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Deltorphins - selective δ-opioid receptor agonists

Deltorfiny – selektywni agoniści receptora δ-opioidowego

Discovery of opioid receptors (μ, κ and δ) and endogenous opioid peptides such as enkephalins, endorphins and dynorphins provoked to search for new opioids. Therefore, Erspamer and co-workers focused research on amphibian peptides based on the knowledge that amphibian skin contains a wide variety of opioid peptides [28].

The first peptide family of amphibian opiates was discovered in 1981 and named dermorphins. Eight years later, deltorphins were isolated from the skin of the Amazonian frogs *Phyllomedusa sauvagei* and *Phyllomedusa bicolor*. They were discovered by cloning of the cDNA from the skin of frogs [16]. At first these peptides were known as a "dermorphin gene associated peptide" (Tyr-D-Met-Phe-His-Leu-Met-Asp-NH_2), then as dermenkephalin (a dermal-opioid enkephalin) and finally in 1989 the term deltorphin was introduced (a δ–receptor-preferring peptide). Shortly afterwards, two additional δ-opioid peptides were discovered, Tyr-D-Ala-Phe-Asp-Val-Val-Gly-NH_2 and its Glu4 analogue, [D-Ala$_2$]-deltorphins I and II, which had even greater bioactivity. To simplify the literature, the name deltorphin A was substituted instead of dermorphin gene-associated peptide, deltorphin or dermenkephalin, while deltorphin B and deltorphin C were proposed for [D-Ala$_2$]deltorphins II and I, respectively [17]. Deltorphin B is known to be a δ_2-opioid receptor agonist and deltorphin C as a δ_1 agonist.

DELTORPHINS AND REWARD

It is well known that activation of opioid receptors modify the release of dopamine in the nucleus accumbens and this mechanism plays an important role in the drug reward. Stimulation of δ-opioid receptors by deltorphin II specifically potentiates D1-mediated responses arising from stimulation of δ-opioid receptors and D1 receptors by deltorphin-induced release of dopamine [19]. Some research also suggests dopamine-independent and opioid receptor-independent mechanisms of this phenomenon [26]. This mechanism suggests that δ-opioid receptors and deltorphins may play an important role as modulators in the conditioned reward induced by drugs. Considering the unique properties of deltorphins, numerous investigations took advantage of their δ-opioid receptor selectivity to explore animal behavior. The conditioned place preference test was used to measure the impact of deltorphins on the rewarding effects of drugs. It was recognized that the δ-selective opiate antagonist naltrindole [20] and nonselective naltrexone [2] attenuated the place preference in cocaine conditioned rats and that naltrindole blocked the effects of cocaine. Furthermore, deltorphin II completely generalized the discriminative stimulus effect of cocaine and this data suggest that the rewarding action of deltorphin II may occur through a link with the dopaminergic system [34].These observations suggested a role of δ-opioid receptors in modulation of cocaine addiction.

Observations on the effect of δ antagonist on morphine–induced place preference in μ-receptor deficient mice and wild type mice revealed the complex interactions among opioid receptors. In wild type mice, morphine induced place preference involving both δ- and μ-receptors; however, in the μ-receptor deficient mice a δ-opiate antagonist eliminated morphine-induced place preference while deltorphin II induced this effect [33]. Furthermore, stimulation of δ-opioid receptors enhanced ethanol-induced place preference. Thus the activation of δ-opioid receptors may also play a role in the rewarding mechanism of ethanol [1, 22].

An explanation for the place preference observed in mice in response to delta agonists, may lie in the partial activation of the μ-opioid receptors by δ agonists [13], in modulating dopamine release or functional interactions between μ and δ receptors.

Other examples supporting such a functional interaction between μ and δ receptors include the enhancement place preference when morphine is co-administered with sub-effective doses of delta agonist and the ability of delta opioid receptor antagonist to block the development of tolerance to the antinociceptive responses of μ-opioid agonists [10].

DELTORPHINS AND PAIN

The analgesic efficiency of opioid drugs has been known for over 6000 years and they are still the main medications for treatment of moderate and severe pain. Most research focuses on supra-spinal δ-opioid receptors to explain their role in modulating pain transmission. In mice, deltorphin II at a moderate dose showed time-related antinociceptive effect after intracerebroventricular (i.c.v) administration which was antagonized by δ-opioid receptor antagonist – naltrindole [15].

