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Cholecystokinin Octapeptide Reverses μ-Opioid-Receptor-Mediated Inhibition of Calcium Current in Rat Dorsal Root Ganglion Neurons[1]

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ABSTRACT

Cholecystokinin octapeptide (CCK-8) is reported to antagonize the analgesic effect produced by μ- and κ- but not δ-opioid agonist in spinal cord. However, the mechanisms of interaction remain obscure. In the present study, whole-cell patch-clamp recording was performed on acutely isolated rat dorsal root ganglion (DRG) neurons to evaluate the effects of the highly specific μ-opioid agonist ohmefentanyl and the δ-opioid agonist DPDPE on voltage-gated calcium channels and the possible interaction between CCK-8 receptor and μ- or δ-opioid receptor. The results indicated that ohmefentanyl, but not DPDPE, can suppress the voltage-gated calcium currents elicited in DRG neurons, an effect readily reversed by naloxone or by the antiopioid peptide CCK-8. The effect of CCK-8 can in turn be abolished by the CCK-B receptor antagonist L365,260. CCK-8 used by itself has no enhancing effect, but rather a depressant effect, on calcium currents. However, used simultaneously with ohmefentanyl, CCK-8 shows a clear-cut reversal of depression of the μ-opioid. We conclude that the depressant effect produced by μ-opioid on voltage-gated calcium current in DRG neurons can be antagonized by CCK-8 through CCK-B receptor located in the same neuron. The δ-opioid DPDPE has no direct effect on the voltage-gated calcium current in DRG neurons.

Both *in vivo* studies (Lamotte *et al.*, 1976; Fields *et al.*, 1980; Ninkovic *et al.*, 1982) and *in vitro* studies (Macdonald and Nelson, 1978; Hiller *et al.*, 1978; Mudge *et al.*, 1979; Werz *et al.*, 1987) have shown that DRG neurons and their processes are equipped with opioid receptors. All three types of opioid receptors, the μ-, δ- and κ-opioid receptors, have been found on the somata of DRG neurons (Werz and MacDonald, 1982b, 1983a,b, 1984b, 1985; Werz *et al.*, 1987). *Kappa* receptors were demonstrated to couple to voltage-gated calcium channels (Werz and MacDonald, 1984a; Schroeder *et al.*, 1991), and activation of κ-opioid receptor was shown to reduce the calcium currents of DRG neurons (Schroeder *et al.*, 1991). Most of the studies of the μ-opioid receptors (Werz and MacDonald, 1983a,b; North *et al.*, 1987; North, 1993) revealed that they are coupled to potassium channels, although evidence was presented that they may also couple to calcium channels (Seward, 1991). Activation of δ-opioid receptor was reported to have no effect on calcium current (Schroeder *et al.*, 1991), although some other reports suggested that δ-opioid receptor may couple to calcium channels (North, 1993). This issue was reevaluated in the present study by using the novel, highly selective μ-opioid agonist OMF (Xu *et al.*, 1985) as well as the classical δ-selective agonist DPDPE in the acutely dissociated DRG preparation. It is generally believed that the binding of opiates to opioid receptors in the cell body produces a reduction of calcium entry (Mudge *et al.*, 1979; Werz and MacDonald, 1982a,b, 1985; Werz *et al.*, 1987; Chalazonitis and Crain, 1986) and that the binding of opiates to opioid receptors in the primary afferent terminals results in a depression of neurotransmitter release (MacDonald and Nelson, 1978; Mudge *et al.*, 1979), which seems to underlie opioid analgesia at the spinal level.

CCK-8, as an endogenous antiopioid peptide (Han *et al.*, 1985), is prevalent in many parts of the central nervous system, notably in the substantia gelatinosa of the spinal cord of a wide variety of species (Hill *et al.*, 1990; Ghilardi *et al.*, 1992). There is evidence that CCK-8 not only produces neuronal excitation in the spinal dorsal horn (Jeftinija *et al.*, 1981) but also acts to antagonize the antinociceptive effect produced by morphine and endogenous opioids (Han, 1992; Wang *et al.*, 1990). Although binding studies and morpholog-

