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Cholecystokinin fails to block the spinal inhibitory effects of nociceptin in sham operated and neuropathic rats

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Abstract

Cholecystokinin (CCK) has a number of roles in the central nervous system and can reduce the analgesic effect of activation of mu (μ), delta (δ) and kappa (κ) opioid receptors. CCK has been proposed to be a major reason for reduced effects of morphine after nerve injury. This study examines if CCK modulates the effect of the Opioid Receptor Like-1 (ORL1) agonist, nociceptin on dorsal horn neurone activity in vivo in the spinal nerve ligation model of neuropathic pain compared with sham-operated and naive rats. In naive and neuropathic rats nociceptin alone inhibited the C-fibre evoked response, post-discharge, wind-up and input, while in sham operated rats nociceptin did not cause any inhibition but by contrast caused a facilitation of post-discharge and wind-up. CCK alone had no significant effect, although did cause slight facilitation in the three groups. In the presence of CCK the inhibitory effect of nocieceptin was blocked in naive animals, but in contrast the inhibitory effect of nociceptin was enhanced by CCK in sham and neuropathic rats. These results emphasize the differences between ORL1 and other opioid receptors. This loss of the inhibitory effect of CCK on nociceptin after nerve injury may be of clinical interest in the treatment of neuropathic pain.

Keywords: Cholecystokinin; Nociceptin/orphanin FQ; Spinal cord; Neuropathic; Electrophysiology; (Rat)

1. Introduction

The predominant form of cholecystokinin (CCK) found in the mammalian central nervous system is the carboxy-terminal octapeptide (CCK-8). CCK-8 is the ligand for the CCK receptor, there are two types of receptor, CCK_A or CCK_1 (peripheral type) and CCK_B or CCK_2 mainly present in the central nervous system (Moran et al., 1986), especially in the rat (Hill and Woodruff, 1990).

The wide distribution of CCK in the central nervous system (Williams et al., 1987; Schiffmann and Vanderhaeghen, 1991), in particular, in the superficial laminae of the dorsal horn of the spinal cord, suggests an important role of this peptide in the modulation of nociceptive transmission. In the first studies defining the physiological role for CCK as a functional antagonist of opioid-induced analgesia (i.e. 'anti-opioid') it was demonstrated that CCK significantly reduces morphine analgesia in the rat tail flick test (Faris et al., 1983). In addition, CCK receptor antagonists induce enhancement of opioid analgesia (Watkins et al., 1985; Stanfa et al., 1994).

In most experiments CCK does not alter baseline pain thresholds, so it is clear that the blockade of morphine analgesia is not due to a direct hyperalgesic effect of CCK (Xu and Wiesenfeld-Hallin, 1997). Although the mechanism is not fully understood by which CCK antagonises opioid analgesia, there is good evidence that CCK counteracts intracellular events subsequent to opioid activation through calcium levels (Wang et al., 1992).

The cloning of the three original opioid receptors μ, δ and κ led to the discovery of the orphan or the ORL1 receptor (Mollereau et al., 1994; Wang et al., 1994). Whilst the ORL1 receptor seems to couple to very similar effect or mechanisms as the classical opioid receptors, functionally it has effects that differ from and even oppose those of opioids (see Calo' et al., 2000, review). The heptadeca-peptide, orphanin FQ/nociceptin (hereafter nociceptin) is an endogenous ligand for the ORL1 receptor (Meunier et al., 1995; Reinscheid et al., 1995; Wang et al., 1994), and has a number of actions that differ from those mediated by the μ-opioid receptor. Thus this peptide can cause peripheral excitations (Inoue et al., 1999; Carpenter et al., 2000) and supraspinal anti-opioid effects

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(Meunier et al., 1995; Reinscheid et al., 1995) yet at spinal levels causes a clear analgesia (Stanfa et al., 1996; Candeletti et al., 1998; Carpenter et al., 2000).

The interaction between CCK and the ORL1 receptor has never been tested at spinal levels where nociceptin is found in neurones that are distinct but adjacent to those containing the opioid ligands for the other receptors. CCK may play a role in the physiological determination of opioid actions since in a rat model of neuropathic pain, there is an increase in spinal CCK and a reduction in the potency of spinal morphine (Xu et al., 1993). This may partly explain cases of reduced opioid sensitivity of neuropathic pain in man. We have therefore tested the CCK-ORL1 interaction after nerve injury.

