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Electrophysiological Responses of δ-Opioid Receptor Expressed on HEK293 Cells

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=ABSTRACT=

To explore electrophysiological properties of the δ-opioid receptors artificially expressed in the mammalian cell, effect of an opioid agonist DPDPE (1 μM) on the voltage-sensitive outward currents was examined in the HEK293 (human embryonic kidney) cells transfected with δ-opioid receptor cDNA cloned from NG-108-15 (neuroblastoma × glioma hybrid) cDNA library. Also studied were effects of 8-bromo-cyclic AMP and naloxone on DPDPE-induced changes in the voltage sensitive outward current. The voltage sensitive outward currents were recorded using perforated patch technique at room temperature. In the non-transformed HEK293 cells, DPDPE did not alter voltage sensitive outward current, indicating that no native δ-opioid receptor had been developed. However, 1 μM DPDPE remarkably increased the voltage sensitive outward current in the transformed HEK293 cells. The increment in voltage sensitive outward current peaked in 7~10 minutes after DPDPE application, and the maximum DPDPE-activated outward current (313.1±12.3 pA) was recorded when the membrane potential was depolarized to +70mV. Following pretreatment of the transformed HEK293 cells with 1 mM 8-bromo-cyclic AMP, DPDPE failed to increase the voltage sensitive outward currents. On the other hand, naloxone completely abolished DPDPE-activated voltage sensitive outward current in the transformed HEK293 cells. The results of present study suggest that in the transformed HEK293 cells an activation of the δ-opioid receptors by an opioid agonist DPDPE increases the voltage-sensitive potassium current as a result of decrement in cyclic AMP level.

Key Words: δ-opioid receptor, HEK293 cell, NG108-15 cell

INTRODUCTION

Since the opioid receptor of the animal brain was first verified in 1973, several subtypes including μ- and δ-opioid receptors have been found in a variety of animal tissues (Pert, 1973; Simon et al., 1973; Terenius, 1973). Opioids affect the functioning of the central nervous system by interacting with membrane receptors. Activation of opioid receptor has been shown to inhibit spontaneous firing of action potential and release of neurotransmitter in various brain areas (Nicoll et al., 1977; Duggan, 1984). In contrast, an increment of spontaneous firing rate was observed in the hippocampus and spinal cord (Nicoll et al., 1977).

Stimulation of μ- and δ-opioid receptors is known

to cause hyperpolarization of neuronal membrane by an increase in an inwardly rectifying K^+ conductance (Morita, 1982; Cherubini, 1985). The coupling of the opioid receptor to the inwardly rectifying K^+ channel appears to be mediated by G-proteins.

Recently, amino acid sequences of various kind of opioid receptors have been cloned. A typical G-protein coupled receptor, δ-opioid receptor is composed of 372 amino acids and shows 7 transmembrane domains (Strader et al., 1994). In 1992 Kieffer et al. and Evans et al. isolated cDNA of δ-opioid receptor from cDNA library of NG-108-15 (neuroblastoma × glioma hybrid) cell and succeeded in expressing receptor on the COS cell.

Also, δ-opioid receptor was expressed in HEK293 (human embryonic kidney) cell. However, electrophysiological properties of this expressed δ-opioid receptors have yet not been thoroughly investigated.

In the present study we examined electrophysiological responses of the expressed δ-opioid receptor to an opioid agonist DPDPE to determine whether these δ-opioid receptors expressed on HEK293 cell are functionally coupled to any effector system. The δ-opioid receptor cDNA used for expression was isolated from cDNA library of NG-108-15 cell and confirmed to be identical with cDNA of Kieffer and Evans (Bzdega et al., 1993).

MATERIALS AND METHOD

Expression of δ-opioid receptor and culture of HEK293 cells

Compementary DNA of δ-opioid receptor was isolated from cDNA library of neuroblastoma × glioma NG108-15. The HEK293 cells were plated onto coverslips and transfected with pRC/CMV containing the δ-opioid cDNA. To select the permanently transformed cells, HEK293 cells were kept in culture in DMEM media supplemented with neomycin for more than 2 weeks.