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ABBREVIATIONS: CCK-8, sulfated cholecystokinin octapeptide; OMF, ohmefentanyl; NX, naloxone; TTX, tetrodotoxin; TEA-Cl, tetraethylammonium chloride; DRG, dorsal root ganglion; EGTA, ethylene glycol bis(-aminoethyl ether)-N,N,N′,N′-tetraacetic acid; DMEM, Dulbecco's Modified Eagle Medium; HBSS, Hank's balanced salt solution; DMSO, dimethyl sulfoxide; ANOVA, analysis of variance.

ical observations have demonstrated that μ- and/or δ-opioid receptors and CCK-B receptors are localized on the DRG neurons (Fields *et al.*, 1980; Ninkovic *et al.*, 1982; Egan and North, 1981; Ghilardi *et al.*, 1992; Dado *et al.*, 1993; Zhang *et al.*, 1993), there is no evidence to demonstrate precisely that the interaction between CCK receptor and μ- or δ-opioid receptor takes place in one and the same neuron. In the present study, we used the whole-cell patch-clamp recording technique on acutely dissociated DRG neurons to assess the interaction between CCK-receptor and μ- or δ-opioid receptor as manifested on the activities of voltage-gated calcium channels.

Materials and Methods

Cell preparation. Single DRG neurons were acutely isolated by enzymatic dispersion of the ganglion taken from male Wistar rats (200–300 g). The method used here was similar to that described by Ikeda *et al.* (1986), except that we used trypsin type I-S (Sigma, 0.56 mg/ml) and collagenase type IA (Sigma, 1.2 mg/ml) incubated at 37°C for 35 min. After the incubation, the enzyme in solution containing the dispersed cells was inhibited by the addition of soya bean trypsin inhibitor type II-S (Sigma, 1.5 mg/ml) and incubated for 10 more min. In addition, we used DMEM instead of HBSS. A phase-contrast microscope (OLYMPUS, eyepiece 10×, object lens 20×) was used to visualize the cells. Neurons isolated in this manner were usually spherical, 10 to 50 μm in diameter and generally devoid of processes. Diameter was defined as the average of the distances along the longest and shortest axes of each cell body. Only cells with relatively small (19–32 μm) and medium-sized (33–38 μm) diameters (Scroggs *et al.*, 1994) were used for clamping study. Most recordings were made between 2 to 8 h after plating.

Whole-cell patch clamp. Patch pipettes with impedance of 2 to 3 MΩ contained a solution consisting of (in mM) 100 CsCl, 2 TEA-Cl, 5 $MgCl_2$, 40 HEPES, 10 EGTA, 2 Mg^{++}-ATP and 0.25 cAMP (titrated to pH 7.2 with CsOH). The cells were allowed to adhere to a plastic coverslip and perfused at room temperature (18°C–20°C) with media containing (in mM) 150 NaCl, 5 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$, 10 HEPES and 10 D-glucose (titrated to pH 7.4 with NaOH). After the whole-cell recording mode was attained, a control solution containing (in mM) 140 TEA-Cl, 9 $BaCl_2$, 1 $MgCl_2$, 5 CsCl, 10 HEPES, 10 D-glucose and 0.001 TTX (titrated to pH 7.4 with CsOH) was applied to the cells to isolate barium currents passing through calcium channels. Currents were recorded in standard whole-cell patch-clamp mode (Hamill *et al.*, 1981) using a EPC-9 patch-clamp amplifier, filtered at 3 kHz with a 4-pole Bessel filter, digitized (5 kHz), stored and analyzed by a microcomputer (Macintosh IIci) using the program Pulse+PulseFit (HEKA elektronik). Test pulses 100 ms in duration were applied every 8 to 20 s to avoid accumulating inactivation. Capacity and series resistance compensation were performed in AUTO mode by EPC-9. Leak and capacity currents were subtracted by computer.

Administration of drugs. For application of drugs, six microtubes (200 μm I.D.) were glued together side by side. Solutions were fed from separated reservoirs by gravity. The microtubes were mounted on a micromanipulator, which was used for moving the tubes to aim at the cell for changing solutions.

Drugs. CCK-8 (Squibb & Sons, Inc.) and DPDPE were prepared with control solution, aliquoted and stored at −20°C. On the day of the experiment the aliquots were diluted in the control solution. L365,260 (Merck Sharp & Dohme) was dissolved in DMSO and 1,2-propanediol (volume of DMSO : 1,2-propanediol = 4 : 1) and diluted in the control solution. OMF (Shanghai Pharmacological Research Institute, Chinese Academy of Sciences), NX and L365,260 were stored at 4°C. Unless otherwise noted, all drugs were from Sigma.

