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Modulation of opioid antinociception by CCK at the supraspinal level: evidence of regulatory mechanisms between CCK and enkephalin systems in the control of pain

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1 Much evidence in the literature supports the idea that cholecystokinin (CCK) interacts with opioids in pain mechanisms. In this work, we have investigated the supraspinal interactions between enkephalins and CCK, using the hot plate test in mice.

2 Intracerebroventricular (i.c.v.) administration of BDNL (a mixed CCK_A/CCK_B agonist) induced dose-dependent antinociceptive responses on both paw lick and jump responses. In contrast, using the same test, the i.c.v. injection of BC 264 (a selective CCK_B agonist) induced a hyperalgesic effect, which was restricted to paw licking and occurred only at a high dose of 2.5 nmol.

3 In addition, i.c.v. administration of BDNL potentiated the antinociceptive effects of the mixed inhibitor of enkephalin degrading enzymes, RB 101 and of the μ-agonist, DAMGO, while BC 264 reduced these effects.

4 Furthermore, at a dose where it interacts selectively with δ-opioid receptors, the opioid agonist BUBU reversed the hyperalgesic responses of BC 264 (2.5 nmol) but was unable to modify the effects induced by BDNL.

5 Taken together, these results suggest the existence of regulatory mechanisms between CCK and enkephalin systems in the control of pain. These regulatory loops could enhance the antinociceptive effects of morphine allowing the opiate doses used to be reduced and thus, possibly, the side-effects to be minimized.

Keywords: CCK system; opioid system; mouse; hot plate test; selective agonists; mixed enkephalin-metabolizing enzyme inhibitor, RB 101

Introduction

Cholecystokinin (CCK) was first recognized as a gastrointestinal hormone and later discovered in very high concentrations in the brain. Several forms of CCK have been detected, including the sulphated octapeptide C terminal, CCK_8, which is the most predominant form in the central nervous system (CNS) (Vanderhaeghen *et al.*, 1975). The unsulphated form of CCK_8 as well as the metabolites CCK_5 and CCK_4 are also found and may also have physiological roles. Analysis of the binding properties of these compounds has provided evidence for the existence of at least two CCK receptor types: CCK_B receptors, the predominent form found in the brain, and CCK_A receptors abundant in peripheral tissues (Moran *et al.*, 1986). CCK_A binding sites have also been found in a few discrete regions of the rat brain and CCK_B receptors have been shown to resemble closely gastrin receptors (Moran *et al.*, 1986; Hayward *et al.*, 1991). CCK_8 interacts at nanomolar affinity with both types of CCK receptors, while the other smaller fragments have selectivity for CCK_B receptors (Innis & Snyder, 1980; Gaudreau *et al.*, 1983; Durieux *et al.*, 1988).

Anatomical studies have shown that enkephalins and CCK_8 have a strikingly similar distribution within many regions of the CNS. This overlapping distribution of both the peptides and their respective receptors in the brain and spinal cord (Stengaard-Pedersen & Larsson, 1984; Crawley, 1985; Gall *et al.*, 1987; Pohl *et al.*, 1990), has focused attention on the role of CCK in nociception. Several studies have reported a naloxone reversible antinociceptive effect of CCK_8 or its analogues in several antinociceptive tests, such as the hot plate, writhing, and tail-flick tests (Zetler, 1980; Jurna & Zetler, 1981; Barbaz *et al.*, 1986; Hill *et al.*, 1987; Pittaway *et al.*, 1987; Hong & Takemori, 1989; Baber *et al.*, 1989).

However, it has also been suggested that CCK_8 has anti-opioid activity (Itoh *et al.*, 1982; Faris *et al.*, 1983). Faris *et al.* (1983) found that CCK reduced the antinociceptive effects produced by the release of endogenous opioids, and did not modify non-opiate responses induced by hind paw foot shock. In addition, numerous studies have shown that peripherally administered CCK antagonists potentiate opioid antinociceptive responses, confirming a functional antagonism of the opioid system by the CCK system (Watkins *et al.*, 1985; Hendrie *et al.*, 1989; Dourish *et al.*, 1990; Wiesenfeld-Hallin *et al.*, 1990; Lavigne *et al.*, 1992). It has been hypothesized that CCK down-regulates opioid effects through activation of CCK_B receptors. However, MK329, a selective CCK_A antagonist, also potentiated the analgesic effects of opiates (Rattray *et al.*, 1988; Hendrie *et al.*, 1989).