Electrophysiology

Macroscopic currents were recorded under perforated whole cell patch clamp. The electrode were pulled from borosilicated glass capillary and fire polished to a 3~5 MΩ resistance without any further treatment. The composition of the internal solution used in all the experiments was 125mM KCl, 2mM $MgCl_2$, 1mM $CaCl_2$, 11mM EGTA, 10mM HEPES and pH 7.2. For the perforation of cell membrane, the tips of electrodes were filled with internal solution containing 500 μg/ml nystatin and 500 μg/ml pluronic. The composition of the external solution was 140mM NaCl, 5.4mM KCl, 2mM $CaCl_2$, 2mM $MgCl_2$, 10mM HEPES, 10 mM glucose and pH 7.4. To record and analyze the membrane currents, Axoclamp-2A and pClamp software were used (both from Axon Instruments).

Chemicals

DPDPE ([D-Pen2,5]enkephalin) and 8-bromo-cyclic AMP were from RBI. Naloxone-HCl and all other chemicals were from Sigma.

RESULTS

Effects of DPDPE on the non transformed HEK 293 cells

To determine whether the non-transformed HEK 293 cell has any native δ-opioid receptor, the effects of δ-opioid receptor specific agonist DPDPE (1 μM) on the voltage sensitive outward current was observed. The membrane potential of the cell was held at - 70 mV and depolarized in step to - 80 ~ +70 mV. Outward current started to appear when the cell was depolarized in steps from holding potential (- 70 mV) to - 50 mV. Along with increment of depolarization the amplitude of outward current was increased. This outward current shows the outward rectification. Fig. 1 and 2 show that 1 μM DPDPE has an effect on membrane currents of the non-trans-

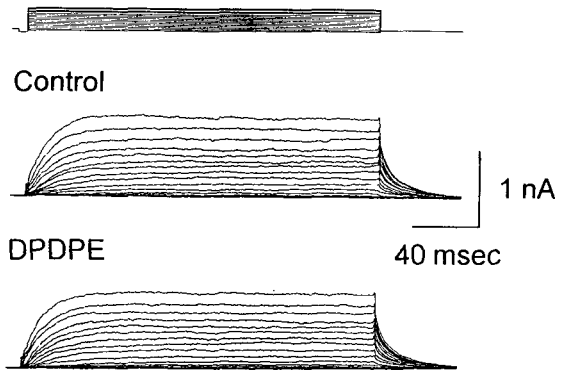

Fig. 1. Effect of DPDPE (1 μM) on the voltage-sensitive outward (VSO) current in the non-transformed HEK293 cells.

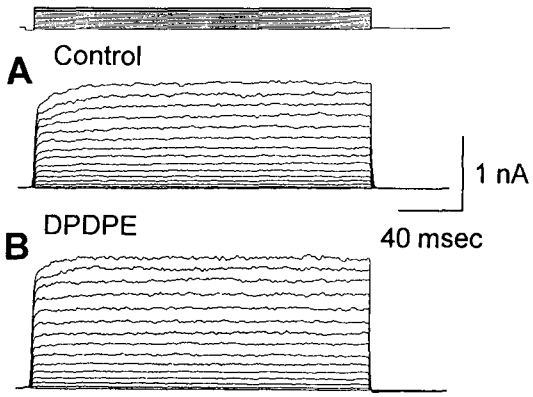

Fig. 3. Effect of DPDPE (1 μM) on the voltage-sensitive outward current in the HEK293 cell transfected with δ -opioid receptor cDNA.

