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Research report

Cholecystokinin octapeptide reverses the κ-opioid-receptor-mediated depression of calcium current in rat dorsal root ganglion neurons

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Abstract

Although the cholecystokinin octapeptide (CCK-8) is reported to antagonize the κ-opioid-receptor-mediated analgesic effect in spinal cord, its mechanism and sites of action remain obscure. In the present study, the whole-cell patch-clamp recording technique was employed to examine the effect of κ-opioid agonist U50488H on voltage-gated calcium channels and the interaction between the CCK-8 and U50488H in acutely isolated rat dorsal root ganglion neurons. The results indicate that the calcium currents elicited in dorsal root ganglion neurons can be depressed by U50488H, an effect readily reversed by the κ-opioid receptor antagonist Nor-BNI or by the antiopioid peptide CCK-8. The effect of the CCK-8 can be abolished by the CCK-B receptor antagonist, L365,260. While CCK-8 showed a potent opioid-reversal effect, it by itself exerted a slight inhibitory effect on calcium current. This novel observation in the dorsal root ganglion neurons indicates that CCK-8 can antagonize the κ-opioid-receptor-mediated depressant effect on voltage-gated calcium current, and this antagonizing effect appears to be mediated via CCK-B receptor.

Keywords: Whole-cell patch-clamp; Voltage-gated calcium current; U50488H; CCK-8; L365,260

1. Introduction

Dorsal root ganglion (DRG) neurons transmit signals of temperature, touch, nociception and muscle or tendon stretch from the periphery to the spinal cord. The 'gate control theory' of Melzack and Wall [17] proposed that presynaptic inhibition of the small DRG neurons selectively reduces the transmission of noxious information through a presynaptic mechanism. Opioid peptides have been shown to be important chemical mediators of this presynaptic inhibition, since they cause both reduction of neurotransmitter release [16] and a decrease of the duration of action potential in DRG neurons [27]. Activation of the κ-opioid receptor is proposed to inhibit Ca^{2+} channels [8], thereby decreasing Ca^{2+}-dependent neurotransmitter release.

The cholecystokinin octapeptide CCK-8, which has been known as an endogenous anti-opioid peptide [10], 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 [7,12]. There is evidence that CCK not only produces neuronal excitation in the dorsal horn but also acts to antagonize the antinociceptive effect produced by morphine and endogenous opioids [10,26].

The sites of opiate–CCK interaction have not been precisely determined, although binding studies suggest that opioid receptors and CCK receptors may be co-localized on the same neuron [2,7,5,18,30]. In the present study we used whole-cell patch-clamp technique on acutely dissociated DRG neurons to assess the possible interaction between CCK- and κ-opioid receptor in controlling the voltage-gated calcium current. The presence of both the κ-opioid receptors and CCK-B (but not CCK-A) receptors in adult rat DRG neurons has recently been demonstrated [7,27].

2. Materials and methods

Single DRG neurons were acutely isolated by enzymatic dispersion from female Wistar rats (200–300 g). The method used here was similar to that described by Ikeda et

Abbreviations: CCK-8, cholecystokinin octapeptide; DRG, dorsal root ganglion; nor-BNI, nor-binaltorphimine dihydrochloride; DMSO, dimethyl sulfoxide; U50, U50488H.

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al. [13], 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 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 minutes. In addition, we used Dulbecco's modified Eagle's medium (DMEM) instead of Hank's balanced salt solution (HBSS). Neurons isolated in this manner were usually spherical, 19–55 μm in diameter and generally devoid of processes. Only cells with relatively small diameter (19–27 μm) as Scroggs and Fox [22] defined were used for clamping study. Most recordings were made between 2 and 8 h after plating.

Patch pipettes with impedances of 2–3 MΩ contained the following media (in mM): 100 CsCl, 2 TEA-Cl, 5 $MgCl_2$, 40 HEPES, 10 EGTA, 2 Mg^{2+} ATP and 0.25 cAMP (titrated to pH 7.2 with CsOH). The cells were allowed to adhere to a plastic coverslip and perfused slowly with media at room temperature (18–20°C) containing (in mM): 150 NaCl, 5 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 10 D-glucose (titrated to pH 7.4 with NaOH). After the whole-cell recording mode was attained, control solution containing (in mM): 140 TEA-Cl, 9 $BaCl_2$, 1 $MgCl_2$, 5 CsCl, 10 HEPES, 10 D-glucose, 0.001 TTX (titrated to pH 7.4 with CsOH) was applied to the cells in order to isolate Ba^{2+} currents through Ca^{2+} channels.

