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REVIEW ARTICLE

Recent developments in the molecular biology of pain

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ABSTRACT Research in understanding the molecular basis of pain has accelerated in the last twenty years. Beside the description of the 'chemical soup' and their receptors in chronic pain, the cloning and role of the TRP (Transient Receptor Potential) channels was also discovered, making the synthesis of new agonists and antagonists possible. However the organism itself is able to antagonize pain by activating endogenous opioid and cannabinoid receptors. New pathways in pain therapy might evolve by exploring these molecular mechanisms further.
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KEY WORDS

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Acute pain is useful for the organism in case of danger; chronic pain however can be harmful to the body. Therefore the former is also called physiologic, because it is basically a protective behaviour, whereas the latter is named pathologic, or in most cases neuropathic pain, characterized by a long-lasting time component usually after injuries or inflammations. The anatomy and physiology of pain were studied in detail in the last two centuries, however the thorough research of the molecular biology of sensory nerves and neurons started only in the twentieth century.

First the so called pain mediators were established, among them biogenic amines (histamine, serotonin), amino acids (glutamate), peptides (substance P, bradykinin, calcitonin gene related peptide (CGRP), prostaglandins, interleukins, cytokines, chemokines, adenine nucleotides. Later their metabolism and the respective receptor were investigated (Fig. 1). The role of ions and the molecular structure of voltage-gated ion channels was also an important step in pain physiology and pathology. Meanwhile the metabolism and the specific receptors of the antinociceptive endogenous substances like the opioid peptides and the anandamides, were also discovered.

Why is it so important to search the molecular background of pain? Because till now only symptomatic drugs existed to treat pain, perhaps the molecular background could help to find a more effective therapy.

Nonspecific ion channels

According the external stimuli pain can be divided into mechanical, chemical and thermal nociception. Mechanical pain caused by pressure or injuries is supposed to be transmitted by the mechanotransduction channels as Asic (acid-sensitive ionic channels) 1,2 and 3, but TRPA1, TRPV2 and TRPV4 as modulators could be also included in this distribution. Voltage-gated potassium, sodium and calcium channels play an important role in the transmission of acute mechanical pain. Chemical noxious nociception is also transferred by the TRP channels (Venkatachalam et al. 2007): TRPV1 (vanillin, capsaicin, resiniferatoxin sensitive), TRPV4 (phorbolesters act as agonists), TRPA1 (isothiocyanate, mustard-oil, icilin, acrolein, etc.),TRPM8 (menthol reactive).Thermal nociception is expressed by the heat sensitive TRPV1, TRPV2, TRPV3 and TRPV4 channels, while cold is transduced by TRPM8 and TRPA1 receptors (Caterina et al.1997 and 2006; Julius et al. 2001; Gunthorpe et al. 2002; Clapham, 2003; Nagy et al. 2004; Szolcsányi et al. 2006; Abrahamsen et al. 2008; Basbaum et al. 2009).Voltage-gated sodium and potassium channels contribute with the TRP and ASIC channels to temperature- and mechanotransduction of pain (Wemme et al. 2006; Zimmermann et al. 2007; Chalfie 2009).

TRP-s are non-selective cation sensitive channels, showing the highest selectivity for calcium permeability. As TRP channels play an important role in all kinds of pain and their structure was investigated only since the 1990s, it seems worth while to take a look at their molecular construction. They contain six transmembrane regions, a pore-loop domain, an intracellular carboxyl and amino termini which assemble as tetramers to form cation-permeable domains. Their amino acid content varies from 761 till 1120. In their N-terminal sequence they display various numbers of ankyrin rings (4-14) except for TRPM8. They are supposed to play a role in trafficking and the formation of homo- or heteromers. The first cloned TRP channel was TRPV1 by Caterina et al. in 1997 (Fig. 2).