The purpose of the present study was to understand more fully the supraspinal interactions between the opioid and CCK systems. With this aim in mind, the effects induced by the CCK_B agonist, BC264 and by mixed CCK_A/CCK_B agonist, BDNL were studied after i.c.v. administration in the hot plate test in mice. In addition, the influence of these CCK agonists on the responses induced by selective opioid-agonists such as DAMGO (μ-agonist) and BUBU (δ-agonist) were determined. RB 101 (N-[(R,S)-2-benzyl-3[(S)(2-amino-4-methylthio)butyldithio]-1-oxopropyl]-L-phenylalanine benzyl ester, Fournié-Zaluski *et al.*, 1992), a mixed inhibitor of enkephalin-degrading enzymes, was also used. This systemically active molecule completely protects the endogenous enkephalins from degradation (Ruiz-Gayo *et al.*, 1992a), allowing their actions with the CCK system to be studied.

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Methods

Animals

Male Swiss mice (20–22 g) (Charles River, France) were used. Animals were housed in groups of 20 for at least two days before the experiments were started, and food and water were available *ad libitum*. Each animal was used only once. Antinociceptive measurements were recorded between 14 h and 20 h.

Administration procedures

Intracerebroventricular (i.c.v.) injections: BC264, BDNL or saline were injected free hand into the left lateral ventricle of a mouse with a modified Hamilton μl syringe in a volume of 10 μl per mouse according to the method of Haley & McCormick (1957).

Systemic injections: DAMGO, BUBU, RB101 or control vehicles were slowly administered intravenously (i.v.) in a volume of 0.1 ml 10 g^{-1}. Naloxone and naltrindole were injected subcutaneously (s.c.) in a volume of 0.1 ml 10 g^{-1}.

The jump responses induced by the thermal nociceptive stimulus were measured at various times, due to the differences in the maximal time-effect of the compounds studied. Thus the dose-response curves of BDNL and BC 264 were determined 15 min after i.c.v. administration. In studies of possible interactions between opioids and CCK-related compounds, BDNL and BC 264 were injected i.c.v. 5 min before i.v. administration of BUBU or 10 min before i.v. administration of DAMGO. Controls were treated with vehicle at the corresponding times. The hot plate test was performed as before, i.e. 15 min after i.c.v. injection of the CCK peptides. In the experiments with RB 101, the CCK agonists were injected i.c.v. 20 min before the hot plate test (i.e. 10 min before i.v. injection of the mixed inhibitor). A longer period (i.e. 10 min instead of 5 min) between the administration of RB 101 and the CCK agonists was required to eliminate a slight 'hypolocomotion' induced by the vehicle used to solubilize the mixed inhibitor.

Hot-plate test

The test was based on that described by Eddy & Leimbach (1953). A glass cylinder (16 cm high, 16 cm diameter) was used to keep the mouse on the heated surface of the plate which was maintained at a temperature of 55 ± 0.5°C using a thermoregulated water circulating pump. The latencies of both forepaw licking (cut-off time: 30 s) and jumping (cut-off time: 240 s) were measured for each animal with a stopwatch. Dose-response curves were established by expressing the data as a percentage of control using the following equation: % of control = (test latency − control latency)/(cut-off time − control latency) × 100.

Chemicals

BDNL (Boc-Tyr(SO_3H)-Nle-Gly-Trp-Nle-Asp-Phe-NH_2, Ruiz-Gayo *et al.*, 1985), BC264 (Boc-Tyr(SO_3H)-gNle-*m*Gly-Trp-(NMe)-Nle-Asp-Phe-NH_2, Charpentier *et al.*, 1988), BUBU (Tyr-D-Ser(O-Tert-Butyl)-Gly-Phe-Leu-Thr(O-Tert-Butyl, Gacel *et al.*, 1988), RB101 (H_2N-CH(CH_2-CH_2-S-CH_3)-CH_2-S-S-CH_2-CH($CH_2\Phi$)-CONH-CH($CH_2\Phi$)-$COOCH_2\Phi$, Fournié-Zaluski *et al.*, 1992) and naltrindole (NTI, 17-cyclopropylmethyl-6,7-dehydro-4,5α-epoxy-3,14-dihydroxy-6, 7,2′,3′-indolomorphinan, Portoghese *et al.*, 1988) were synthesized in the laboratory following previously reported methods. DAMGO (Tyr-D-Ala-Gly-(NMe)Phe-Gly-ol) was purchased from Bachem AG (Switzerland) and naloxone from Mallet SA (France). BDNL, BC264, DAMGO, naloxone and naltrindole were dissolved in saline and BUBU (L-lysine salt) in distilled water. RB101 was dissolved in the following vehicle: ethanol (10%): cremophor EL (10%): distilled water (80%). Cremophor EL was purchased from Sigma (France).

