96	NS
Ptosis		
Naloxone	(4, 32) = 16.95	<0.0001
CCK-8	(4, 32) = 2.41	NS
BC 264	(4, 44) = 1.77	NS
Rearing		
Naloxone	(4, 32) = 4.01	<0.01
CCK-8	(4, 32) = 4.32	<0.05
BC 264	(4, 44) = 15.45	<0.0001
Mastication		
Naloxone	(4, 32) = 42.27	<0.0001
CCK-8	(4, 32) = 2.05	NS
BC 264	(4, 44) = 2.41	NS
Jumping		
Naloxone	(4, 32) = 2.84	<0.05
CCK-8	(4, 32) = 0.65	NS
BC 264	(4, 44) = 0.77	NS

Number of animals per group = 8–11.

to decrease locomotor activity and rearing. In agreement with this result, systemic injection of CCK-8 has been reported to reduce exploratory behavior in mice (9), rats (8), and hamsters (33).

In order to further characterize the significance of the behavioral changes induced by CCK agonists in morphine-dependent rats, the effects of CCK-8 and BC 264 on rats receiving an acute injection of morphine were also investigated. Acute morphine produces a biphasic effect on locomotion, with hyperactivity induced at low doses and hypoactivity at a higher dose. This mixed depressant and stimulant effect has been widely described, and according with the present study the peak effect of hyperlocomotion was observed previously 2 h after IP administration of 5 mg/kg of morphine, and an almost total immobility was reported 2 h after morphine 20 mg/kg (1). Central administration of CCK-8 significantly blocked the enhancement in rearing behavior induced by morphine, and BC 264 antagonized increases in both rearing and locomotor activity. However, both CCK agonists did not change morphine-induced depressant effects. An opposite interaction between CCK and opioid systems on locomotion has been already reported. Thus, peripheral injection of CCK-8 antagonized both hyperactivity and hypoactivity induced by morphine in the hamster (33,34), and the nonselective CCK antagonist proglumide abolished the hyperactivity but enhanced the suppressant effects of morphine on motility in rats (3). Morphine-induced hypoactivity seems to be mediated through μ-opioid receptors, whereas δ receptors are more directly implicated in the hyperactivity response (5). Consequently, the antiopioid effect of CCK agonists on morphine-induced hyperactivity, but not on morphine-depressant effects, may suggest a preferential participation of δ-opioid receptors in the CCK–opioid interaction observed in this experiment. It would be useful to further investigate this point with selective opioid antagonists. The higher effectiveness of BC 264 in reducing morphine-induced hyperlocomotion indi-