Sensitization of TRP channels may give rise to allodynia, hyperalgesia, inflammation and pain. The endogenous mediators of these effects could be prostaglandins, adenosine, ATP, serotonin, bradykinin, CGRP, NGF (Nerve growth factor), Substance P (see also second paragraph), but enzymes as

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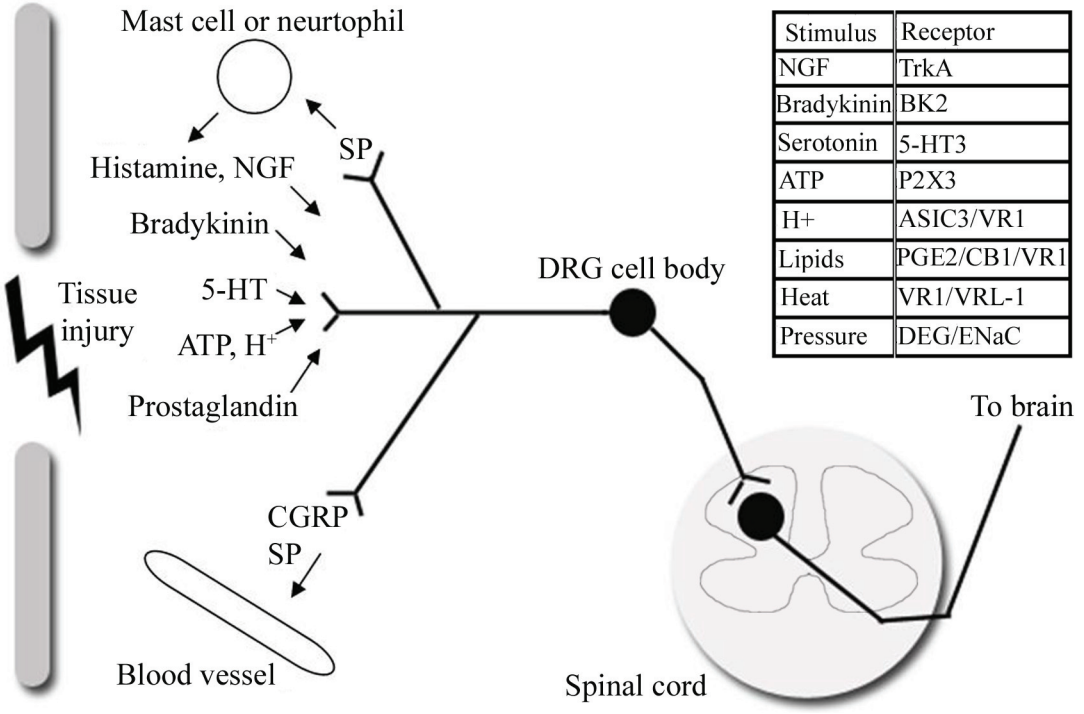

Figure 1. Inflammatory mediators released at tissue injury, adapted from Julius and Basbaum (2001).

protein kinase A and C, and phospholipase C are activators of TRPV too (Oláh et al. 2002; Varga et al. 2006).

Desensitization of the TRP channels occurs when long-lasting action of noxious stimuli persists. A typical example is the desensitization of TRPV1 channels with capsaicin or resiniferatoxin (Jancsó et al. 1977; Szolcsányi et al. 1990). External application of some of these agonists was already used in pain therapy in the form of plasters on the skin. A lot of TRPV1 inhibitors are in clinical testing phase too, but side effects as for example high fever in some of them, render their introduction in therapy difficult. Centrally acting inhibitors achieve a maximal action (Patapoutian et al. 2009). Blocking of $Ca2^{+}$ penetration in most TRPV channels is also a possibility for pain reduction. Calmodulin is able to link to the C-terminal and to the first ankyrin ring amino acids, decreasing in such manner $Ca2^{+}$ actions on TRPV1.

The application of molecular, genetic (in situ hybridization, knockout animals) and immunological (immunohistochemical and fluorescent) and cell culture methods facilitated the localization of TRP channels and other pain related receptors in the central and peripheral nervous system. The detailed application of these methods were able to prove the presence of TRPV1 and other pain receptors not only in the dorsal root ganglion, where they were localized first owing to their high concentrations, but also in the CNS, skin and somatic organs as well (Mezey et al. 2000). These methods enabled the localization of various receptors not only in the different organs, but also within the sensory neurons, nerve endings or glial cells. The liberated pain mediators from immune cells, mast cells, platelets and macrophages act as an 'inflammatory soup' on their specific receptors (see also Fig. 1). Therefore it is not easy to inhibit some or all of them, without damaging their physiological role (Patapoutian et al. 2009).

