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

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SINCE the characterization of orphanin FQ (OFQ), the endogenous ligand of ORL1 receptor, much work has focused on its physiological functions. OFQ was reported to antagonize the effect of opioid-induced antinociception, although its mechanism remains obscure. In the present study, whole-cell patch clamp recording technique was used to observe if OFQ can reverse the inhibition of calcium current produced by the κ-opioid agonist U50,488H (U50) in acutely dissociated rat DRG neurons. The concentrations of OFQ and U50 were 50 nM and 10 μM, respectively. Among 49 cells recorded, the calcium channel currents of 37 (75.5%) cells were inhibited by U50, of which 30 (81.1%) cells could be reversed by OFQ. It was interesting to note the similarity between OFQ and the well characterized anti-opioid peptide CCK-8 in that it reversed κ-opioid receptor agonist induced suppression on calcium channel current, while by itself showed a calcium channel suppressive effect. Thus OFQ may be regarded as another anti-opioid peptide. *NeuroReport* 9: 2095–2098

Key words: Calcium current; Opioid receptor; Orphanin FQ; U50,488H

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Introduction

Since the cloning of the three major opioid receptors in the early 1990s,[1–3] a novel opioid receptor (ORL1) was recently discovered based on the consensus sequence of the mouse δ-opioid receptor.[4–6] This receptor displays a unique yet overlapping mRNA distribution with the other opioid receptors.[4,5,7] *In situ* hybridization analysis revealed that ORL1 mRNA is densely expressed in the cerebral cortex, thalamus, subfornical organ, habenulae, hypothalamus, central gray, dorsal raphe, locus coeruleus, and also in the dorsal horn of the spinal cord[4] and dorsal root ganglia.[8] ORL1 maybe involved in a wide variety of physiological functions, especially in nociception and its modulation.

It was reported that OFQ could elicit a potent antinoceptive effect at the spinal level.[9–11] Zhu *et al.*[12] demonstrated in behavioral studies that OFQ was able to antagonize opioid analgesia mediated by μ and δ-opioid receptors in the brain and by μ- and κ-opioid receptors in the spinal cord. In the present work we studied the effect of OFQ per se on calcium channel current in acutely dissociated DRG neurons and its influence on the suppressive effect of calcium channel currents induced by the activation of κ-opioid receptors.

Materials and Methods

Cell preparation: Male Wistar rats (200–300 g) were quickly decapitated, and the DRGs were dissected out. The method for preparing DRG neurons was identical to those described previously.[13,14] The DRGs were initially incubated in trypsin type I-S (Sigma, 0.56 mg/ml) and collagenase type IA (Sigma, 1.2 mg/ml) at 37°C for 35 min, then 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 another 10 min. After the incubation, the individual DRG neurons display three different diameter ranges according to Scroggs and Fox:[15] small (18–27 μm), medium (33–37 μm) and large (44–54 μm). Diameter was defined as the average of the distance along the longest and shortest axis of each cell body. Only the small and the medium-sized cells were used for clamping in the present study. The recording was made between 2 and 8 h after plating.

Whole-cell patch clamp: Patch pipettes with resistance of 2–3 MΩ were filled with the following media (in mM): 100 CsCl, 2 TEACl, 5 $MgCl_2$, 40 HEPES, 10 EGTA, 3 Mg^{2+}-ATP (titrated to pH 7.2 with CsOH). The cells were allowed to adhere to a 35 mm

culture dish and perfused slowly with media at room temperature (19–21°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, a control solution containing (in mM) 147.5 TEACl, 5 $BaCl_2$, 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 barium current through calcium channels. Currents were recorded in standard whole-cell patch-clamp mode[16] using an EPC-9 patch-clamp amplifier, filtered at 3 kHz with a 4-pole Bessel filter, digitized (5 kHz), stored and analysed by a personal computer (Macintosh IIci) using the program Pulse+PulseFit (HEKA Electronik). In case of rundown, percentage inhibition induced by chemicals was calculated as 100[(C-D)/C], in which D is the peak current in the presence of chemicals, and C is the peak current averaged before and after chemicals. Only those cells that had a substantial recovery are included in this paper. Test pulses 100 ms in duration were applied every 8–20 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: Six microtubes (200 μm i.d.) were glued together side by side to apply drugs. 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: OFQ (a product of Phoenix, CA, USA) was dissolved in the control solution described above, aliquoted and stored at –20°C. On the day of the experiment the aliquots were diluted in the control solution. U50,488H (Upjohn Company, USA) was stored at 4°C. Unless otherwise noted, all drugs were purchased from Sigma.