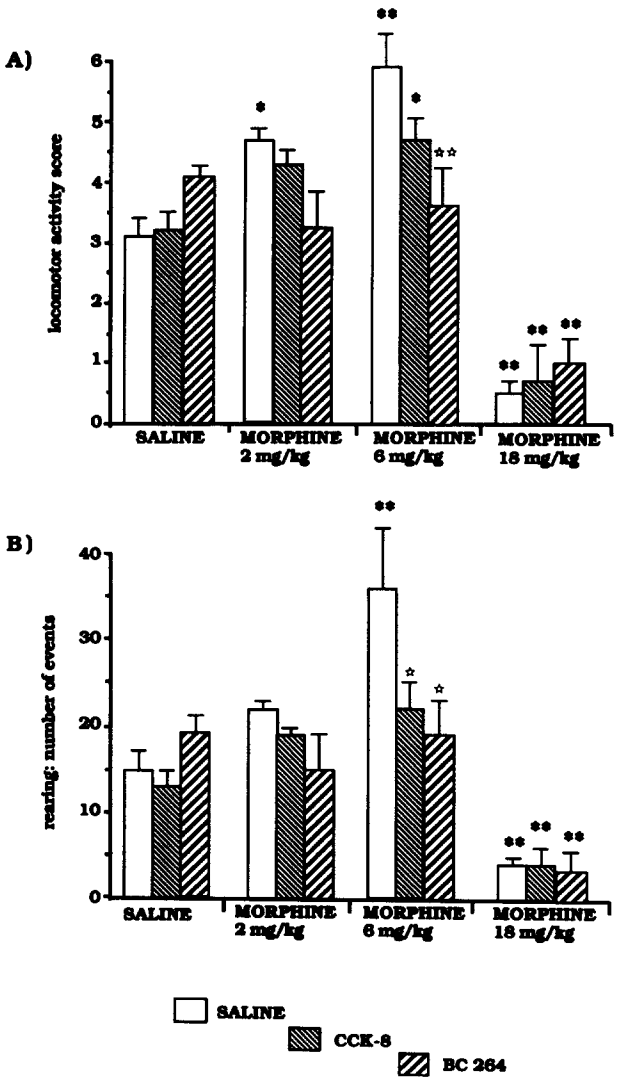

FIG. 2. Locomotor activity (A) and rearing behavior (B) induced by acute IP administration of saline or morphine and ICV injection of saline, CCK-8, or BC 264. Abscissa represents the different treatments. Ordinate expresses the values of the mean ± SEM for each group. Number of animals per group = 9–10. *$p < 0.05$, **$p < 0.01$ vs. saline control. ☆$p < 0.05$, ☆☆$p < 0.01$ vs. corresponding dose of morphine (Newman-Keuls test).

cates that this antiopioid effect seems to be mediated through CCK-B receptors, at least at the central level. This is in agreement with previous studies reporting a selective participation of CCK-B receptors on the modulation produced on the CCK system on exogenous and endogenous opioids induced antinociception (12,23,26).

Several hypotheses based on all of these results may explain the behavioral changes induced by CCK agonists, and particulary by BC 264, after chronic morphine treatment. Indeed, BC 264 administration in morphine-dependent rats only induced nonspecific signs of withdrawal, such as increase in locomotor activity and rearing behavior, and the presence of wet dog shakes, which also appeared in nondependent rats. The hyperactivity induced by BC 264 might be, first, the result of a selective interaction on locomotor activity. Indeed, the effects induced by morphine on locomotor activity are different in dependent and nondependent animals. A tolerance to the suppressant effects and a potentiation of the stimulant effects have been reported in morphine-dependent rats (4,22). Accordingly, in our study the administration of 30 mg/kg of morphine induced a slight trend to increase locomotor activity in dependent rats (i.e., the opposite effect than in naive animals). BC 264 also induced an opposite effect, enhancement or decrease of activity after acute or chronic morphine, respectively, and consequently the hyperactivity in dependent animals could have a significance similar to the acute antiopioid interaction. However, this hypothesis seems unlikely, since BC 264 did not increase activity after acute injection of different doses of morphine, and the only antiopioid effect induced by CCK agonists on locomotor activity after acute morphine was the decrease in morphine-induced hyperactivity. Furthermore, this acute interaction was observed with both CCK-8 and BC 264, whereas in chronic morphine rats only BC 264 was able to increase activity.

Both CCK-B receptor activation by BC 264 and blockade of opioid receptors by naloxone induced a similar hyperactivity in morphine-dependent rats. Consequently, a second hypothesis is that this hyperactivity may reflect an antiopioid effect induced by BC 264 (i.e., the expression of a mild morphine-withdrawal syndrome). Thus, the activation of CCK receptors could be insufficient to induce the presence of major withdrawal signs in morphine-dependent rats, but sufficient to induce the presence of some minor signs, such as the increase in locomotion and rearing behavior. Whatever the significance of these behavioral changes, the role played by the CCK system on the expression of physical opioid dependence seems to be minor. Otherwise, the interaction between CCK and the expression of the motivational aspects of morphine dependence has not been elucidated. Physical and motivational components of opiate withdrawal involve different neural pathways: locus coeruleus and mesolimbic systems, respectively (21). Considering the role played by CCK on the control of emotional states (7,31) and the involvement of the mesolimbic system in this effect (11), it is possible that the changes observed in locomotion could be associated with the expression of other motivational aspects of morphine dependence. Further studies will be performed to investigate this point.

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