Opioid peptides, cannabinoids and receptors

The living organism defends itself with endogenous compounds against pain. Such are the opioid peptides (endorphins, enkephalins, endomorphins, dynorphins, nociceptin) and the endogenous cannabinoids (anandamide, N-arachidonoyl-dopamine and 2-arachidonylglycerol).

Opioid peptides act by binding to mu-, delta- and kappa-opioid receptors. Endomorphin-1 and -2 are mu-specific, met- and leu-enkephalins are mu- and delta specific,-beta-endorphin is also similarly mu- and delta specific, dynorphin (1-19) is kappa-opiate receptor specific (for reviews see Wollemann 1990; Kieffer 1995). The opiate receptors activate inhibitory guanine nucleotide binding proteins (G_i), their effect results in blocking adenylate cyclase and protein kinase A. The inhibition of proteinkinase A or C results in a decrease of TRPV1 effect, therefore pain is diminished (Vetter et al. 2006). The reverse reaction, *i.e.* inhibition of

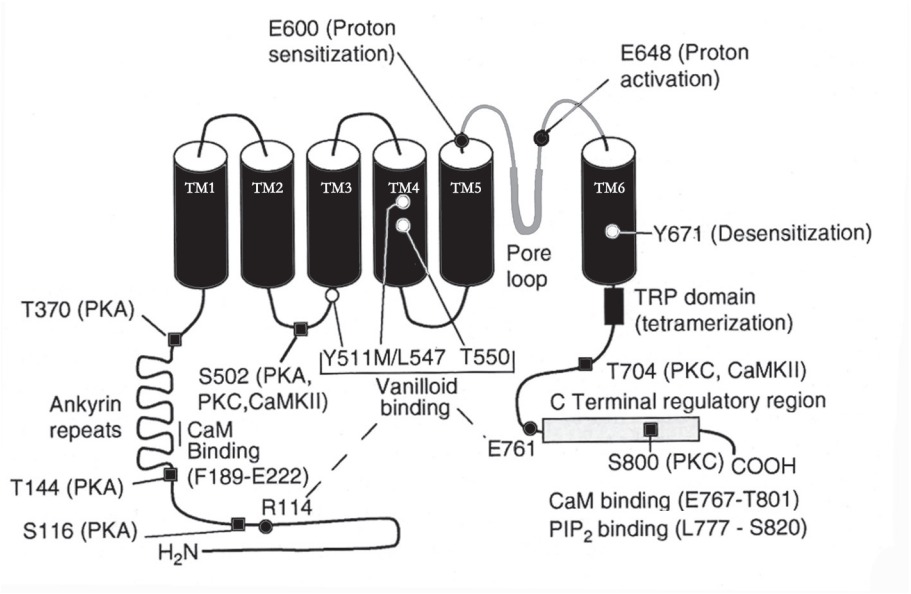

Figure 2. Topological model of TRPV1 domains. PKA: proteinkinase A, CaM: calmodulin ,CAMKI, CAMKII: calmodulin kinase I and II, PIP2: phosphatidylinositol-diphosphate ,PKC: proteinkinase C, adapted from Caterina and Park (2006).

opiate receptor mu and kappa binding by capsaicin occurs also *in vitro* and was suspended by capsazepine, but it is not a direct action on mu-opiod receptors, because in CHO cells stably expressing mu opioid receptors and lacking TRPV1, the inhibition of capsaicin was not present. The effect was exerted most probably through the activation of protein kinases (Wollemann et al. 2008). In addition opiate receptors act through G_i on ion channels by increasing K+ and decreasing $Ca2^+$ conductance, which brings about the reduction of membrane permeability.