Results

U50 reduced Ca^{2+} channel current: Previous work in our laboratory has provided a detailed profile of U50[14] on DRG whole-cell patch clamping, which produced a concentration-dependent inhibition on calcium channel currents. Since we found in the present study that 10 μM U50 was more effective than 5 μM (data not shown), the higher concentration was used for the whole study.

OFQ inhibition of Ca^{2+} channel current: The concentration–effect relationship for OFQ inhibition on calcium channel currents was evaluated by applying increasing concentrations of OFQ to the neurons while evoking Ca^{2+} channel currents with –10 mV step potentials from a holding potential of –90 mV. Figure 1 shows that OFQ inhibited the Ca^{2+} currents in a concentration-dependent manner. A maximal inhibitory effect (28.7 ± 4.3%) was induced by 1 μM OFQ. Further increase of the concentration of OFQ to 5 μM produced no greater effect.

OFQ reversed U50-induced inhibition of Ca^{2+} channels: As we have shown, both U50 and OFQ can inhibit Ca^{2+} channel currents. The maximal effective concentration of U50 was 10 μM and the minimal effective concentration of OFQ was 50 nM. These two concentrations were chosen for studying their interaction. After the Ca^{2+} channel currents were inhibited by U50, U50 and OFQ were co-applied and the inhibitory effect was reversed. Figure 2 shows a representative curve where the peak calcium current (6.86 nA) was suppressed by 10 μM U50,488H to 5.26 nA (23% reduction), which was almost completely reversed by OFQ at 50 nM.

Of 49 neurons where satisfactory clamping was performed and 10 μM U50 was added, 37 cells (76%) showed > 10% suppression of calcium channel currents. Addition of OFQ (50 nM) in the presence of U50 produced a reversal of U50 effect in 30 neurons (81%), referred to as the 'OFQ reversible' group (Fig. 4). The extent of reversal varied from 12% to 100% (Fig. 3), with an average of 56.3 ± 4.7%. In seven neurons, OFQ was totally ineffective in reversing U50 effect: this was referred to as the 'OFQ non-reversible' group.

Comparison of the two groups revealed that U50 was effective in suppressing the calcium current to a similar extent (17.7 ± 1.1% in the reversible group and 17.8 ± 1.3% in the non-reversible group,

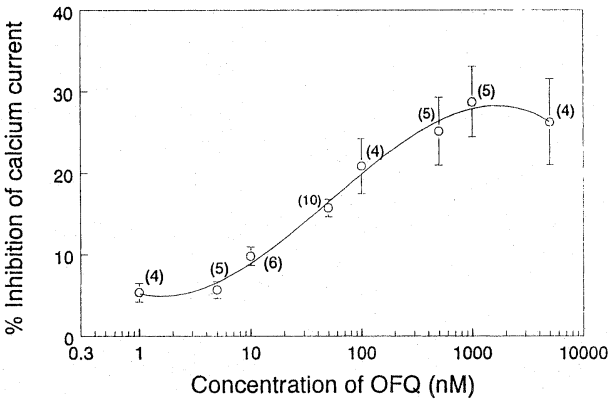

FIG. 1. The effect of OFQ on Ca^{2+} channel currents in DRG neurons. Each point represents the mean ± s.e.m. The number of cells was shown in parentheses, the sigmoidal curve represents a fit through these points by least square.