Currents were recorded in standard whole-cell patch-clamp mode [9] using 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). In case of rundown, percentage inhibition induced by drugs is calculated as $100[(C-D)/C]$, where D is the peak current in the presence of drugs, and C is the peak current averaged before and after drugs, and only those cells that had a substantial recovery are included in this paper. Test pulses of 100 ms duration were applied every 8–20 s, those of 500 ms duration were applied every 20–40 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. We should point out that some cells had a problem of voltage clamp for the misadjustment of % R_s compensation.

For drug application, a series of 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 for aligning the flow of solution from the tubes relative to the cells in order to change solutions.

Sulfated cholecystokinin octapeptide (CCK-8, Squibb and Sons) and Nor-BNI (RBI) were prepared in control solution, aliquoted and stored at −20°C. On the day of the experiment, the aliquots were diluted in the control solution. L365,260 was dissolved in DMSO/1,2-propanediol (4:1) and diluted in the control solution. U50488H (Upjohn Company, USA) and L365,260 were stored at 4°C. Unless otherwise noted, all drugs were purchased from Sigma.

3. Results

3.1. U50 reduced Ca^{2+} channel current

We were unable to construct a smooth dose–response curve because of the cell-to-cell variation (data not shown) and the limited recording time from any one cell due to run down of Ca^{2+} current. However, we did find from Fig. 1 that, while 10 nM U50 showed no inhibition of Ca^{2+} current, 100 nM U50 inhibited Ca^{2+} current in the same cell, and that 5 μM was a saturating concentration since the response to 10 μM was never greater. Therefore we chose 5 μM as the concentration of U50 in the following experiments.

The I–V curves in Fig. 2a show that voltage-gated Ca^{2+} currents was depressed by 5 μM U50. The depressant effect was overcome by high depolarization above +60 mV, displaying voltage-dependent inhibition similar to that first described by Bean [1]. In 10 cells tested, the depression of Ca^{2+} current by 5 μM U50 is blocked by highly selective κ-opioid receptor antagonist Nor-BNI (5 μM), as shown in Fig. 2b. Nor-BNI per se has no effect on Ca^{2+} current (data not shown).

3.2. CCK-8 reversed U50-induced inhibition of Ca^{2+} channels

In the previous study [15], we found that the inhibitory effect of CCK-8 on calcium currents and the reversal of

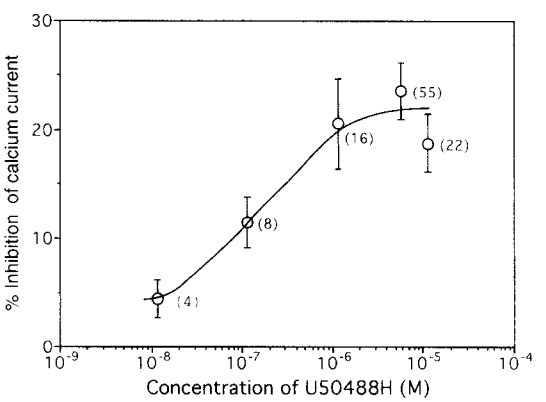

Fig. 1. Dose relationship of U50488H (U50)-induced inhibition on Ca^{2+} current. Concentration–response relationship for the U50-induced inhibition of peak Ca^{2+} current. Each point represents the mean ± S.E.M. The number of experiments was shown in parentheses. Percent inhibition is calculated as $100[(C-D)/C]$, where D is the peak current in the presence of U50, and C is peak current averaged before and after U50 when there was a good recovery. The response was measured during voltage steps which elicited the largest Ca^{2+} current. The solid line represents the fit of the data to the Hill equation.

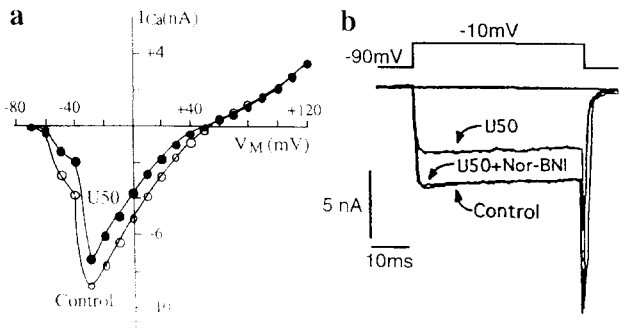

Fig. 2. Inhibition of calcium current in DRG neurons by κ-selective opioid agonist U50. a: U50-inhibited voltage-gated Ca^{2+} currents. Peak current–voltage plots were derived from currents recorded in the absence and presence of 5 μM U50. b: the inhibition on Ca^{2+} current by 5 μM U50 was completely blocked by 5 μM Nor-BNI.