The amino acid structure of opioid receptors was discovered in the 1990s using cloning methods (Kieffer et al.1992). They contain an extracellular N-terminal and an intracellular C-terminal. Between them seven transmembrane regions are located, which include the most hydrophobic amino acids with the highest homology among the three opioid receptor types (Chen et al. 1993). The overall homology is around 60% (Fig. 3.). The third transmembrane region is most important in drug binding, the third intracellular region between the fifth and sixth transmembrane regions is the binding site of G_i and G_o proteins. The phosphorylations of serines and threonines by protein kinase C on the C-terminal and on the third intracellular parts result in desensitization. The arginines in the N-terminal region serve as potential glycosylation sites. The cysteines in the opioid receptors can be palmitoylated which might serve as a connection to the membrane (Chen et al. 1998). The strongest pain inhibitory action is exerted across the mu receptors. The antinociceptive action of endogenous opioid peptides however disappears rapidly due to brain peptidase enzymes.

A Janus-faced opiate receptor like protein (OPRL) was the latest discovered among the opiate receptors (Meunier et al.1995, Reinscheid et al.1995). Its structure and peptide affinity resembles somewhat to the kappa-opioid receptor. The specific ligand nociceptin causes in low concentration analgesia, but in higher amounts pronociceptive actions occurred. The antinociceptive action is localizable in the spinal cord, whereas the hyperalgesic effect is occurring supraspinally (Meunier 1997, Meunier et al.2000, Reinscheid et al. 1998, Reinscheid et al. 2000).

One of the oldest pain killing methods used first in ancient China was opium smoking, which had a central painkilling and euphoric effect. The active principle of the smoke is the different opiate alkaloids in the poppy-head. Opioid alkaloids were extracted from the latex of poppy heads later in the XIXth century, among them the most important painkiller was morphine, a phenanthrene alkaloid. The advantage of drugs acting on mu-opiate receptor is, that they act not only at the peripheral nervous system, but they also stop the consciousness of pain in the central nervous system. A lot of synthetic antinociceptive opioid compounds are now used in therapy, but they all have more or less serious side-effects during long application like tolerance, habituation, addiction and withdrawal symptoms. After chronic opioid therapy withdrawal induces also hyperalgesia and activation of glial cells. Glial cells, mainly astrocytes and microglia, are activated also during neuropathic pain. They are able to reverse the effect of opioids contributing to opioid tolerance, dependence and withdrawal (Watkins et al.2005, 2007). The selective agonists of the kappa-opioid receptor (benzeneacetamidine, benzomorphan, cyclazocine) induce dysphoria and hypothermia. They are therefore not used as painkillers; nevertheless the

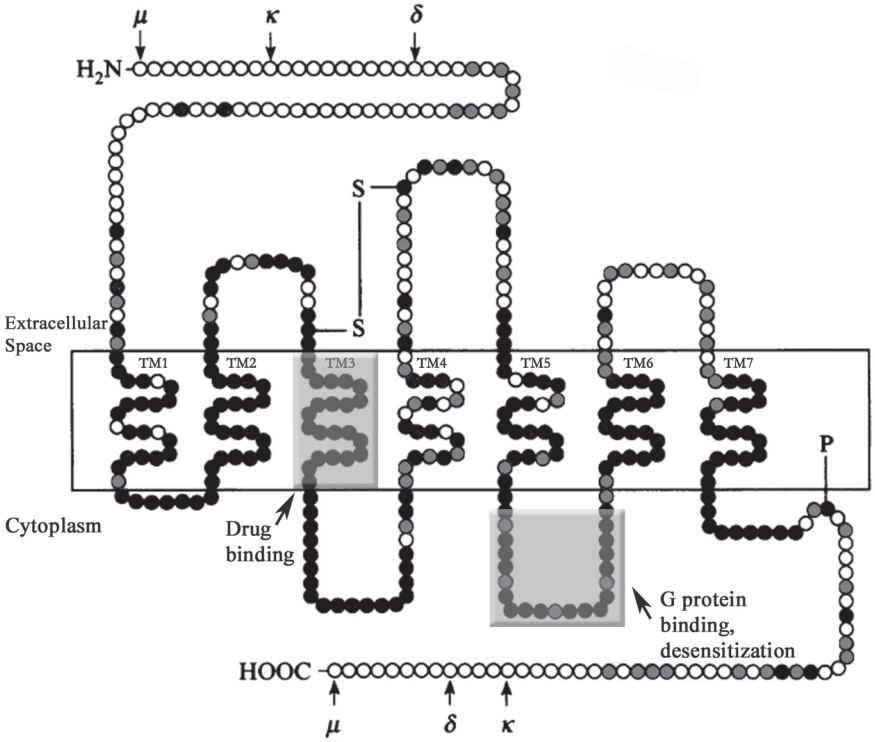

Figure 3. Protein structure and similarity distribution among the three opioid receptors. Adapted from Chen et al. (1993). The N- and C-termini of each opioid receptor are marked by arrows. P: palmitoylation site. Black shading: identical among all three receptors, grey shading: identical in two out of three receptors, o: unique for each receptor.