Statistical analysis

Data were analysed with a one-way analysis of variance (ANOVA). *Posthoc* comparisons were made with Dunnett's *t* test for dose-response curves or Newman-Keuls test for multiple comparisons. The level of significance was set at $P<0.05$.

Results

Effects induced by i.c.v. administration of the mixed CCK_A/CCK_B agonist (BDNL) and of the selective CCK_B agonist (BC264) in the hot plate test in mice

As shown in Table 1, BDNL produced significant antinociceptive effects in both paw lick and jump responses ($F(3,28) = 10.88$, $P<0.01$ for the lick; $F(3,28) = 17.07$, $P<0.01$ for the jump) 15 min after i.c.v. administration.

In contrast, i.c.v. injection of the selective CCK_B agonist, BC264, only at a dose of 2.5 nmol, induced a significant decrease in the paw lick latency, 15 min after administration ($F(4,45) = 3.40$, $P<0.05$). No modification of the jump response ($F(4,45) = 0.85$, $P>0.05$) was observed after i.c.v. administration of BC264 (Table 1).

Effects of BC264 (i.c.v.) on DAMGO-induced analgesia (i.v.)

DAMGO administered i.v. (2 mg kg^{-1}) 5 min before the hot plate test induced antinociceptive effects on both paw lick (Figure 1a) and jump (Figure 1b) latencies. The increases in the threshold were diminished, but not suppressed, by prior injection of BC264 (15 min before testing) at a dose which

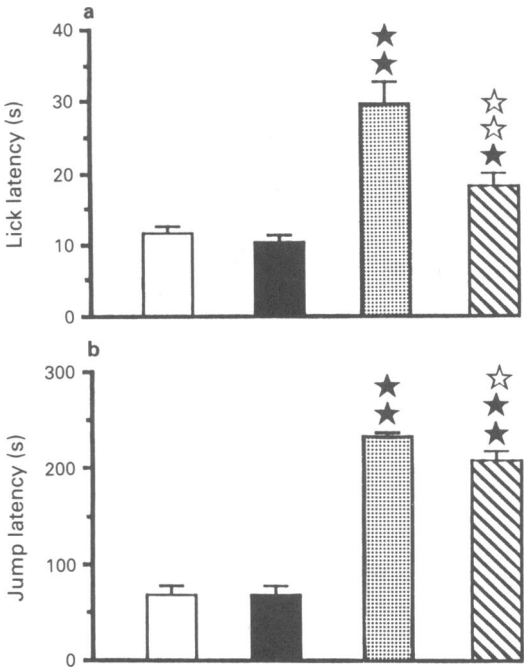

Figure 1 Effects of DAMGO (2 mg kg^{-1}, i.v., 5 min before the test), BC264 (1 nmol, i.c.v., 15 min before testing), and a combination of the two drugs in the hot plate test in mice. (a) Paw lick latencies. (b) Jump latencies. Control, open column; BC264, solid column; DAMGO, stippled column; and DAMGO + BC264, hatched column. The results are expressed as means ± s.e.mean. ($n = 8–10$ mice for each group). ★$P<0.05$; ★★$P<0.01$ as compared to control group, and ☆$P<0.05$; ☆☆$P<0.01$ as compared to DAMGO-treated group (Newman Keuls test).

Table 1 Hyperalgesic or antinociceptive effects observed 15 min after i.c.v. administration of BC264 or BDNL in the hot plate test in mice