Results

OMF inhibited calcium channel current. DRG neurons contain several types of calcium channels. The low-threshold, T-type calcium channel is easily distinguished because it is activated by relatively negative voltages and rapidly inactivated during a maintained stimulus (Carbone and Lux, 1984). Figure 1a shows that the T-type (left) and high-threshold calcium currents (right) were completely blocked by 1 mM $CdCl_2$. Figure 1b shows 1 of 12 cases of current-voltage curves with holding potential set at −90 mV. The highly selective μ-opioid receptor agonist OMF was shown to inhibit calcium currents at 1 μM, and the inhibitory effect could be completely removed when OMF was washed away (data not shown). The variations of the inhibition by 1 μM OMF of calcium currents are shown in figure 2b. Figure 1c shows that the inhibition of calcium current produced by OMF could be reversed by the μ-opioid receptor antagonist 10 μM NX. NX at 10 μM by itself had no effect on calcium current (data not shown).

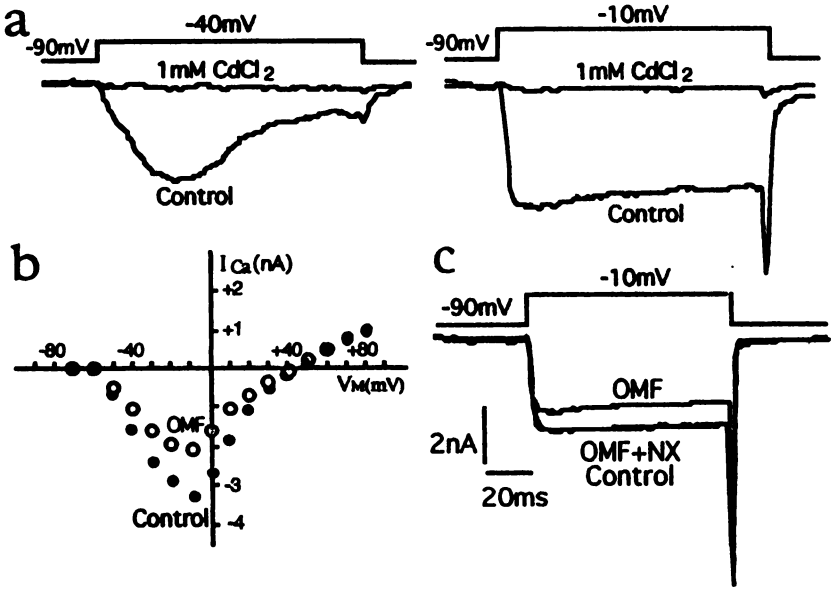

Fig. 1. Inhibition of calcium current in DRG neurons by the μ-selective opioid agonist OMF. a) Low-threshold (left) and high-threshold (right) calcium currents were elicited by steps to −40 mV and −10 mV, respectively, from −90 mV in the same cell. Both currents were blocked by 1 mM $CdCl_2$. b) Current-voltage curve of the OMF inhibition of calcium currents. Peak current-voltage plots were derived from currents recorded in the absence and presence of 1 μM OMF. The recovery curve from OMF inhibition is not shown. c) The inhibition of calcium current by 1 μM OMF was almost completely blocked by 10 μM NX.