2. Methods

2.1. Spinal nerve ligation

Experiments were carried out on 31 male Sprague–Dawley rats (University College animal house) and all the procedures were approved by the Home Office and follow the guidelines of the International Association for the Study of Pain (Zimmerman, 1983). Rats were divided into three experimental groups: spinal nerve ligation, sham operated and naive rats. One neurone was studied in each animal in the various experimental groups.

The spinal nerve ligation and sham surgery used rats weighing 130–150 g and all animals weighed 220–250 g at the time of the electrophysiology.

The spinal nerve ligation model of neuropathic pain first described by Kim and Chung (1992) was used (Suzuki et al., 1999). Briefly, rats were first anaesthetised with a mixture of halothane (3.5% for induction, 1.5% for maintenance) and a 1:3 flow ratio of N_2O/O_2. The left side of the spinal nerves L5 and L6 were exposed and tightly ligated using 6–0 silk thread and the L4 sciatic nerve branch was left uninjured.

Sham operations were performed to produce a control group, whereby the surgical procedure was identical to that of the experimental group, but spinal nerve ligation was omitted. Hemostasis was confirmed, the wound sutured and the animal recovered from anaesthesia.

2.2. Behavioural studies

After a recovery period, successful reproduction of the neuropathic model was confirmed by assessing the behavioural sensitivity to mechanical and cooling stimuli performed on days 2, 4, 7, 9, 12 and 14 postoperative.

Mechanical stimuli: foot withdrawals to trials of ascending von Frey filaments (bending forces 1, 5 and 9 g: 9.9, 49.5 and 89.1 mN, respectively), considered non-noxious under normal circumstances, were quantified. In a trial a single filament was applied 10 times to the plantar surface of the foot, through the metal mesh floor, for 2–3 s each time. Cooling stimuli: was tested by the application of a drop of acetone onto the plantar region of the foot. In this case each trial consisted of five applications of acetone, each separated by a period of 5 min. A withdrawal response was observed as a shaking, flicking or licking of the paw following acetone application. In both behavioural tests rats show stable allodynia within 2 days of surgery and this persists for 5–10 weeks (Garry and Tanelian, 1997).

2.3. Spinal cord electrophysiology

Subsequent to behavioural testing (from post-operative day 14), operated rats were used for electrophysiology studies as were normal rats without any surgery (weighing 220–250 g). Animals were anaesthetised with 3.5% halothane in 66% N_2O:33% O_2, anaesthesia was maintained throughout the experiment via cannula inserted into the trachea. The animals were secured in a stereotaxic frame, the vertebrae rostral and caudal to vertebra L1–L3 were clamped and a laminectomy was performed to expose the spinal cord and the level of halothane was finally reduced to 1.5%. Body temperature was maintained at 37 °C with the heating blanket coupled to the rectal probe. A parylene coated tungsten electrode was then descended into the cord and recordings made from neurones in segments L4–L5, which receive afferent input from the toe region. The depth of the recording was detected from the microdrive readings.

Extracellular recordings were made of single dorsal horn neurones that either receive input from the toe region ipsilateral to the spinal nerve ligation or sham procedure, or from either side of spinal cord in non-operated rats.

The selected neurone must respond to both noxious and non-noxious stimuli. Two fine needles, attached to a stimulus isolator module, were applied to the receptive field, to allow transcutaneous electrical stimulation. The C-fibre threshold was determined by giving single, electrical pulses of amplitude 0.1–3.3 mA incrementally until a C-fibre latency response was evoked. Tests consisted of a trial of 16 electrical stimuli, frequency 0.5 Hz, 2 ms pulse duration at three times the threshold required to evoke a C-fibre response. The action potentials evoked by each stimulus were superimposed and constructed into post-stimulus histograms by the Spike2 software. These were then separated on the basis of latency, into total Aβ-fibres (0–20 ms), Aδ-fibres (20–90 ms) and C-fibres (90–300 ms) action potentials. Action potentials arriving 300–800 ms were classed as 'post-discharge' and wind-up was the different between the total number of action potentials (90–800 ms) produced the 16 stimuli and the input × 16 (Suzuki et al., 1999).

The interactions between CCK and nociceptin were studied in normal ($n=6$), sham-operated ($n=6$) and spinal nerve ligated rats ($n=6$). In addition we also studied a group of normal ($n=6$), sham ($n=3$) and spinal nerve ligation rats ($n=4$) treated only with nociceptin.