	Paw licking		*Jumping*	
	Latency (s)	*% of control*	*Latency* (s)	*% of control*
BC264				
Control	9 ± 0.9		69.7 ± 8.9	
0.1 nmol	8.4 ± 0.8	− 2.8 ± 3.9	68.5 ± 6.2	− 0.7 ± 3.7
1 nmol	7.4 ± 0.9	− 7.6 ± 4.1	81.4 ± 12.6	6.9 ± 7.4
2.5 nmol	5.9 ± 0.7*	− 14.8 ± 3.3	72.1 ± 4.6	1.4 ± 2.7
5 nmol	7.9 ± 0.8	− 5.5 ± 4.0	60.9 ± 7.1	− 5.1 ± 4.2
BDNL				
Control	8.1 ± 1.0		83.1 ± 5.4	
0.05 nmol	8.1 ± 0.9		92.3 ± 14.2	5.9 ± 9.0
0.1 nmol	11.0 ± 0.9	13.2 ± 4.3	84.7 ± 8.2	1.0 ± 5.2
0.2 nmol	20.0 ± 3.4**	54.3 ± 15.5	150.7 ± 9.1**	43.1 ± 5.8
0.5 nmol	26.8 ± 4.1**	85.4 ± 18.8	153.7 ± 14.0**	45.0 ± 8.9

Values are mean ± s.e.mean.
*$P<0.05$; **$P<0.01$ as compared to control group (Dunnett's test).

had no effect on either paw lick or jump latency (1 nmol) ($F(3,30) = 22.45$, $P<0.0001$ and ($F(3,30) = 93.79$, $P<0.0001$ for paw lick and jump latencies, respectively).

Effects of BC264 (i.c.v.) on RB101-induced analgesia (i.v.)

As shown in Figure 2b, the mixed inhibitor RB101 (20 mg kg^{-1}) produced a significant increase in jump latency, 10 min after i.v. injection. This antinociceptive response observed was diminished, but not fully antagonized, by prior administration of BC264 (1 nmol, i.c.v.), 20 min before the hot plate test ($F(3,29) = 15.3$, $P<0.0001$). On the paw lick latency no modifications were observed after administration of RB101, or the CCK_B agonist BC264 or after co-administration of the two drugs (Figure 2a) ($F(3,29) = 1.19$, $P>0.05$).

Effects of BUBU (i.v.) on BC264-induced hyperalgesia (i.c.v.)

As shown in Figure 3a, i.c.v. administration of the highly selective CCK_B agonist, BC264, administered at 2.5 nmol, 15 min before the hot plate test, decreased the threshold of the paw lick latency, as compared to controls. This hyperalgesia was antagonized by BUBU administered 10 min before the hot plate test at a dose (1 mg kg^{-1}) reported to be δ-selective (Figure 3a) ($F(3,33) = 3.90$, $P<0.01$). On the jump response, no modifications were observed either after administration of the agonists alone, or after their co-administration (Figure 3b) ($F(3,33) = 0.58$, $P>0.05$).

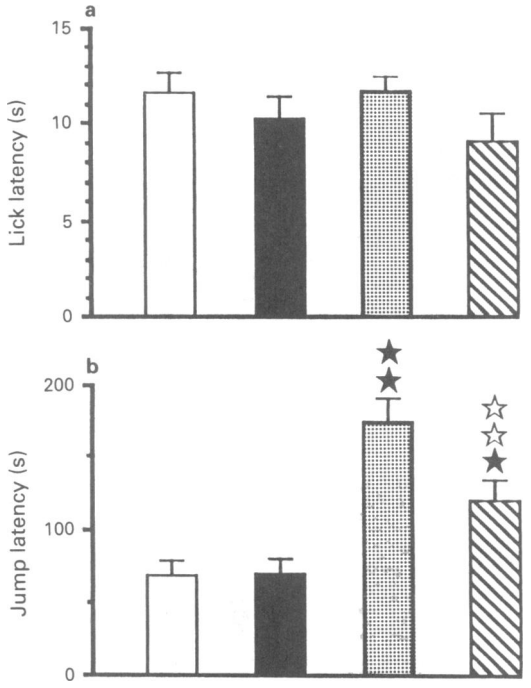

Figure 2 Effects of RB101 (20 mg kg^{-1}, i.v., 10 min before the test), BC264 (1 nmol, i.c.v., 20 min before testing), and a combination of the two drugs in the hot plate test in mice. (a) Paw lick latencies. (b) Jump latencies. Control, open column; BC264, solid column; RB101, stippled column; and RB101 + BC264, hatched column. The results are expressed as means ± s.e.mean. ($n = 8-9$ mice for each group). ★$P<0.05$; ★★$P<0.01$ as compared to control group, and ☆☆$P<0.01$ as compared to RB101-treated group (Newman Keuls test).