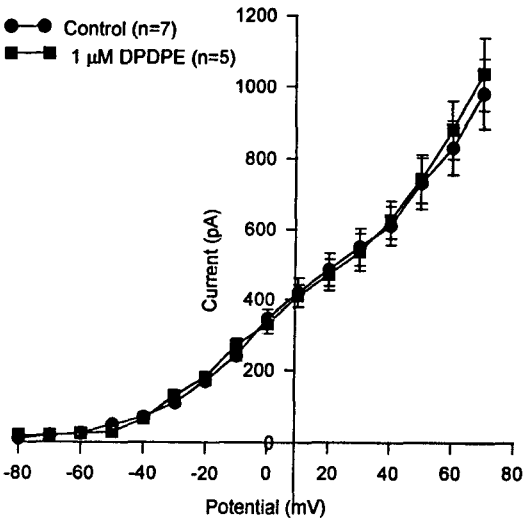

Fig. 2. Current-voltage relations of the voltage-sensitive outward current before and after application of DPDPE (1 μM) in the non-transfmed HEK293 cells.

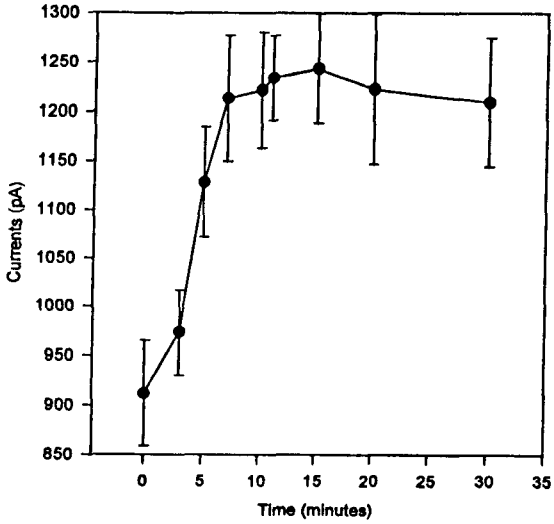

Fig. 4. Time course of change in the DPDPE-activated voltage-sensitive outward current in the HEK293 cell transformed with δ-opioid receptor cDNA.

formed HEK293 cells.

Effects of DPDPE on the transformed HEK293 cells

Fig. 3 shows the effect of DPDPE on the transformed HEK293 cell. As with the non-transformed cells an outward current was also recorded when the transformed HEK293 cells were depolarized in steps. In the transformed HEK293 cells, 1 μM remarkably increased this voltage-sensitive outward current (Fig. 3B). The DPDPE-induced increment in voltage sensitive outward current peaked (313.1 ±

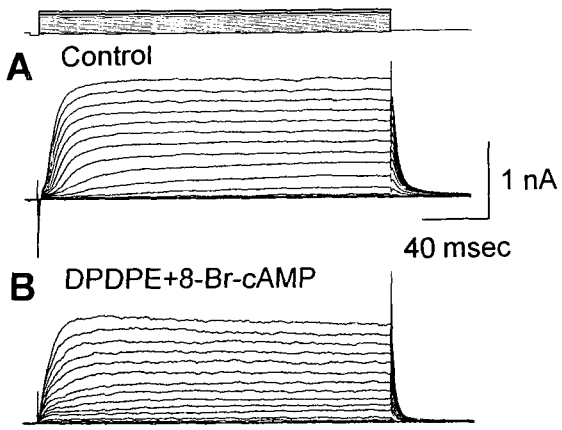

Fig. 5. Inhibition of the opioid agonist-activated outward current by 8-bromo-cAMP in the HEK293 cells transfected with δ-opioid receptor cDNA.