CCK 1 μg in 50 μl saline was given intrathecally 20 min before the application of 250 μg in 50 μl saline of nociceptin and the neuronal responses were followed until maximum

inhibition was achieved. Responses were followed for 1 h, while the additional group was given only 250 μg/50 μl of nociceptin and activity followed for 1 h. Intrathecal injection of nociceptin 250 μg/50 μl is the top dose which induces maximum effect as seen in preliminary studies and by (Carpenter et al., 2000). Previous studies have shown that intrathecal CCK 1 μg produces small facilitatory effect which returned to control levels within 40 min (Magnuson et al., 1990).

The results are presented as mean ± standard error of mean (S.E.M.). Drug effects are expressed as mean maximal percentage of the pre-drug control value and presented as a percentage of inhibition. Statistical analysis of dose effects were performed with Mann–Whitney unpaired test where $P \leq 0.01$ and $P \leq 0.05$ were taken as significant using InStat software.

2.4. Materials

Drug used in this study are cholecystokinin sulphated octapeptide (CCK-8) and nociceptin, both products of Tocris, UK.

3. Results

Overall 31 dorsal horn neurones were studied and their mean depth was 750 ± 50 μm from the surface of the spinal cord and this did not differ between groups.

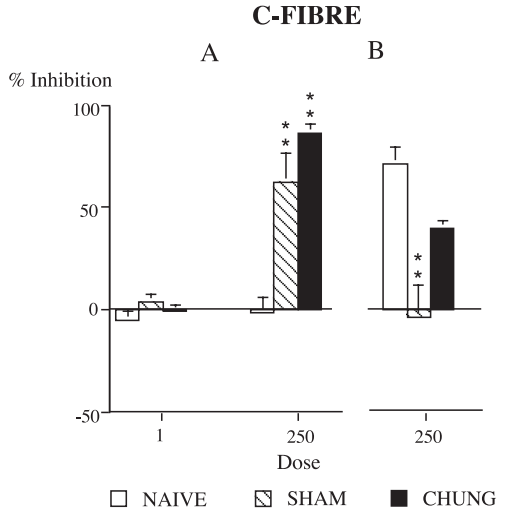

Fig. 1. Effect of intrathecal (i.t.) administration of (A) 1 μg/50 μl CCK (studied for 20 min) followed by 250 μg/50 μl nociceptin (1 h) on the C-fibres evoked responses in naive, sham operated and neuropathic rats. CCK alone did not cause any significant changes in all the three groups. CCK in presence of nociceptin increases the inhibitory effect of nociceptin in sham operated and neuropathic rats, while in naive rats the inhibitory effect of nociceptin was blocked by CCK. (B) Intrathecal administration of nociceptin 250 μg/50 μl followed for 1 h induces inhibition on C-fibres in naive and neuropathic rats, but had no effect in sham operated rats. Data are expressed as the % maximal inhibition ± S.E.M. Significance is made in respect of the naive group (**$P \leq 0.01$).

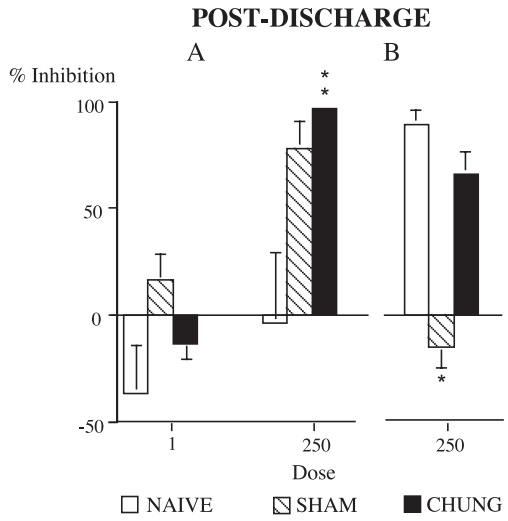

Fig. 2. Effect of intrathecal (i.t.) administration of (A) 1 μg/50 μl CCK (studied for 20 min) followed by 250 μg/50 μl nociceptin (1 h) on the post-discharges responses in naive, sham operated and neuropathic rats. CCK alone did not cause any significant changes in all the three groups. CCK in presence of nociceptin increases the inhibitory effect of nociceptin in sham operated and neuropathic rats, contrarily in naive rats the inhibitory effect of nociceptin was blocked by CCK. (B) Intrathecal administration of nociceptin 250 μg/50 μl followed for 1 h induces inhibition on post-discharges responses in naive and neuropathic rats, but had a slight facilitatory effect in sham operated rats. Data are expressed as the % maximal inhibition ± S.E.M. Significance is made in respect of the naive group (**$P \leq 0.01$, *$P \leq 0.05$).