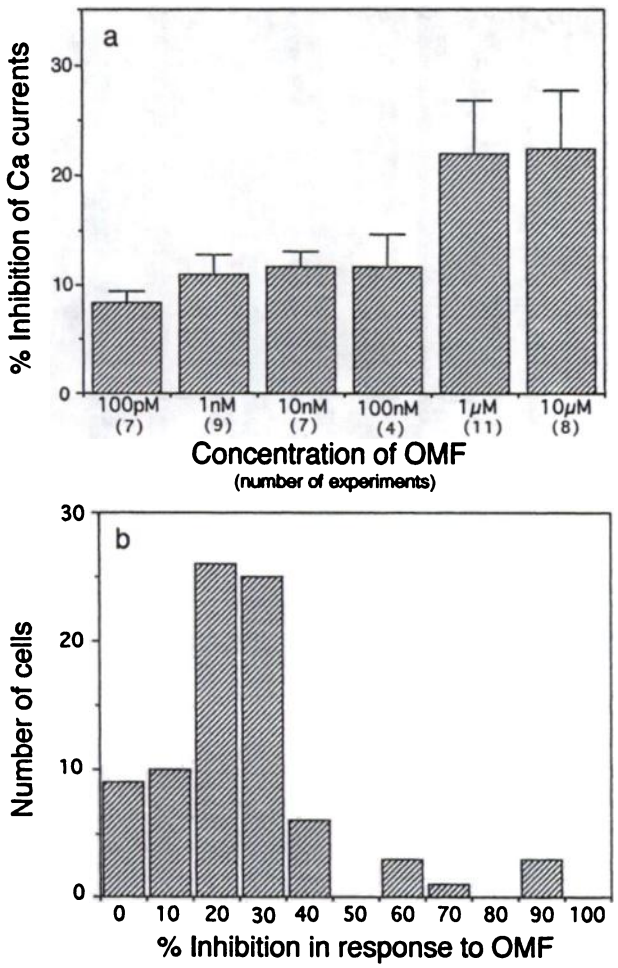

Fig. 2. Dose-response relationship and the variation of OMF-induced inhibition of calcium currents. a) Concentration-response relationship for the OMF-induced inhibition of peak calcium current. Vertical bar represents S.E.M. The numbers of experiments are shown in parentheses. Percentage inhibition was calculated as $100[(C - D)/C]$, where D is the peak current in the presence of OMF, and C the peak current averaged before and after OMF when there was a good recovery. The response was measured during voltage steps that elicited the largest calcium current. b) Histogram showing distribution of calcium current inhibition on 83 different cells in response to 1 μM OMF. Calculation of percentage inhibition is the same as in figure 2a.

We were unable to construct a smooth dose-response curve of OMF on calcium current. This is probably because of the cell-to-cell variation and the limited recording time from any one cell due to run-down of calcium current. However, we did see that the inhibitory effect of OMF on calcium currents increased with the concentration of OMF from 100 pM to 10 μM. The inhibitions of 1 μM and 10 μM were significantly greater than that of 100 pM ($P < .05$, ANOVA followed by Duncan's Multiple Range test, $dF = 5,40$) (fig. 2a). Because 1 μM concentration elicited almost the maximal response, we chose to use this concentration in the following experiments on the inhibition by OMF of calcium currents. Figure 2b is a histogram showing the range of responses to 1 μM OMF among 83 cells. In 9 cells, calcium currents were essentially unaffected (less than 5% depression) by OMF. The extent of inhibition varied widely among sensitive cells, but in the majority of cases the inhibitory effect of OMF did not exceed 40%.

CCK-8 reversed OMF-induced inhibition of calcium channels. Figure 3 shows that 1 μM OMF inhibited calcium currents, an effect that could be almost completely reversed by 20 nM CCK-8. This effect of CCK-8 was in turn reversed by 1 μM L365,260, an antagonist of CCK-B receptor. Application of 20 nM CCK-8 by itself also produced an inhibitory effect on calcium currents that could be readily reversed by co-application of L365,260 (fig. 4a). L365,260 by itself had no effect on calcium currents (data not shown). Figure 4b shows the dose-response relationship of the CCK-8-induced inhibition of calcium currents. The inhibition elicited by 20 nM CCK-8 is significantly greater than that elicited by 10 pM, 100 pM and 1 μM, and the data exhibit a bell-shaped curve.

Figure 5 shows that the reversal of OMF-induced inhibition by CCK-8 also exhibits a bell-shaped dose-dependent curve, and the maximal effect occurs at 2 nM and 20 nM.

Out of 40 cells in which OMF (1 μM) exerted an inhibitory effect on calcium currents, 31 cells (77.5%) were sensitive to CCK-8 (20 nM), and the inhibitory effect of OMF was almost completely reversed. In the other 9 cells (22.5%), the effect of OMF was not at all affected by CCK-8 (fig. 6).

DPDPE, a selective agonist of δ-opioid receptor, has no effect on calcium currents. Thirty-three cells were tested with DPDPE (10 nM, 7 cells; 100 nM, 7 cells; 1 μM, 19 cells). Only four cells displayed a response, and not one exceeded 5% depression of calcium currents (data not shown). Because no obvious effect of DPDPE occurs, no antagonist of δ-opioid receptor was used.