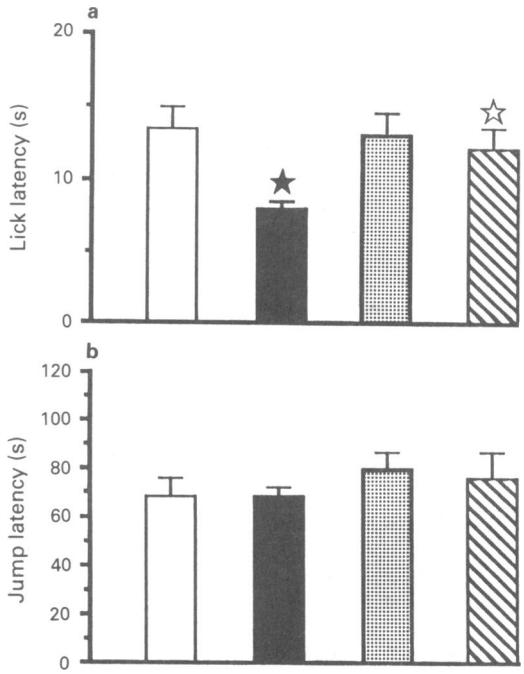

Figure 3 Effects of BUBU (1 mg kg^{-1}, i.v., 10 min before the test) on the responses observed after the i.c.v. administration of BC264 (2.5 nmol, 15 min before testing) in the hot plate test in mice. (a) Paw lick latencies. (b) Jump latencies. Control, open column; BC264, solid column; BUBU, stippled column; and BUBU + BC264, hatched column. The results are expressed as means ± s.e.mean. ($n = 9-10$ mice for each group). ★$P<0.05$ as compared to control group, and ☆$P<0.05$ as compared to BC264-treated group (Newman Keuls test).

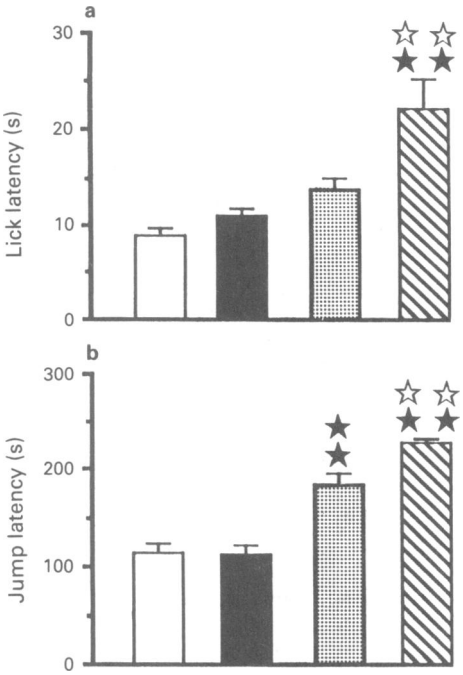

Figure 4 Effects of DAMGO (0.5 mg kg^{-1}, i.v., 5 min before the test), BDNL (0.1 nmol, i.c.v., 15 min before testing), and a combination of the two drugs in the hot plate test in mice. (a) Paw lick latencies. (b) Jump latencies. Control, open column; BDNL, solid column; DAMGO, stippled column; and DAMGO + BDNL, hatched column. The results are expressed as means ± s.e.mean. ($n = 9-11$ mice for each group). ★★$P<0.01$ as compared to control group, and ☆☆$P<0.01$ as compared to DAMGO-treated group (Newman Keuls test).