thermic effect could be related with the more efficient use of kappa ligands against pain caused by burning. These effects on temperature regulation are partly antagonized by naloxone, some of them are naloxone resistant (Martin 1984). It is known, that for the painkiller activity of delta opioid agonists the presence of mu-opioid receptors is also essential. They form probable heteromers with the delta-opioid receptor to be more effective. According to Scherrer et al. (2009) delta opioid receptors are responsible for antinociception of mechanical and mu-opioid receptors of heat pain-killing in the dorsal root ganglion.

The synthesis of the endogenous cannabinod anandamide is catalysed by the enzymes N-acyltransferase and phospholipase D in the presence of Ca2+ (Liu et al. 2008). The metabolism is achieved by a fatty acid amide hydrolase (FAAH), resulting in arachidonic acid and ethanolamide (Cravatt et al. 1996). The inhibition of FAAH activity increases the effect of anandamide (Jhaveri et al. 2006). 2-arachidonylglycerol is metabolized by monoacylglycerol lipase (MAGL). Genetic disruption of this enzyme causes elevation of MAGL in the brain which can lead to desensitization of cannabinoid receptors (Lichtman et al. 2010). Anandamides are formed from the neuron membrane and they are not stored in synaptic vesicles, but act directly on cannabis receptors. The cannabis receptors are also coupled to $G_{i/o}$ proteins like the opioid receptors. They have two types, CB1 which is present in the central nervous system and CB2 which occurs in the periphery and acts mainly through the immune system. The final cannabis action is perfected by the closing of Ca2+ ion channels and the opening of K+ ion channels of the cells. The physiological analgetic, sedative, and addictive actions of the endogenous cannabinoids are somewhat similar, but less strong than those of the opiates, however the amount of CB receptors in the brain is higher than those of opioid receptors (Christie 2006, Maldonado et al. 2006). Anandamide in higher concentrations is also able to activate and desensitize TRPV1 (Di Marzo et al. 2002, Akopian et al. 2009). Another endogenously acting agonist is NADA (N-arachydonoyl-dopamine), which is cited together with anadamide as endogenously occurring ligands of 'ionotropic cannabinoid receptors' (i.e.TRPV-s) (Akopian et al.2009). Cannabinoid alkaloids are present in the plant Indian hemp *Cannabis sativa.* Among phytocannabinoids (about 80) the most effective is delta9-tetrahydro-cannabinol. The plant is desiccated and pulverized, finally smoked in the form of cigarettes. Their introduction as analgetics is employed in carcinomatous patients, affective and neurodegenerative diseases (Izzo et al. 2009).

Conclusions

In conclusion one can admit, that pain is a very complicated category, not only from the physiological (acute) and pathological (chronic), but also from the molecular aspect. Based on this dual concept not alone should the physiological mechanims be solved, but the pathological one, being far more intricate and even much more distant to clear up. The new concept of genomics and proteomics could be extended quite well on pain as painomics (painomix?). Nevertheless the scientific effort put in this important symptom for mankind should go further until it is solved.

Beside physical pain, psychical suffering might also be a tormenting factor of the pain syndrome, but even less molecular events are known compared with the somatic symptoms, yet a disorder in the metabolism of brain neurotransmitters (serotonin, dopamine) are supposed to be a cause or consequence of it.

Among the greatest writers, Dante Alighieri characterized this sort of pain in his Divina Commedia with the following lines: 'Nessun maggior dolore che ricordarsi del tempo felice nella miseria' (There is no greater pain to remember a happy time when one is in misery) Inferno, Canto V. linea 121-123.