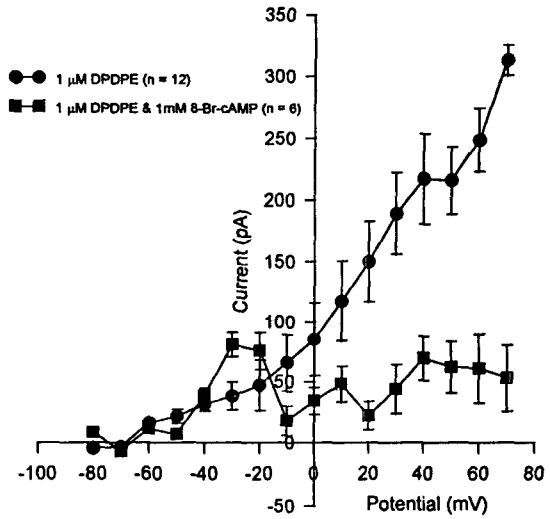

Fig. 6. Current-voltage curves of the DPDPE-activated voltage-sensitive outward current before and after application of 8-Bromo cAMP (1 mM) in the transformed HEK293 cells.

12.3 pA) in 7～10 minutes after the agonist application (Fig. 4).

Effect of 8-bromo-cyclic AMP on the δ-opioid agonist activated outward current

8-Bromo-cyclic AMP is a cAMP analogue and

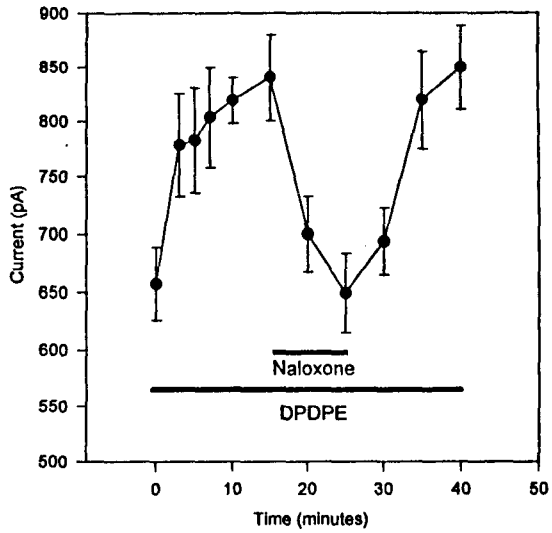

Fig. 7. Effect of naloxone (500 nM) on the opioid agonist-activated outward current in the HREK293 cell transfected with δ-opioid receptor cDNA.

permeable to cell membrane. In the cell, cAMP is hydrolyzed by cyclic nucleotide phosphodiesterase, but 8-bromo-cyclic AMP is not disintegrated by the phosphodiesterase. To determine the effect of 8-bromo-cyclic AMP on the change of voltage sensitive outward current induced by δ-opioid agonist DPDPE, transformed HEK293 cell was treated with 8-bromo-cyclic AMP before the application of DPDPE. Fig. 5 shows that 8-bromo-cyclic AMP abolishes the effect of DPDPE on voltage dependent outward current. Difference between the voltage sensitive outward currents recorded after and before the application of DPDPE represents DPDPE-induced net outward current that is, DPDPE-activated voltage sensitive outward current. Fig. 6 shows I-V curves of the DPDPE- activated voltage sensitive outward current and the effect on this current of 8-bromo-cyclic AMP. 8-Bromo-cyclic AMP abolished the DPDPE-activated net outward current.

Effect of naloxone on the δ-opioid agonist-activated outward current

To determine the effect of naloxone on δ-opioid receptor expressed on the membrane of HEK293 cells, change of DPDPE induced outward current was observed after adding 500 μM naloxone to the perfusate. When the DPDPE-activated voltage sensitive outward current reached the maximal value, naloxone was added to the perfusate. Fig. 7 shows that DPDPE-activated outward current was almost completely abolished by naloxone and recovered after removal of naloxone from the perfusate.

DISCUSSION

HEK293 cell used in the present study is a cell line derived from the human embryoic kidney cell and permanently transformed. In the cell membrane of HEK293 adenylate cyclase is activated by VIP (vasoactive intestinal polypeptide) but not by glucagon or secretin (Simmons, 1990). In the experiment to determine whether δ-opioid receptor is naturally expressed on the non-transformed HEK 293 cell, DPDPE did not increase the voltage dependent outward current. This result indicates that no native δ-opioid receptor was expressed on HEK293 cell.