Discussion

This study describes the ability of the neuropeptide CCK-8 to reverse the inhibition of voltage-dependent calcium current by the highly selective μ-opioid agonist OMF in DRG neurons acutely dissociated from the rat spinal cord. This work represents an extension of *in vivo* investigations performed by this and other groups (Jurna and Zetler 1981; Faris *et al.*, 1983; Han *et al.*, 1985, 1992; Dourish *et al.*, 1988; Wang *et al.*, 1990; Dickenson, 1992), which established that CCK-8 could reverse or antagonize the analgesic effect in-

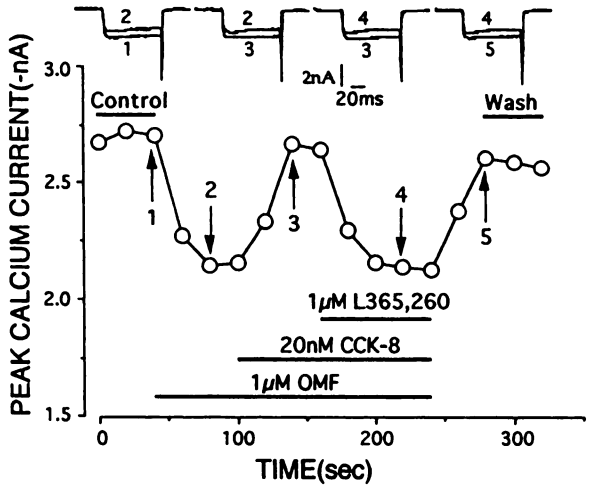

Fig. 3. CCK-8 reverses OMF-induced inhibition on calcium currents. Calcium current (traces) was elicited by steps to +10 mV from −90 mV at times indicated in the respective graphs of peak current *vs.* time. Bars indicated the time of drug application and the concentration of drugs.

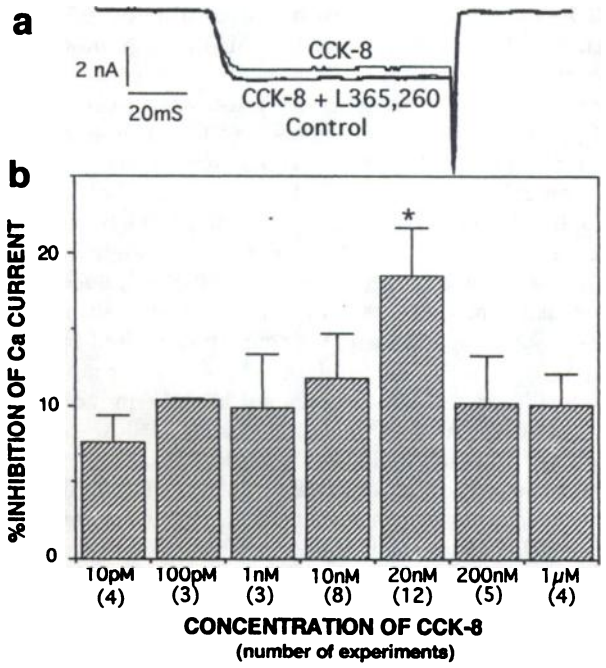

Fig. 4. Dose-dependent inhibition of calcium current by CCK-8. a) The inhibition of calcium current by 20 nM CCK-8 is blocked by 1 μM CCK-B antagonist L365,260. Calcium currents were elicited by steps from −90 mV to −10 mV. b) Histogram showing the dose dependence for the CCK-8-induced inhibition of peak calcium current. Method for calculation of percentage inhibition is the same as in figure 2a. Each column represents the mean ± S.E.M. Shown in parentheses are the numbers of experiments. * $P < .05$, ANOVA followed by Duncan's Multiple Range test compared with the 10-pM group ($dF = 7,34$; $D = 3.468$).