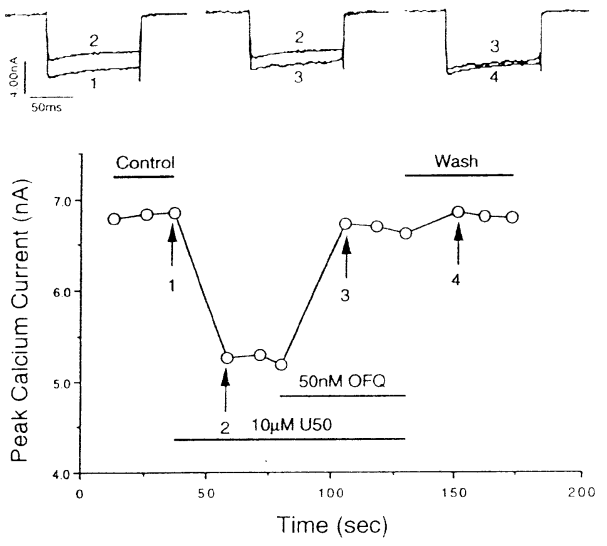

FIG. 2. OFQ reverses U50,488H-induced inhibition of calcium current. Calcium channel currents were elicited by steps to –10 mV from –90 mV at times indicated in the respective graphs of peak current *vs* time. Bars indicate the time of drug application and concentration of the drug was shown above the bar.

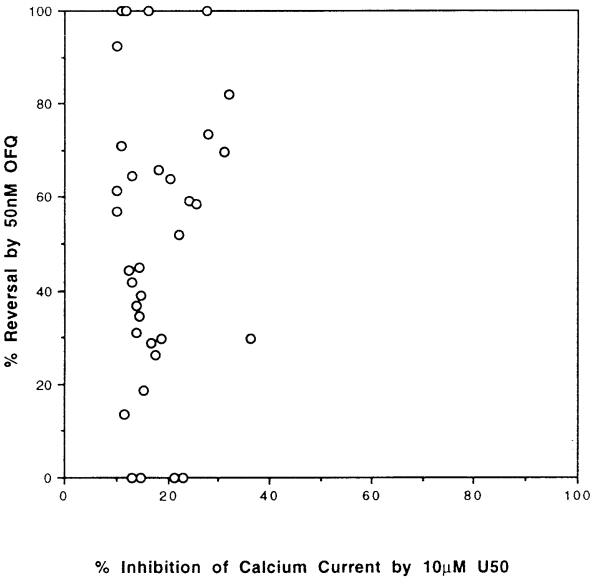

FIG. 3. The relationship between percentage inhibition of calcium currents by 10 μM U50 alone *vs* percentage reversal of the effect of 10 μM U50 by 50 nM OFQ for all 37 DRG neurons where the combination was tested.

respectively). The peak calcium current between the two groups revealed no significant difference.

Discussion

Previous work in our laboratory has focused on the interactions between opioid peptides and anti-opioid peptides at different levels of the neuronal activities. The study at ion channel showed that both opioid

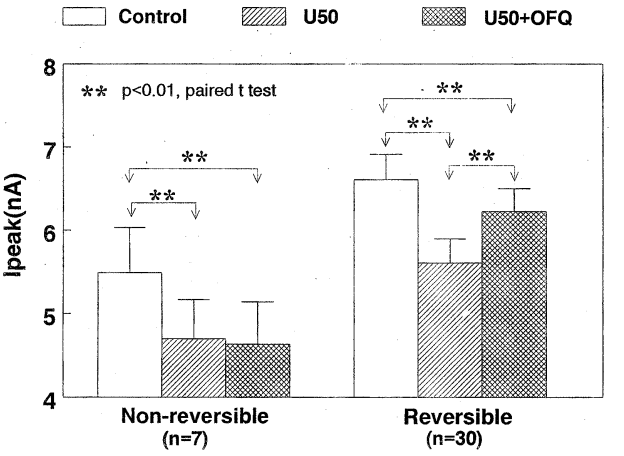

FIG. 4. Suppression of calcium current by the κ-opioid receptor agonist U50,488H (10 μM) and its reversal by OFQ (50 nM). In a total of 37 neurons, 30 were reversed by OFQ and seven were non-reversible. Statistical analysis showed that there was no significant difference between the control currents of the groups. In a poured sample of 37 cells, the grand average showed a statistically significant reversal of U50,488H effect by OFQ (data not shown). Each column represents the mean ± s.e.m. The number of experiments is shown in parentheses.

peptide and CCK-8 can inhibit the voltage gated calcium currents in rat DRG neurons. However, while 1 μM OMF (μ-opioid receptor agonist) and 5 μM U50,48SH produce suppressive effects through their respective receptors, these effects can be reversed by CCK-8 (20 nM)[13] in an all or none manner. Among the cells which were sensitive to μ-opioid receptor agonist, the inhibitory effect of 78% cells could be reversed by CCK-8, and the effect of 59% of the cells which were sensitive to κ-opioid receptor could be reversed by CCK-8. The results indicate the co-existence of opioid receptor and CCK-receptor on these cells.