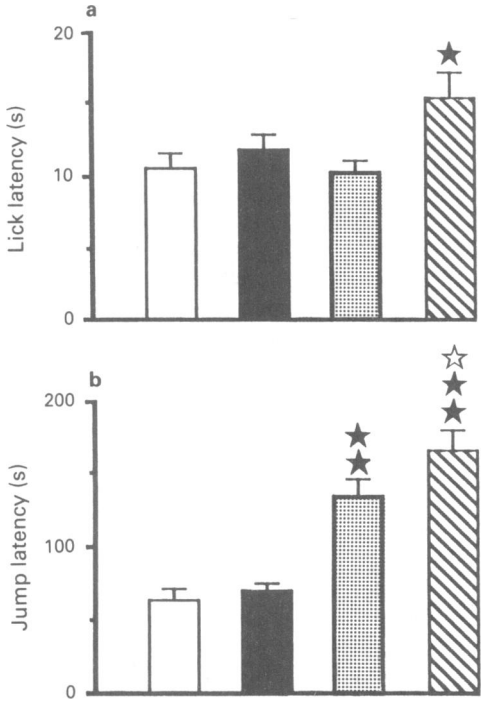

Figure 5 Effects of RB101 (10 mg kg^{-1}, i.v., 10 min before the test), BDNL (0.1 nmol, i.c.v., 20 min before testing), and a combination of the two drugs in the hot plate test in mice. (a) Paw lick latencies. (b) Jump latencies. Control, open column; BDNL, solid column; RB101, stippled column; and RB101 + BDNL, hatched column. The results are expressed as means ± s.e.mean. ($n = 9-11$ mice for each group). ★$P<0.05$; ★★$P<0.01$ as compared to control group, and ☆$P<0.05$ as compared to RB101-treated group (Newman Keuls test).

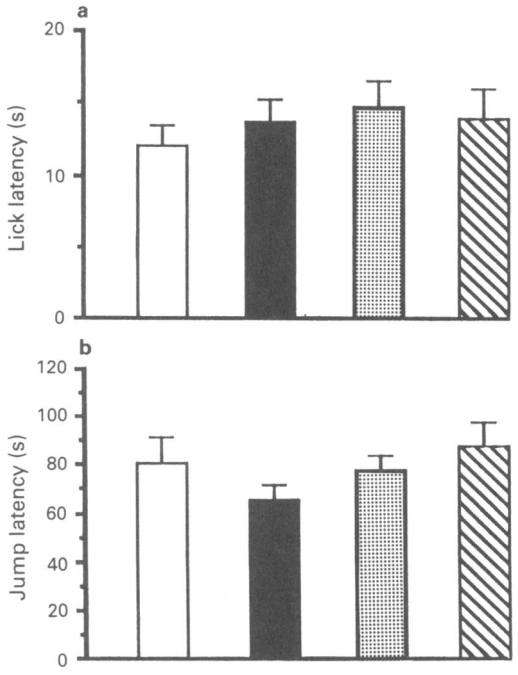

Figure 6 Effects of BUBU (1 mg kg^{-1}, i.v., 10 min before the test), BDNL (0.1 nmol, i.c.v., 15 min before testing), and a combination of the two drugs in the hot plate test in mice. (a) Paw lick latencies. (b) Jump latencies. Control, open column; BDNL, solid column; BUBU, stippled column; and BUBU + BDNL, hatched column. The results are expressed as means ± s.e.mean. ($n = 8-10$ mice for each group).

Effects of BDNL (i.c.v.) on DAMGO-induced analgesia (i.v.)

DAMGO, administered 5 min before the hot plate test (0.5 mg kg^{-1}, i.v.), increased the latency of the jump response (Figure 4b). This antinociceptive effect was enhanced by i.c.v. administration of BDNL (0.1 nmol) 15 min before the hot plate test (Figure 4b) ($F(3,37) = 42.92$, $P<0.0001$). Moreover, although neither BDNL nor DAMGO had any effect on the paw-lick latency, co-administration of the two agonists led to a significant increase in the threshold latency as compared to the control group (Figure 4a) ($F(3,37) = 10.00$, $P<0.0001$).

The antinociceptive effects induced by co-administration of DAMGO and BDNL were significantly antagonized by naloxone (0.1 mg kg^{-1}, s.c.) ($F(5,42) = 7.3$ and ($F(5,42) = 26.36$ for paw lick and jump latencies, respectively) but not by the selective δ-antagonist, naltrindole (0.1 mg kg^{-1}, s.c.) ($F(5,42) = 7.9$ paw lick, ($F(5,42) = 22.75$ jump) (data not shown).

Effects of BDNL (i.c.v.) on RB101-induced analgesia (i.v.)

As shown in Figure 5b, RB101 (10 mg kg^{-1}, i.v.) administered 10 min before the hot plate test, increased the jump latency threshold. This increase was enhanced by prior i.c.v. administration of BDNL (0.1 nmol), 20 min before the test ($F(3,37) = 25.80$, $P<0.0001$). On the paw lick response, i.v. administration of RB101 (10 mg kg^{-1}, 10 min before the test) had no effect. However, co-administration of RB101 and BDNL (0.1 nmol, i.c.v., 20 min before testing) led to an enhancement of the latency as compared to the control group (Figure 5a) ($F(3,37) = 3.58$, $P<0.05$).