References

Abrahamsen B, Zhao J, Asante CO,Cendan CM, Marsh S, Martinez-Barbera JP, Nassar MA, Dickenson AH, Wood JN (2008) The cell and molecular basis of mechanical, cold, and inflammatory pain. Science 321:702-705.

Akopian AN, Ruparel NB, Jeske NA, Patwardhan A Hargreaves KM (2009) Role of ionotropic cannabinoid receptors in peripheral antinociception and antihyperalgesia. Trends in Pharmacol Sci 30:79-84.

Basbaum AI, Bautista DM, Scherrer G, Julius D (2009) Cellular and molecular mechanism of pain. Cell 139:267-284.

Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D (1997) The capsaicin receptor: A heat activated ion channel in the pain pathway. Nature 389:816-824.

Caterina MJ, Park,U (2006) A polymodal sensor in the nociceptor terminal. Current Topics in Membranes 57:113-150.

Chalfie M (2009) Neurosensory mechanotransduction. Nat Rev Mol Cell Biol 10:44-52.

Chen Y, Mestek A, Liu J, Yu L (1993) Molecular cloning of a rat kappa opioid receptor reveals sequence similarities to the mu and delta opioid receptors. Biochem J. 295:625-628.

Chen C, Shahabi V, Xu W, Liu-Chen LY (1998) Palmitoylation of the rat mu opioid receptor. FEBS Letters 441:148-152.

Christie MJ (2006) Opioid and cannabinoid receptors: friends with benefits or just close friends? Br J Pharmacol. 148:385-388.

Clapham DE (2003) TRP channels as cellular sensors. Nature 426:517-524.

Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB (1996) Molecular characterization of an enzyme that degrade neuromodulatory fatty-acid amines. Nature 384:83-87.

Di Marzo V, Blumberg PM, Szallasi A (2002) Endovanilloid signaling pain. Curr Op in Neurobiol.12:372-379.

Gunthorpe MJ, Benham CD, Randal A, Davis JB (2002) The diversity in the vanilloid (TRPV) receptor family of ion channels. Trends Pharm Sci. 23:183-191.

Izzo AA, Borrelli F, Capasso R, Di Marzo V, Mechoulam R (2009) Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb. Trends Pharm Sci 30:515-527.

Jancsó G, Király E, Jancsó-Gábor A (1977) Pharmacologically induced selective degeneration of chemosensitive primary sensory neurons. Nature 270:741-743.

Jhaveri MD, Richardson D, Kendall DA, Barrett DA, Chapman V (2006) Analgesic effects of fatty acid amide hydrolase inhibition in a rat model of neuropathic pain. J Neurosci 26:13318-13322.

Julius D, Basbaum AI (2001) Molecular mechanisms of nociception. Nature 413:203-210.

Kieffer BL, Befort K, Gaveriaux-Ruff C, Hirth CG (1992) The delta-opioid receptor isolation of a cDNA by expression cloning and pharmacological characterization. Proc Natl Acad Sci USA 89:12048-12052.

Kieffer BL (1995) Recent advances in molecular recognition and signal transduction of active peptides: receptors for opioid peptides. Cell Mol Neurobiol.15:615-635.

Lichtman AH, Blankman JL, Cravatt BF. (2010) Endocannabinoid overload. Mol Pharmacol. 78:993-995.

Liu J, Wang L, Harvey-White J, Huang BX, Kim HY, Luquet S, Palmiter RD, Krystal G, Rai R, Mahadevan A, Razdan RK, Kunos G (2008) Multiple pathways involved in the biosynthesis of anandamide. Neuropharmacology 54:1-7.

Maldonado R, Valverde O, Berrendero F (2006) Involvement of the endocannabinoid system in drug addiction. Trends in Neurosci 29:225-239.

Martin WR (1984) Pharmacology of opioids. Pharmacol Rev. 35:283-323.

Meunier JC, Mollereau C, Toll L, Suaudeau C, Moisand C, Alvinerie P, Butour JL, Guillemot JC, Ferrara P, Monsarrat B, Mazarguil H, Vasart G, Parmentier M, Costentin J (1995) Isolation and structure of the endogenous agonist nociceptin. Nature 377:532-535.