3.1. Nociceptin alone

In all three animal groups intrathecal injection of nociceptin 250 μg/50 μl did not cause any changes in Aβ and Aδ induced activity.

In naive and neuropathic rats nociceptin caused a clear inhibition of C-fibre evoked responses (naive 70.8 ± 9%, neuropathic 39.3 ± 4%), post-discharge (naive 89 ± 6%, neuropathic 65.7 ± 10%), wind-up (naive 72.9 ± 9%, neuropathic 69 ± 11%) and input (naive 87 ± 10%, neuropathic 41 ± 22%) see Figs. 1–4B. Although there was a tendency for the effects of the nociceptin to be reduced after nerve injury this was not significant. Contrarily in sham operated rats nociceptin did not have any inhibitory effect but rather facilitated the wind-up by 64 ± 9% and post-discharges increased by 15 ± 10%, see Figs. 1–4B.

3.2. CCK and nociceptin

The application of 1 μg of CCK did not cause a significant effect in any of the three groups although there was a tendency towards a mild facilitation, see Figs. 1–4A.

After the application of 250 μg of nociceptin in the continued presence of CCK we observed that in the naive animals, there was no change in the lack of significant effect of nociceptin on Aβ and Aδ fibre-evoked responses compared to either sham or neuropathic rats. However, in naive

animals the marked inhibitory effect of nociceptin alone on C-fibre, post-discharge, wind-up and input responses was abolished in the presence of CCK (see Figs. 1–4, respectively). Thus, CCK acts as an anti-opioid at the ORL1 receptor in normal rats as shown by its block of the effect of nociceptin.

In sham operated rats where the application of nociceptin alone did not cause any inhibition, nociceptin in the presence of CCK caused a significant ($P \leq 0.01$) inhibition of the C-fibre-evoked responses from $+1.9 \pm 7.6\%$ facilitation in naive to $62 \pm 14\%$ inhibition in sham animals with CCK/nociceptin (see Fig. 1A). Also wind-up was significantly ($P \leq 0.01$) inhibited to a much greater extent comparing $27 \pm 35\%$ facilitation in naive to $80 \pm 7\%$ inhibition in sham rats with CCK plus nociceptin (see Fig. 3A). There was also significant ($P \leq 0.05$) inhibition of input responses in sham rats after the combination with respect to lack of effect the naive animals (see Fig. 4A).

The inhibitory effect of nociceptin after CCK on the C-fibre, post discharge, wind-up and input, response was considerably greater in neuropathic rats and caused an almost complete block of activity ($86 \pm 4\%$, $97 \pm 2\%$, $88 \pm 6\%$ and $96 \pm 3\%$, respectively) leading to a significant inhibition ($P \leq 0.01$ in all cases), see Figs. 1–4A.

Comparison of the effect of 250 μg of nociceptin in sham and the neuropathic group both pre-treated with 1 μg of CCK reveals no difference between the groups although they have opposite responses with nociceptin only.