These effects were antagonized by naloxone (0.1 mg kg^{-1}, s.c.) ($F(5.42) = 2.469$, paw lick; $F(5,42) = 20.835$, jump) but not by NTI ($F(5,42) = 5.269$ and $F(5,42) = 28.284$ for paw lick and jump responses, respectively) (data not shown).

Effects of co-administration of BDNL (i.c.v.) and BUBU (i.v.)

Co-administration of BDNL (0.1 nmol, i.c.v. 15 min before the hot plate test) and BUBU (1 mg kg^{-1}, i.v. 10 min before the test) did not modify either paw lick or jump latencies (Figure 6) ($F(3,32) = 0.41$, $P > 0.05$ and $F(3,32) = 1.68$, $P > 0.05$, for paw lick and jump responses, respectively).

Discussion

Intracerebroventricular administration of BDNL, a peptidase-resistant CCK_8 analogue which has mixed CCK_A/CCK_B agonist activity, significantly increased both paw lick and jump latencies in the hot plate test in mice, confirming previous reports suggesting that CCK has antinociceptive properties (review in Pittaway & Hill, 1987). In contrast, with the same test, i.c.v. injection of the selective CCK_B agonist, BC264 at a single dose of 2.5 nmol, induced a slight hyperalgesic effect, restricted to paw lick. Derrien *et al.* (1993) have demonstrated that the antinociceptive effects of BDNL involve activation of CCK_A binding sites, and result from an indirect involvement of μ-opioid receptors. The present results could indicate that the CCK_A agonist activity of BDNL is much greater than its CCK_B activity.

In order to investigate the role of the endogenous opioid peptides and which type of opioid receptor (μ or δ) is involved in both paw lick and jump latencies changes induced by the CCK agonist, BDNL and BC264 were administered to mice at non-active doses to avoid possible additive effects. This was followed by i.v. injection of either the μ-agonist, DAMGO, or the mixed inhibitor, RB101, at doses giving weak antinociceptive effects. Under these conditions, BDNL potentiated the antinociceptive effects of RB101 and DAMGO, while BC264 reduced them. Furthermore, the use of high doses of DAMGO (2 mg kg^{-1}, i.v.) or RB101 (20 mg kg^{-1}, i.v.) which elicit strong antinociceptive responses showed that BC264 does not completely antagonize the opioid antinociceptive effects. Moreover, the potentiation of the effects of DAMGO or RB101 by BDNL were fully antagonized by prior injection of naloxone, but not by the δ-selective antagonist NTI. Taken together, these results suggest the occurrence of regulatory mechanisms between CCK and opioid systems in the control of thermal nociception, and provide evidence that CCK can differentially modulate nociceptive perception in mice through at least two types of CCK receptors.

Schematically, activation of CCK_B receptors by BC264 could reduce the levels of endogenous enkephalins released into the extracellular space, while the reverse situation could occur by stimulation of CCK_A binding sites by BDNL, as previously suggested (Cesselin *et al.*, 1984; Hagino *et al.*, 1991). This is in agreement with a previous study, which showed that the antinociceptive effects of CCK_8 were both naloxone-reversible and potentiated by simultaneous administration of the peptidase inhibitors bestatin, thiorphan and captopril (Hill *et al.*, 1987) suggesting that the octapeptide produces its antinociceptive effects in an indirect manner by increasing the release of endogenous opioids.

Furthermore, several electrophysiological studies have demonstrated the existence of regulatory mechanisms between opioid and CCK systems in the control of pain. Indeed, CCK_8 has been found to prevent the inhibition of C-fibre evoked activity of dorsal horn nociceptive neurones resulting from injection of DAMGO (Magnuson *et al.*, 1990; Kellstein *et al.*, 1991) but had no effect on δ-mediated inhibition induced by δ-opioid agonists (Magnuson *et al.*, 1990). Thus, stimulation of CCK_B receptors by BC264, leading to a decrease in the antinociceptive responses observed after μ-opioid receptor activation by DAMGO or RB101, could be related to a diminution of the inhibition of C-fibre firing. The reverse situation could occur after administration of the mixed CCK_A/CCK_B agonist, BDNL.

Another possibility is that CCK peptides produce an allosteric change in the opioid receptors leading to a post-receptor change which counters the opioid effector system. Indeed, Wang & Han (1990) have shown in *in vitro* studies that CCK_8 modifies the binding of μ-opioid agonists to their receptors. This result is in agreement with behavioural studies reported by the same authors, showing that the analgesia produced by injection of μ-agonists is markedly antagonized by injection of CCK_8 (Wang *et al.*, 1990).