In the present study, the similar method was used to observe the effect of OFQ *per se* and the interaction between OFQ and U50,488H (κ-opioid agonist) on voltage gated calcium channels in rat DRG neurons. Among 49 neurons, the voltage gated calcium currents of 37 (76%) neurons could be inhibited by U50,488H, of which the effect of 81% (30/37) cells could be blocked by OFQ (50 nM). The results indicate the co-existence of κ-opioid receptor and ORL1 receptor on small and medium sized DRG neurons. Unlike CCK-8, the reversal effect produced by OFQ was not all or none in type.

Figure 3 showed that in most cases, the blocking effect of OFQ was partial rather than complete. It needs to be further investigated whether the variation was due to the difference in the number of the ORL1 receptors on each cell or the difference in the affinity of the receptors.

It should be noted that the effect of OFQ itself on voltage-gated calcium channel was suppressive, similar to the opioid effect. It has been demonstrated

that OFQ can inhibit calcium channel in the SH-SY5Y cell line[17] and in acutely isolated hippocampus neurons.[18,19] How can we, then, explain the blocking effect of OFQ on opioid effects by U50? First, the result did not support the possibility of OFQ being a partial agonist, i.e., agonist at lower concentration and antagonist at higher concentration. In our experiment, however, the concentration of OFQ was the same (50 nM) and it inhibited the Ca^{2+} current when it was used alone, but reversed opioid effect when it was used in combination with opioids. Theoretically, the interaction can occur at pre-synaptic, synaptic and post-synaptic level. First, a pre-synaptic interaction, i.e., antagonism occurs at chemical level, rather than in biological systems, could be excluded. Because the reversal effect could be recorded in only part of the DRG neurons, if it is a chemical reaction, it should be detected in all cells. Second, as to the interaction at synaptic level, it is known that OFQ shows no affinity with κ-opioid receptor,[20] hence, the reversal effect of OFQ could not be produced through κ-opioid receptor. Third, it has been reported that opioid receptors[21] and the ORL1 receptors[18] are coupled to G-protein to produce their effects. The existence of minor differences in receptor G-protein coupling may cause competitive antagonism between U50 and OFQ at G-protein level. Certainly, it needs to be further studied. Our preliminary data indicate that OFQ could reverse the inhibitory effect induced by its highly selective μ-opioid receptor agonist ohmefentanyl.

The notion that OFQ can block the suppressive effect of calcium current induced by U50 seems difficult to reconcile with the results obtained from some experiments at behavioral level. Mogil *et al.*[22] reported that i.c.v injection of OFQ can block the analgesic effect mediated by μ, δ, and κ-opioid receptors. Grisel *et al.*[23] further demonstrated that OFQ shows anti-opioid action in the brain but not in the spinal cord. The results at behavioral level may not definitely correspond with the results at channel level, as there are much more complicated factors determining their effects *in vivo*. However, Zhu *et al.*[12] reported that OFQ could antagonize opioid analgesia mediated by μ- and δ-opioid receptors in the brain and by μ- and κ- but not δ-opioid receptors in the spinal cord, which is the same as our results at the calcium channel level. In the present study we used the DRG neurons, its afferent fiber terminates at spinal cord and contacts with dorsal raphe neurons, thus functionally DRG neurons should belong to the spinal cord at least partially. The discrepancy made us consider that there may exist two opposite mechanisms in the synapses at spinal level.

Conclusions

The results of the present study demonstrate that OFQ can block the inhibitory effect produced by κ-opioid receptor agonist at calcium channel level of rat DRG neurons which is similar to the profile of another anti-opioid peptide CCK-8 first reported from this laboratory. The similarity and difference between the well characterized antiopioid peptide CCK-8 and the OFQ as a new candidate of anti-opioid family deserve further explanation.

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