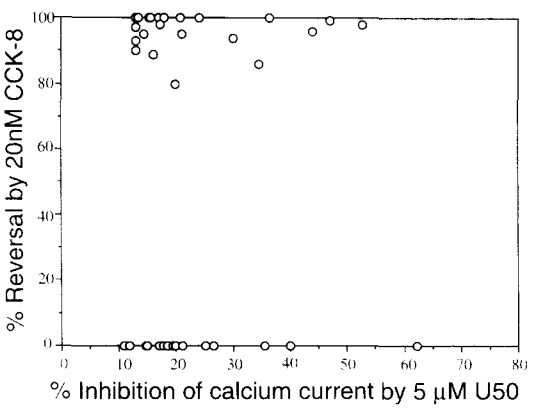

Fig. 4. The relationship between percent inhibition of calcium currents by 5 μM U50 alone and the percent reversal of the effect of 5 μM U50 by 20 nM CCK-8 for all 37 DRG cells where the combination was tested.

ohmefentanyl-induced inhibition by CCK-8 were both bell-shaped. The optimal concentrations of both are 20 nM. Hence we chose 20 nM as the concentration of CCK-8 to test. The results showed (Fig. 3) that the inhibitory effect of U50 was reversed by 20 nM CCK-8, and that this effect of CCK-8 was completely reversed by 1 μM L365,260. Application of 1 μM L365,260 itself had no significant effect. The results suggest that CCK-B receptor is involved in antagonizing κ-opioid-mediated inhibitory effect on Ca^{2+} channel function.

Fig. 4 shows that, out of 37 cells in which U50 (5 μM) exerted an inhibitory effect on Ca^{2+} currents, 22 cells (59%) were sensitive to CCK-8 (20 nM) which antagonized the inhibitory effect of U50, while 15 cells (41%) were not. For the 22 cells sensitive to CCK-8, the inhibition on Ca^{2+} currents by U50 was almost completely reversed, while the inhibition of the other 15 cells was not affected by CCK-8. Ten cells, out of the 22 cells sensitive to CCK-8, were tested with L365,260 following the CCK-8 application. The results showed that all of the CCK effect

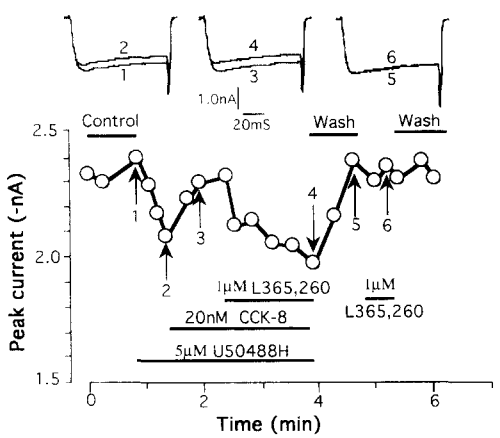

Fig. 3. CCK-8 reverses U50-induced inhibition of calcium current. Ca^{2+} currents (traces) were elicited by steps to −10 mV from −90 mV at times indicated in the respective graphs of peak current versus time. Bars indicate the time of drug application and concentration of drugs.

of the 10 cells were removed by L365,260 (data not shown).

3.3. The effect of CCK-8 itself on calcium current

Application of 20 nM CCK-8 itself produced a reduction rather than an enhancement of the Ca^{2+} current. This effect of CCK-8 was dose-dependent (Fig. 5b) and could be readily reversed by co-application of the CCK-B antagonist L365,260 (Fig. 5a). The inhibition of Ca^{2+} current by CCK-8 was also found to be voltage-dependent (data not shown). This was unexpected, since an anti-opioid peptide would not have been expected to mimic the action of opioids.

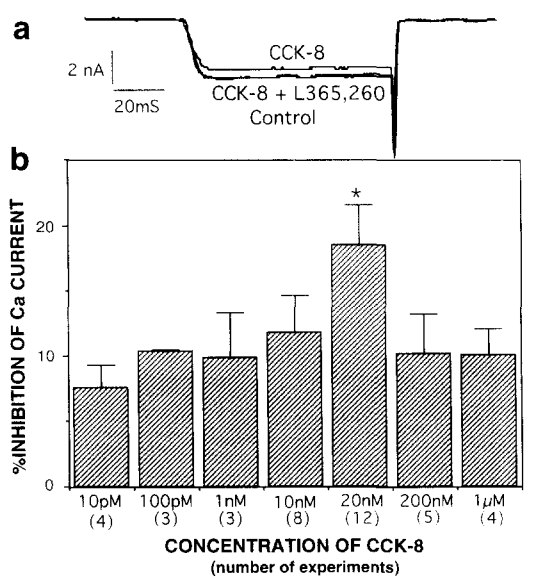

Fig. 5. Dose-dependent inhibition of Ca^{2+} current by CCK-8. a: the inhibition on Ca^{2+} current by 20 nM CCK-8 is blocked by 1 μM L365,260. Ca^{2+} currents were elicited by step from −90 mV to −10 mV. b: histogram showing the dose dependence for the CCK-8-induced inhibition of peak Ca^{2+} currents. Calculation of percent inhibition is the same as in Fig. 1. Each column represents the Mean ± S.E.M. The number of experiments is shown in parentheses.