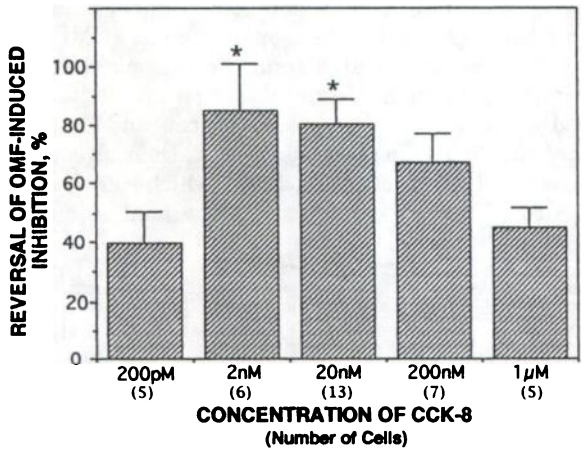

Fig. 5. Concentration-reversal relationship of CCK-8 on OMF-induced inhibition. Percentage of reversal was calculated as $100[(A-B)/A]$, where B is the peak current in the presence of CCK-8, and A is the peak current averaged from that before the application of OMF and after the washing of OMF and CCK-8 when there was a good recovery. The response was measured during voltage steps that elicited the largest calcium current. Each bar represents the mean ± S.E.M. The numbers of experiments are shown in parentheses under each bar. * $P < .05$, ANOVA followed by Duncan's Multiple Range test compared with the 200-pM group ($dF_1 = 4,33$; $D_1 = 3.512$; $dF_2 = 5,33$; $D_2 = 3.481$).

duced by opioid agonists. It is one of the first studies to elucidate the mechanism of this opioid/CCK interaction in single cells *in vitro*.

OMF is a novel μ-opioid agonist developed at the Shanghai Institute of Materia Medica by Xu and co-workers (1985).

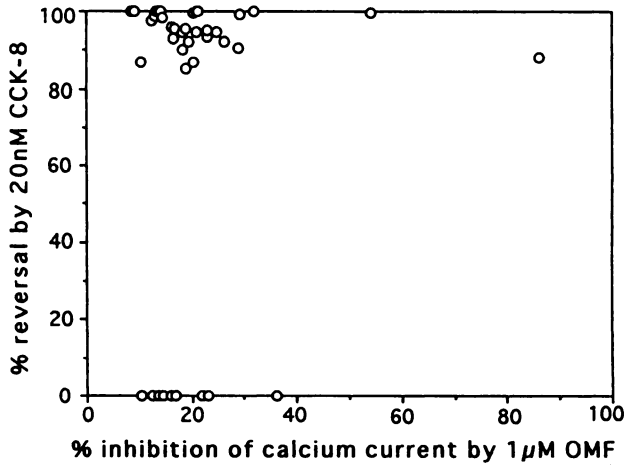

Fig. 6. The relationship between percent inhibition of calcium current by 1 μM OMF alone and the percent reversal of the effect of 1 μM OMF by 20 nM CCK-8 for all 40 DRG cells where the combination was tested.

According to Goldstein and Naidu (1989), OMF was ranked as having the highest selectivity for μ-opioid binding sites. Its nonpeptide chemical nature makes it free of the problem of enzymatic degradation that occurs with most peptide opioid agonists.

In a previous study performed in this laboratory (Xu *et al.*, accepted in Neuroscience), we found that CCK-8 was capable of reversing the inhibition of voltage-dependent calcium current induced by the κ-selective opioid agonist U50488H in the same DRG preparation. It was interesting to find that the κ-agonist-induced inhibition of calcium current could be readily blocked by the highly κ-selective opioid antagonist nor-BNI at 5 μM, but not by NX at 10 nM, 100 nM, 1 μM or 10 μM. It is thus safe to use a full dose of NX without worrying about its influence on the κ-opioid effect up calcium channels. The possibility that the effect of OMF might have been mediated by δ-opioid receptors can also be ruled out, because in the present preparation the most selective δ-opioid agonist, DPDPE, did not show any significant suppression of the calcium current at 10 nM, 100 nM or 1 μM, an effect comparable to that reported by Schroeder *et al.* (1991). These results suggest that the δ-opioid receptor is not coupled to the calcium channels, although it is well known that the δ-opioid receptors are coupled to potassium channels (North, 1993) and that this coupling can lead to a secondary inhibitory effect on calcium channels (Werz and MacDonald, 1983a; North, 1993). This may explain the results obtained by McDonald's group in the 1980s: that activation of δ-opioid receptors in DRG neurons secondarily results in the inhibition of calcium channels. In our preparation, the potassium channels have been blocked, so this secondary effect can be ruled out. The assumption of opioid/CCK interaction in the present study was made on 40 DRG neurons that had already been shown to respond to μ-opioid OMF. However, the group data shown in figure 6 reveal that this interaction existed in only 31 out of 40 neurons (77.5%). This all-or-none distribution may be explained that among DRG neurons equipped with μ-opioid receptors, only 77.5% of them show a coexistence of CCK receptor. In addition, in the population of small and medium-sized DRG neurons, there seems to be a heterogeneous distribution of the density of μ-opioid receptors, as reflected by the wide variety in the degree of suppression of

calcium current in response to the same concentration of OMF (fig. 2b). This may be one of the reasons for the variability in the dose-response curve (fig. 2a).