All deltorphins have negligible κ-opioid receptor affinity, and this fact excludes an involvement of κ-opioid receptors in antinociceptive or locomotor effects of these peptides. The analysis of correlation between activity of deltorphins and their affinities for μ- and δ-opioid receptors showed that the antinociceptive potency of these peptides increases with their μ-affinity. However, these peptides have antinociceptive potency higher than would have been expected from their μ-affinity. Interestingly, δ-mediated analgesia was significantly decreased in μ-deficient mice and unaltered in the absence of κ receptors or in mice lacking endogenous opioids [12].

The published data suggest that the δ-agonists play predominantly a modulatory role in nociception rather than a primary role. However, other findings showed that the intensity of δ-opiate analgesia depends on coactivation of μ-opiate receptors by endogenous or exogenous opiates [27].

It has been indicated that short-term morphine pretreatment can increase δ-opioid receptor mediated antinociception by promoting the translocation of δ-opioid receptors from cytoplasm to the cell surface. Injection of deltorphin after chronic morphine pretreatment produced a mild antinociception and caused a decrease in locomotor activity of rats. These observations indicate that chronic morphine administration has influence on δ-opioid receptor-mediated effects. However, activation of these receptors does not appear to compensate for the decrease in antinociception caused by morphine tolerance [24].

DELTORPHINS AND LOCOMOTION

All the deltorphin analogs had a dose-dependent, biphasic effect on locomotor activity: low doses mostly induce stimulation, larger doses induce initial suppression, followed by hyperactivity. The initial depressive phase is accompanied by antinociception and catalepsy. It was observed that locomotor activity arose when catalepsy had faded and analgesia begun to decrease. Injections of the

highly selective δ-opioid receptors agonist, deltorphins, into the rat brain ventricles, ventral tegmental area, and nucleus accumbens invariably increase locomotor activity and induce stereotyped behavior [29].

Locomotor stimulation was antagonized by the δ-opioid receptor antagonist, naltrindole, thereby confirming intervention of the δ-opioid system. This stimulation was also antagonized by high doses of naloxone but was unaffected by the μ_1-selective antagonist, naloxonazine [18].

It has been indicated that the degree of early hypolocomotion induced by deltorphins depends upon the greater or lower dominance of the inhibitory μ_2-opioid receptor system over the excitatory δ-opioid receptor system.

DELTORPHINS AND TEMPERATURE

Endogenous opioids are involved in body temperature regulation in mammals. In rodents, systemic and central injections of opiates and opioid peptides produce profound body temperature changes [7], presumably through activation of opioid receptors within the preoptic area of the anterior hypothalamus, the primary temperature control center in mammals. Three opioid receptors are localized within the preoptic area of the interior hypothalamus [21]. This suggests that all three receptors (μ, δ and κ) may mediate temperature effects of opiates and opioid peptides [9].

The selective δ-opioid receptor agonist deltorphin II produced biphasic effects on core temperature in rats, in which hypothermia was followed by hyperthermia. The treatment with the selective δ-opioid receptor antagonist naltrindole blocked the hypothermia, whereas the hyperthermia was unaffected, or slightly potentiated. The non-selective opioid receptor antagonist naloxone acts contrariwise. These effects are in agreement with a negative interaction between δ-opioid receptors and an additional opioid receptor. In support of a specific role of μ-opioid receptors in such effects, the selective μ-opioid receptor antagonist β-funaltrexamine potentiated hypothermia and blocked hyperthermia of deltorphin II. Altogether, these effects strongly support the notion of a functional antagonism between δ- and μ-opioid receptors underlying the biphasic temperature effects of deltorphin II [17].

The κ-opioid receptors are not likely relevant for this deltorphin effect, as activation of this opioid receptor results in hypothermia [4] and hypothermia produced by deltorphin II is related to the activation of δ-opioid receptors.

POTENTIAL CLINICAL AND THERAPEUTIC APPLICATIONS

There is considerable interest in developing agonists for clinical uses towards opioid receptors other than μ-opioid receptors. This is because undesirable effects, including respiratory depression and physical dependence, are associated with the use of μ-opioid receptors agonists [32]. Deltorphins are interesting in this context, as animal experiments have supported beneficial properties, like stimulatory effects on respiration, analgesia and minimal abuse liability, of this compounds [17]. Generally, such effects of deltorphin analogs are attributed to the activation of δ-opioid receptors. Therefore the δ-opioid receptor agonists as nonaddicting analgesic drugs may offer an excellent means to counteract acute or chronic pain [25] while circumventing the known effects on gastrointestinal function or respiratory depression associated with treatment by alkaloid opiates.