4. Discussion

All three types of opioid receptors (μ-, δ-, κ-) have been found in DRG neuron [5,27]. While the μ- and δ-receptors are proposed to affect either Ca^{2+} channels or K^+ channels, the κ-receptor acts exclusively on Ca^{2+} channels [19]. Our finding that the inhibitory effect produced by U50 was completely blocked by the κ-selective antagonist Nor-BNI lends support to the view that the effect of U50 on Ca^{2+} current is a result of its interaction with the κ-opioid receptor. That the influence of CCK-8 in antagonizing the effect of U50 could be completely blocked by CCK-B selective antagonist (L365,260) is in line with the finding that only CCK-B but not CCK-A binding sites are present in rat DRG neurons [7]. It is also very similar to the previous study performed in this laboratory [15] that CCK-8 was capable of reversing the inhibition of voltage-dependent calcium current induced by μ-selective opioid agonist ohmefentanyl in the same DRG preparations.

The assumption of opioid–CCK interaction in the present study was made in 37 DRG neurons that had already been shown to respond to κ-opioid U50. However, the group data shown in Fig. 4 reveal that this interaction existed in only 22 out of 37 neurons (59%). This all-or-none distribution may be explained that among DRG neurons equipped with κ-opioid receptors, only 59% of them show a coexistence of CCK receptor.

It is peculiar that CCK-8 antagonized the κ-receptor mediated suppression of Ca^{2+} current in DRG cell, yet CCK-8 itself inhibited the Ca^{2+} current (Fig. 5). However, similar phenomena have been found in ^{45}Ca-uptake experiments where CCK-8 antagonized the inhibitory effect of κ-opioid on ^{45}Ca uptake in rat dorsal horn synaptosomal preparation, yet CCK-8 itself inhibited the ^{45}Ca uptake [25]. This dilemma may have the same underlying mechanism as another equally peculiar finding, namely that CCK-8 antagonizes opioid analgesia, but itself may produce analgesia after intrathecal [14] or subcutaneous [29] injection. These questions may best be addressed once the mechanisms underlying CCK- and opioid-receptor-mediated effects on ion channels are clarified.

Both the κ-opioid-induced and CCK-8-induced inhibition of Ca^{2+} current in rat DRG neurons could be overcome by large membrane depolarizations. A similar voltage-dependent inhibition of Ca^{2+} currents has been observed for μ-opioid-receptor [23], α_2-adrenoreceptor [1] and LHRH [4]. Voltage dependence may not, however, be a property common to all receptor-mediated inhibition of Ca^{2+} current, since voltage-independent inhibition has been reported for both $GABA_B$ [21] and α_2-adrenoreceptor [24]. The results suggest that κ-opioid receptor and CCK-B receptor may share a common mechanism to inhibit Ca^{2+} current in rat DRG neurons.

From a methodological point of view, it is certainly more convenient to test the opioid–CCK-8 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 Ca^{2+} current 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, since the release of neuropeptides occurs in the peripheral as well as at the central terminals [20], and opioid-receptor and CCK-receptor are present on DRG neuron both at their perikarya and central terminals [2,5,7,18,30].

Opioid- and CCK-receptors are predominantly located in the substantia gelatinosa, where the afferent fibers terminate. It is interesting to know whether the opioid/CCK-8 interaction occurs at pre- or post-synaptic sites, or a combination of both. In an in vitro longitudinal slice preparation of the dorsal horn, Dickenson et al. [3] applied glutamate to excite neurons directly via pressure ejection, thereby bypassing the afferent terminals. No functional interaction was found between morphine and CCK, suggesting that the site of action of CCK- and opioid-receptor may be at the presynaptic terminals of the afferent fibers. Our data in the present study from rat DRG neurons, which provide a model for presynaptic opioid- and CCK-receptor interaction, suggest that κ-opioid receptor and CCK-B receptor may co-exist at least in a subpopulation of the DRG neurons. Hence, the interaction between opioid- and CCK-receptor may well be located presynaptically.

It is likely that sensory modality varies among DRG cell bodies of different diameter. Rapidly conducting A_α- and A_β-type DRG neurons have the largest cell bodies, whereas slower conducting A_α- and C-type DRG neurons have smaller cell bodies [11]. Most A_α-type DRG neurons are committed to the transmission of proprioceptive and tactile information, whereas A_δ- and C-type DRG neurons most frequently transmit pain and thermal information [6,28]. Thus, the results obtained from small and medium 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.

Acknowledgements

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