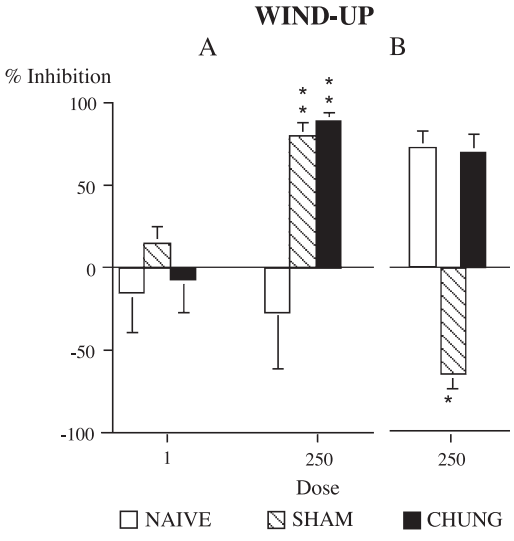

Fig. 3. Effect of intrathecal (i.t.) administration of (A) 1 μg/50 CCK (studied for 20 min) followed by 250 μg/50 μl nociceptin (1 h) on the wind-up responses in naive, sham operated and neuropathic rats. CCK alone did not cause any significant changes in all the three groups. CCK in presence of nociceptin increases the inhibitory effect of nociceptin in sham operated and neuropathic rats, while in naive rats the inhibitory effect of nociceptin was blocked by CCK. (B) Intrathecal administration of nociceptin 250 μg/50 μl followed for 1 h induces inhibition on wind-up responses in naive and neuropathic rats, but had a facilitatory effect in sham operated rats. Data are expressed as the % maximal inhibition ± S.E.M. Significance is made in respect of the naive group (**$P \leq 0.01$, *$P \leq 0.05$).

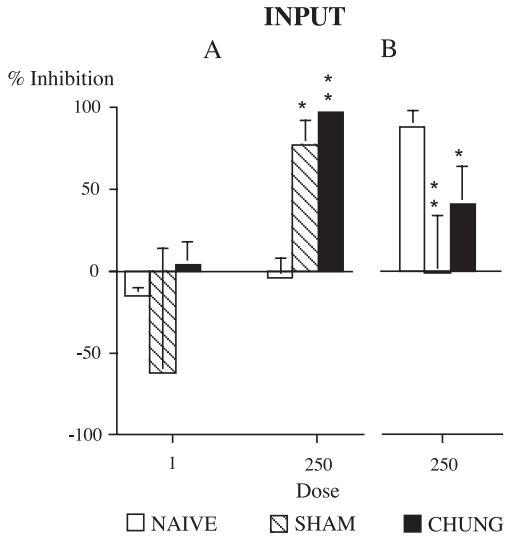

Fig. 4. Effect of intrathecal (i.t.) administration of (A) 1 μg/50 μl CCK (studied for 20 min) followed by 250 μg/50 μl nociceptin (1 h) on the input responses in naive, sham operated and neuropathic rats. Also here CCK alone did not cause any significant changes in all the three groups although there was a facilitatory effect in sham operated rats. CCK in presence of nociceptin increases the inhibitory effect of nociceptin in sham operated and neuropathic rats, contrarily in naive rats the inhibitory effect of nociceptin was blocked by CCK. (B) Intrathecal administration of nociceptin 250 μg/50 μl followed for 1 h induces inhibition on input responses in naive and neuropathic rats, but had a slight facilitatory effect in sham operated rats. Data are expressed as the % maximal inhibition ± S.E.M. Significance is made in respect of the naive group (**$P \leq 0.01$, *$P \leq 0.05$).

Overall, in the presence of CCK, the inhibitory effect of nociceptin on C-fibres, post-discharge, wind-up and input, is blocked in normal animals. On the other hand, in the presence of CCK the effect of nociceptin is not only restored in sham rats but is enhanced in the neuropathic group.

4. Discussion

Cholecystokinin has been shown to be a functional anti-opioid peptide at the spinal level, an area where both nociceptin and other opioids acting on μ and δ receptors elicit analgesia. There are a number of important functional differences between the ORL1 receptor and other opioid receptors and there have been no previous studies on the interaction between CCK and the ORL1 receptor using nociceptin in the dorsal horn of the spinal cord. In initial behavioural studies, the intracerebroventricular (i.c.v.) injection of nociceptin caused hyperalgesia (Meunier et al., 1995; Reinscheid et al., 1995). However, others reported the i.c.v. injection of nociceptin caused a biphasic response, a brief period of hyperalgesia followed by a prolonged period of naloxone-reversible analgesia (Rossi et al., 1996). This has been attributed to the fact that the i.c.v. injection may elicit opioid-mediated analgesia which outlasts the transient

reversal and hyperalgesia caused by nociceptin (Mogil et al., 1996). Supraspinal nociceptin does not have anti-opioid effects in motivational paradigms, and does not precipitate a withdrawal syndrome in morphine-dependent rats (Tian et al., 1997). In fact, i.c.v. injection of nociceptin was found to be motivationally neutral in the rat place preference/aversion test (Devine et al., 1996).