The fact that the μ-opioid suppressive effect on calcium current can be reversed by CCK-8 may be explained in several ways: 1) CCK-8 may interact directly with the opioid receptor. However, in order to reverse the opioid effect, the affinity of the opioid receptor toward CCK-8 should be much higher than that for opioids, which seems unreasonable. Another point in favor of the CCK effect being mediated through activation of a CCK, and not a μ-opioid, receptor is that the effect was blocked by a CCK antagonist. 2) The interaction of CCK-8 with the CCK-receptor may induce an increase of the calcium current that counteracts the opioid effect. This seems not to be the case, however, because CCK-8 by itself produced a decrease rather than an increase of the calcium current, as shown in figure 4. Miller and Luppica (1994) reported, in an *in vitro* study, that CCK-8 could antagonize the effect of morphine only if it was presented before the opioid, which indicates that the opioid and CCK receptors may share the same signal transduction pathways. If this mechanism works in our case, we should see a failure of CCK-8 to influence opioid effect rather than a reversal of the opioid effect. 3) CCK-8 interacts with CCK receptors that cross-talk with the opioid receptor. This interpretation is supported by the experimental result that the CCK-8 effect can be totally abolished by the CCK-B receptor antagonist L365,260 (fig. 3). Binding studies have shown that activation of CCK receptor may induce a decrease in the number (B_{max}) of μ-opioid binding sites without altering their affinity (Wang and Han, 1990), although the details of this receptor/receptor interaction remain obscure.

At the present time we are not able to provide a satisfactory explanation for the peculiar phenomenon that although both OMF and CCK-8 suppressed calcium current, the effect of OMF could be reversed by CCK-8. However, similar phenomena have been found in ^{45}Ca uptake experiments, wherein CCK-8 antagonized the inhibitory effect of morphine on ^{45}Ca uptake in rat brain (not spinal cord) synaptosomal preparation, yet CCK-8 itself inhibited the synaptosomal ^{45}Ca uptake (Wang *et al.*, 1989). This dilemma may have the same underlying mechanism as another, equally peculiar finding: that CCK-8 antagonizes opioid analgesia (at nanogram dose) yet by itself at high (microgram) dose produces analgesia after its intrathecal (Jurna and Zetler, 1981) or s.c. (Zetler, 1980) injection. Figures 4 and 5 reveal that the inhibitory effect of CCK-8 on calcium currents and the reversal of OMF-induced inhibition by CCK-8 are both bell-shaped; the optimal concentrations were 20 nM and 2 and 20 nM, respectively. Thus the concentration of the agonist used in the study should be taken into consideration as an important factor. We believe that these discrepancies will not be resolved until we know, in terms of molecular mechanisms, how CCK receptor and opioid receptor are interconnected and how are these receptors are linked to ion channels.

It should be mentioned that the functional implications of a suppression of calcium channels in DRG neurons by CCK include a decrease in neuronal firing. However, previous investigations have reported that CCK-8 caused an increase in population spike amplitude in dorsal horn neurons (Jeftinija *et al.*, 1981) and in the hippocampal slices (Bohme *et al.*, 1988). The differences between these two events are at least 3-fold: 1) The effect of CCK-8 on dorsal horn neurons may not work through calcium channels (Sah, 1990), and the effect of CCK-8 on hippocampal slice was a result of its modulation of potassium channels (Buckett and Saint, 1989; Saint and Buckett, 1991) whereas in the DRG preparation the potassium channels were fully blocked. 2) The group firing recordings made in the brain slices would certainly be more complicated than the single-neuron patch-clamp recording. 3) The mechanism whereby the CCK-receptor connects to the calcium channel may be different between the dorsal horn neurons and the pyramidal neurons.