Meunier JC (1997) Nociceptin/orphanin FQ and the opioid receptor-like ORL1 receptor. Eur J Pharmacol. 340:1-15. Meunier JC, Mouledos I, Topham CM (2000) The nociceptin (ORL 1) receptor molecular cloning and functional architecture. Peptides 21:893-900.

Mezey E, Tóth ZE, Cortright DN, Arzubi MK, Krause JE, Elde R, Guo A, Blumberg PM, Szállási A (2000) Distribution of mRNA for vanilloid receptor subtype 1 (VR1) and VR1-like immunoreactivity, in the central nervous system of the rat and human. Proc Nat Acad Sci USA 97:3655-3660.

Nagy I, Sántha P, Jancsó G, Urbán L (2004) The role of the vanilloid (capsaicin) receptor (TPRV1) in physiology and pathology. Eur J Pharmacol. 500:351-369.

Oláh Z, Karai L, Iadarola MJ (2002) Protein kinase Calpha is required for vanilloid receptor 1 activation. Evidence for the multiple signaling pathways. J Biol Chem. 277:35752-35759.

Patapoutian A, Tate S, Woolf CJ (2009) Transient receptor potential channels: targeting pain at the source. Nat Rev.Drug Discov. 8:55-68.

Reinscheid RK, Nothacker HP, Bourson A, Ardati A, Henningsen RA, Bunzow JR, Grandy DK, Langen H, Monsma FJ, Civelli O (1995) Orphanin FQ: A neuropeptide that activates opioidlike G protein-coupled receptors. Science 270:792-794.

Reinscheid RK, Nothacker HP, Civelli O (2000) The orphan FQ/nociceptin gene structure, tissue distribution of expression and functional implications obtained from knockout mice. Peptides 21:901-906.

Scherrer G, Imamachi N, Cao YK, Contet C, Mennicken F, O`Donnel D, Kieffer BL, Basbaum AI (2009) Dissociation of the opioid receptor mechanisms that control mechanical and heat pain. Cell 137:1148-1159.

Szolcsányi J, Szállási A, Szállási Z, Joó F, Blumberg PM (1990) Resiniferatoxin: an ultrapotent selective modulator of capsaicin-sensitive primary afferent neurons. J Pharmacol Exp Ther. 255:923-928.

Szolcsányi J, Pethő G (2006) History of ion channels in the pain sensory system. Current Topics in Membranes 57:21-72.

Varga A, Bölcskei K, Szőke É, Almási R, Czéh G, Szolcsányi J, Pethő G (2006) Relative roles of protein kinase A and protein kinase C in modulation of transient receptor potential vanilloid type 1 receptor responsiveness in rat sensory neurons in vitro and peripheral nociceptors in vivo .Neuroscience 140:645-657.

Venkatachalam K, Montell C (2007) TRP Channels. Ann Rev Biochem 76:387-417.

Vetter I, Wyse BD, Montheit GR, Roberts-Thompson SJ, Cabot PJ (2006) The mu opioid agonist morphine modulates potentiation of capsaicin-evoked TRPV1 responses through a cyclic AMP-dependent protein kinase A pathway. Mol Pain 2:22,1-16

Watkins LR, Hutchinson MR, Johnston IN, Maier SF (2005) Glia: novel counter-regulators of opioid analgesia. Trends Neurosci 28:661-669.

Watkins LR, Hutchinson MR, Milligan ED, Maier SF (2007) „Listening" and „talking" to neurons: Implication of immune activation for pain control and increasing the efficacy of opioids. Brain Res Rev 56:148-169.

Wemme JA, Price MP, Welsh MJ (2006) Acid-sensing ion channels: advances, questions and therapeutic opportunities.Trends Neurosci 29:578-586.

Wollemann M (1990) Recent developments in the research of opioid receptor subtype molecular characterization J.Neurochem 54:1095-1101.

Wollemann M. (2008) Capsaicin inhibits the in vitro binding of peptides for mu- and kappa-opioid and nociceptin receptors. Brain Res Bull. 77:136-142.

Zimmermann K, Leffler A, Babes A,Cendan CM, Carr RW, Kobayashi J, Nau C, Wood JN, Reeh PW (2007) Sensory neuron sodium channel Nav1.8 is essential for pain at low temperatures. Nature 447:855-858.