At the level of the spinal cord, nociceptin behaves as an analgesic. Spinal administration of nociceptin inhibits the C-fibre-dependent, post discharge, wind-up and input of dorsal horn neurones, whilst sparing the A-fibre evoked responses (Stanfa et al., 1996; Carpenter et al., 2000). Other studies have supported this with evidence that C-fibre evoked responses are inhibited in preference to A-fibre-evoked responses (Faber et al., 1996), so that the peptide has selective effects on noxious evoked activity and hyperalgesia (Hao et al., 1998). This suggests a selective action of nociceptin on noxious evoked responses in agreement with previous studies (Carpenter et al., 2000; Candeletti et al., 1998; Faber et al., 1996). Here we also found that nociceptin has the expected spinal analgesic effect in normal and neuropathic rats, although we did not observe any analgesic effect in sham operated rats. Clearly there is plasticity in the effects of nociceptin and although the sham surgery is an essential control for the spinal nerve ligation surgery, we have observed changes in the effect of another peptide, galanin, in this condition (Flatters et al., 2002). There clearly are persistent changes that may represent post-operative pain conditions, after the sham surgery which since it involves anaesthesia, exposure of nerve, suturing and recovery is possibly not surprising.

In this study, we found that application of CCK did not cause a significant effect in any of the three groups in agreement with the results of Stanfa and Dickenson (1993) in normal animals and after carrageenan inflammation. In normal animals CCK had an expected 'anti-opioid' action since it blocked the effects of nociceptin in a similar manner to that reported for μ-opioid receptor agonists such as morphine (Faris et al., 1983; Barber et al., 1989; Magnuson et al., 1990).

However, we found that CCK enhanced the inhibitory effects of nociceptin in neuropathic and in sham rats, comparing to rats treated with only nociceptin. Thus CCK does not act as an anti-opioid after either sham surgery or the subsequent nerve injury but rather enhances the action of nociceptin. After inflammation, which is likely to be present after the sham surgery that involves exposure of the nerve, the effects of morphine (Stanfa and Dickenson, 1993) and nociceptin (Carpenter et al., 2000) are enhanced and this has been attributed to decreases in the availability of endogenous CCK (Stanfa and Dickenson, 1993). Thus there are similarities between spinal μ-opioid and ORL1 receptor function in normal animals and after tissue injury. However, after nerve injury CCK had no 'anti-opioid' action since it failed to block the effects of nociceptin and in fact, tended to augment the inhibitory effects of the peptide. This is entirely different from morphine since in a rat model of neuropathic pain, there is an increase in spinal CCK and a reduction in the potency of spinal morphine (Xu et al., 1993). Other studies have also shown reduced effects of systemic but not spinal morphine after nerve injury in this electrophysiological model (Suzuki et al., 1999). Since this profile of the CCK-nociceptin interaction is very different from that of morphine (Stanfa and Dickenson, 1993), the results suggests a plasticity in the actions of CCK/nociceptin and further emphasises that the ORL1 receptor has very different characteristics from the μ-opioid receptor at spinal levels. It is interesting to note that these marked changes in the inhibitory effect of nociceptin in the presence of CCK in neuropathic and sham rats occurs in situations were there is an upregulation of both the CCK receptor and ORL1 receptor (Xu et al., 1993; Briscini et al., 2002), respectively.

Work from this laboratory has also shown that gabapentin, an anti-convulsant used for neuropathic pain patients also has enhanced effects after both sham and nerve injury as compared to normal animals (Chapman et al., 1998). One of the most widely proposed mechanisms of CCK is an involvement in calcium signalling. Intracellular calcium is mobilised by CCK, which opposes the suppression mediated by opioids of the increase in intracellular calcium. Furthermore, CCK reverses the suppression of the K^+-induced increase in intracellular calcium produced by opioids (Wang et al., 1992). Since gabapentin is thought to modulate calcium channel activity and there is an upregulation of the alpha 2 delta subunit of calcium channels after nerve injury and enhanced actions of both N (nerve injury) and P (tissue damage) after pathological changes (Suzuki et al., 2002), this suggests that the CCK/ORL1 interaction, as suggested for μ-opioid receptors in normal animals may involve intracellular calcium. Another possible mechanism is that CCK, acting on CCK_B receptors, decreases the availability of the enkephalins (Nicholas et al., 1996).

Whatever the form of the interaction, in normal animals CCK plays a physiological role in negatively modulating antinociceptive action of opioid agonists in the spinal cord. Since this role is altered after nerve injury and in presence of surgery, the ORL1 receptor may be a target for the treatment of nerve injury and other pain states but this will require production of a non-peptide agonist at this receptor.

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