Neurotransmitters are known to modulate the excitability of neurons by affecting ion chennels, K^+ channel being one of the primary targets of such modulation. In fact, many receptors of neurotransmitters have been shown to couple to the potassium condutance in neurons. In locus ceruleus opioid and α_2 adrenergic receptors activate the same type of K^+ channel through the identical signal transduction pathway. μ-opioid agonists were reported to hyperpolarize arcuate neuron of the hypothalamus as a result of the activation of inwardly rectifying K^+ channels (Loose, 1990). Recent cloning technique of opioid receptor provided an opportunity to examine the molecular mechanism of signal transduction pathway of opioid receptor. In the experiment in which μ-opioid receptor and the G protein-coupled K^+ channel isolated from the heart atrial cells were coexpressed in Xenopus oocyte, μ-opioid was found to be functionally coupled to the expressed G-protein-coupled K^+ channel (Chen, 1994). This channel showed the properties of inwardly rectifying K^+ channel. Fan et al. (1992) and Wimpey et al. (1992) have shown that stimulation of μ- or δ-opioid receptors activate the voltage dependent K^+ channels. Therefore it is very likely that the voltage sensitive outward current recorded from HEK293 cell is the potassium current. In this experiment a binding assay was performed to confirm faithful expression of δ-opioid on mammalian cell. Result of binding assay (result is not shown here) indicated the success of expression but the physiological and pharmacological properties of expressed receptors remains to be determined. In present experiment, δ -opioid receptor agonist DPDPE (1 μM) increased the voltage sensitive outward current in transformed HEK293 cells.

It is well known that μ- and δ-opioid receptor is functionally coupled to G proteins and adenylate cyclase (DeVries et al., 1990; McKenzie, 1990; Van Vliet et al., 1990). But the kind of G-protein to be coupled to the opioid receptor has not been fully clarified. However, the δ-opioid receptors expressed on neuroblastoma cell such as NG108-15, NS20Y and N1E115 are coupled to G_{i2}, G_{i3} and G_o (Prather et al., 1994). Many aspect of relationship between the opioid receptors and the activity of ionic channels have been uncovered. Generally stimulation of μ- or δ-opioid receptor deactivates the adenylate cyclase via the action of G-proteins and consequently decreases cAMP level in cytosol and the activity of PKA (cyclic AMP dependent protein kinase A) as well. The dephosphorylation of voltage dependent K^+ channels due to a decreased activity of PKA is known to increase the potassium current through the membrane. It is also known that stimulation of μ-opioid receptor increases the

activity of calcium dependent phosphodiesterase and promotes the destruction of cAMP (Law Loh, 1993). The opioid receptors on the neurons of locus ceruleus of the rat and submucosal plexus of guinea pig activate the inwardly rectifying K^+ channel but not the activity of PKA or PKC. These previously reported experimental results strongly suggest that the membrane delimited pathway exists between receptors and ionic channels (Wimpey, 1992).

In this experiment DPDPE-activated voltage sensitive outward current peaked in 7 to 10 min in the transformed HEK293 cells, indicating that the activation of ionic channel induced by δ-opioid receptor was mediated by a slow signal transduction pathway.

8-bromo-cyclic AMP, non-hydrolysable cyclic AMP analogue, is freely permeable to cell membrane. In the present experiment, pretreatment of transformed HEK 293 cell with 8-bromo-cyclic AMP abolished the DPDPE-activated voltage sensitive outward current. Such results agree with those reported by others experiments. Evans et al. (1992) has demonstrated that the δ-opioid receptors expressed on COS cell decreased the concentration of cytosolic cyclic AMP.

In the present study, the DPDPE-activated voltage sensitive outward current was suppressed following application of naloxone.

The results of present study suggest that in the transformed HEK293 cells an activation of the δ -opioid receptors by an opioid agonist DPDPE increases the voltage-sensitive potassium current as a result of decrement in cyclic AMP level.

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