Deltorphins produce antidepressant-like effects in the forced swim test mediated by the δ-opioid receptor and they also increase brain-derived neurotrophic factor (BDNF) mRNA expression, especially in hippocampus. In addition, BDNF is a growth factor whose expression is increased in

response to brain damage. Therefore, the ability of deltorphins to increase BDNF mRNA expression suggests that these compounds could be important in treating depression, but also in treating patients suffering stroke, head trauma, or possibly other neurodegenerative disorders [36]. They might also find a unique clinical application in additionally attenuating the physical dependence to morphine-, cocaine-, and alcohol abuse [11].

Since it is known that δ agonists play a role in modulating growth hormone during periods of insulin-induced stress, deltorphins may have significant relevance in treating diabetic patients. The combination with other antidiabetic drugs might function synergistically to combat this disease with minimal side effects and to lower the risk associated with current therapeutic regimes [17]. In human subjects, deltorphin inhibits the secretion of growth hormone and ACTH induced by insulin-induced hypoglycemia and modulates the secretion of luteinizing hormone in women [6].

Although deltorphins have a relatively high degree of stability and solubility in biological fluids, time release mechanisms, such as through the use of biologically erodable microspheres [22] would offer distinct therapeutic advantages. Deltorphins conjugated to highly lipophilic triglycerides [30] or glycosylated [35] may improve their transit across the blood-brain barrier to produce analgesia.

OTHER PROPERTIES OF DELTORPHINS

In addition to the antinociceptive and addictive properties of μ-selective alkaloid opiates that cause major and well known side effects, namely respiratory depression and suppression of gastrointestinal motility, deltorphin I at low doses stimulated respiratory activity in fetal lambs [5].

It is interesting that deltorphin II injected i.c.v. or intrathecally inhibits antidiarrheal and colonic antipropulsive effects of opiates [3]. Furthermore, deltorphin II inhibits acidified alcohol-induced gastric mucosal lesions after subcutaneous administration [8], but using the i.c.v. route, it failed to affect gastric secretion [14]. This peptide is also involved in the control of ingestive behavior. It stimulated the intake of food and sucrose [31] and when administered in conjunction with angiotensin II it increased water consumption in rats [37].

CONCLUSIONS

Deltorphins are the most powerful and selective natural, exogenous δ-opioid peptides known to date. Deltorphins activate δ-opioid receptors but also μ-opioid receptors, or the μ/δ-opioid receptor complex. They also enhance the release of dopamine in the mesolimbic dopamine system; therefore they induce the rewarding effect and/or potentiate the rewarding effects of drugs. A recent study also demonstrates that δ-opioid receptors and dopamine D1 receptors are co-localized in individual neurons of the striatum of rats. This opens the possibility for an intracellular mechanism, by which dopamine D1 receptors activation could modulate delta opioid receptor function. The discovery of the amphibian opiate peptides provided likewise models for novel analgesics with enhanced therapeutical benefits and reduced toxicity, and potent antidepressant actions, which could also attenuate the physical dependence to morphine, cocaine and alcohol abuse. Therefore, deltorphins are a promising target for the development of novel treatment for several disorders, including depression.

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SUMMARY

Deltorphins are peptides isolated from the skin of the Amazonian frogs. Although deltorphins were isolated above 20 years ago they are still the most potent and selective δ-opioid receptor agonists available today. They enhance the release of dopamine in the mesolimbic dopamine system and they are involved in reward effects of drugs. The activity of deltorphins also contains dose-dependent stereotyped pattern of locomotor activity, antinociceptive effect, impact on body temperature and production of antidepressant-like effects in the forced swim test in rats. Therefore, deltorphins are a promising target for the development of novel treatment for several disorders, including depression.

STRESZCZENIE

Deltorfiny są peptydami wyizolowanymi ze skóry amazońskich żab. Chociaż zostały wyizolowane ponad 20 lat temu, są one nadal najsilniejszymi i najbardziej selektywnymi dostępnymi agonistami receptora δ-opioidowego. Potęgują uwalnianie dopaminy w dopaminowym układzie mezolimbicznym i są zaangażowane w nagradzające efekty wielu nadużywanych substancji. Aktywność deltorfin obejmuje również zależny od dawki stereotypowy wzór aktywności lokomotorycznej, efekt antynocyceptywny, wpływ na temperaturę ciała i działanie antydepresyjne w teście wymuszonego pływania u szczurów. Zatem deltorfiny są obiecującym celem w rozwoju terapii różnych chorób, włączając depresję.