If stimulation of CCK sites is capable of modulating the opioid system, this system could in turn regulate the release of CCK peptides. In particular, μ-opioid receptor stimulation could modulate the release of endogenous CCK. In agreement with this, the stimulation of μ-opioid receptors has an inhibitory influence on the K^+-evoked release of CCK-like material (CCKLM) at spinal and supraspinal levels (Rattray & De Belleroche, 1987; Rodriguez & Sacristan, 1989; Benoliel *et al.*, 1991; 1992). Thus, the decrease in the opioid antinociceptive responses observed after administration of BC264, could be explained by a decrease in endogenous CCK release.

On the other hand, recent studies *in vitro* have shown that δ-opioid agonists enhance the K^+-evoked release of CCKLM from slices of rat substantia nigra and spinal cord (Benoliel *et al.*, 1991; 1992). Also, the activation of δ-opioid receptors either by BUBU, a highly selective δ-agonist (Gacel *et al.*, 1990), or by endogenous enkephalins, reduces the *in vivo* binding of the CCK_B-selective agonist [^{3}H]-pBC264, following increases in extracellular levels of endogenous CCK (Ruiz-Gayo *et al.*, 1992b). The results obtained in this study have shown that central injections of BDNL and BC264 produce opposing effects on the opioid system through stimulation of CCK_A and CCK_B receptors, respectively. Thus, it could be speculated that the antagonism of the hyperalgesia observed after administration of BC264 by the δ-opioid agonist BUBU, could be due to an increase in endogenous CCK release which could counterbalance the hyperalgesic effects of BC264 through activation of the different receptor types.

In summary, the results obtained provide evidence that the CCK system can modulate pain perception through activation of at least two receptors in thermal nociception leading to opposing effects. This observation could explain the bell-shaped dose-response curves observed in this and other studies using CCK compounds (Dourish *et al.*, 1988; Hendrie *et al.*, 1989). Moreover, the present study supports the existence of regulatory mechanisms between CCK and enkephalin systems. These CCK/opioid interactions may be

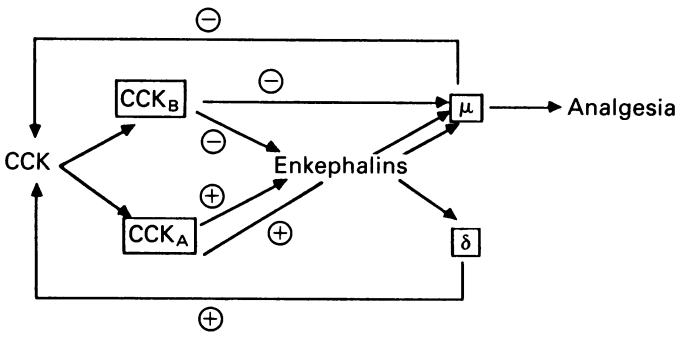

Figure 7 Schematic representation of the proposed regulation loops between the CCK and the opioid systems. The CCK agonists, endogenous and/or exogenous, stimulate the CCK_B and/or the CCK_A receptors which can modulate the opioidergic systems either directly (via binding of opioid agonists or via the C-fibre evoked activity) or indirectly (via the release of endogenous enkephalins). In addition, activation of μ-opioid receptors, which leads to antinociceptive responses, could negatively modulate the release of endogenous CCK, while δ-opioid receptors may enhance it.

mediated supraspinally. Indeed, the responses observed in the hot plate test are believed to be supraspinally organized responses (Schmauss & Yaksh, 1984). However, the results obtained could still be interpreted as spinally mediated effects, since drugs given i.c.v. very rapidly spread into the spinal subarachnoid space.

Schematically, activation of CCK_B receptors could negatively modulate the opioid system either directly (via binding of opioid agonists or C-fibre evoked activity), or indirectly (via release of endogenous enkephalins), while stimulation of CCK_A receptors would enhance opioid release. In addition, it appears that the opioid system can regulate the release of CCK peptides: activation of δ-opioid receptors could increase the synaptic concentration of endogenous CCK, while μ-opioid receptors could decrease it (Figure 7). The existence of these regulation loops between both systems, could mean that the doses of opiates needed to produce analgesia might be reduced by co-administration of CCK agonists. This may reduce the side-effects observed with opiates, although studies are needed to confirm this proposal.

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