From a methodological point of view, it is more convenient to test the opioid/CCK interaction at the soma rather than at the terminals of the DRG neuron. An important issue is whether the same principle applies at the nerve terminal as in the cell body. A tempting speculation is that both calcium currents and the biochemical machinery for their modulation are similar throughout the cell. This may in fact be plausible in the case of the DRG neuron, because the release of neuropeptides occurs in the peripheral as well as in the central terminals (Otsuka and Konishi, 1983), and opioid receptors and CCK receptors are present on DRG neurons both at their perikarya and at their central terminals (Ghilardi *et al.*, 1992; Dado *et al.*, 1993). Furthermore, there is a parallel relationship between the inhibition of calcium entry on the soma and the inhibition of transmitter release from primary terminals (MacDonald and Nelson, 1978; Mudge *et al.*, 1979; Werz and MacDonald, 1982a,b, 1985, 1987; Chalazonitis and Crain, 1986).

Opioid receptors and CCK receptors are located predominantly in the substantia gelatinosa, where the afferent fibers terminate. It is interesting to consider whether the opioid/CCK interaction occurs at pre- or postsynaptic sites or at a combination of both. In an *in vitro* longitudinal slice preparation of the dorsal horn, Dickenson *et al.* (1992) applied glutamate to excite neurons directly through pressure ejection, thus by-passing the afferent terminals. No functional interaction was found between morphine and CCK, which suggests that the site of action of CCK and opioid receptors may be at the presynaptic terminals of the afferent fibers. Our data in the present study provide a model for presynaptic opioid receptor and CCK receptor interaction, which suggests that μ-opioid receptor and CCK-B receptor may coexist, at least in a subpopulation of the DRG neurons. Hence the interaction between opioid and CCK receptors may be located presynaptically.

It is likely that sensory modality varies among DRG cell bodies of different diameter. Rapidly conducting Aα- and A_β-type DRG neurons have the largest cell bodies, whereas slower conducting Aδ- and C-type DRG neurons have smaller cell bodies (Harper and Lawson, 1985). Most Aα-type DRG neurons are committed to the transmission of proprioceptive and tactile information, whereas Aδ- and C-type DRG neurons most frequently transmit pain and thermal information (Fyffe, 1983; Yaksh and Hammond, 1982). Thus the results obtained from small and medium-sized DRG neurons used in the present study may explain, at least in part, the mechanism for the CCK antagonism of opiate analgesia and the development of opioid tolerance.

Because it is well known that $GABA_B$ agonist (Menonjohansson *et al.*, 1993; Gruner and Silva, 1994) and adenosine

A_1 agonist (Sah, 1990) negatively modulate these calcium channels just as μ-opioid does, it will be interesting to learn whether CCK-8 can also antagonize the effect of $GABA_B$ agonist and adenosine A_1 agonist. This issue is related to the specificity of CCK antagonism for opioid receptors. Preliminary results show that the inhibitory effect exerted by the $GABA_B$ agonist baclofen (100 μM, inhibition rate 17.9 ± 2.1, n = 12) and by the adenosine A_1 agonist CPA (1 μM N^6-cyclopentyladenosine, inhibition rate 27.9 ± 3.7, n = 12) on the calcium channel of the DRG neurons can also be antagonized by CCK-8. These effects of CCK can in turn be canceled by the CCK-B receptor antagonist L365,260 (Liu *et al.*, in preparation). The preliminary results suggest that CCK not only antagonizes the inhibition of calcium current mediated by opioids but also antagonizes the inhibition mediated by some other neurotransmitters or neuromodulators, such as adenosine A_1 agonist and $GABA_B$ agonist. It is thus postulated that CCK-8 may act through at least two major mechanisms: 1) Interaction with CCK receptors that cross-talk with certain other co-localized receptors, such as opioid receptors (a process that may also involve adenosine A_1 receptors and $GABA_B$ receptors) to reduce its total number and/or to decrease its affinity (Wang and Han, 1990), serving as the basis for its antiopioid effect. It is not impossible that CCK, through similar antiopioid mechanisms, antagonizes the effect of adenosine A_1 receptor agonist and $GABA_B$ receptor agonist. 2) Mobilization of intracellular calcium storage *via* the IP_3 pathway (Zhang, *et al.*, 1992; Wang, *et al.*, 1992), which would neutralize the $[Ca^{++}]_i$-lowering effect induced by many calcium-channel-inhibitory neurotransmitters. This is certainly an area of intensive pharmacological interest that deserves further investigation.

Acknowledgments

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