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The NOP receptor involvement in both withdrawal- and CCk-8-induced contracture responses of guinea pig isolated ileum after acute activation of κ-opioid receptor

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ABSTRACT

In isolated guinea-pig ileum (GPI), the κ-opioid acute withdrawal response is under the control of several neuronal signaling systems, including the μ-opioid, the A_1-adenosine and the CB_1 receptors, which are involved in the inhibitory control of the κ-withdrawal response. After κ-opioid system stimulation, indirect activation of μ-opioid, A_1-adenosine and CB_1 systems is prevented by the peptide cholecystokinin-8 (CCk-8). In the present study, we have investigated whether the NOP system is also involved in the regulation of the acute κ-withdrawal response. Interestingly, we found that in GPI preparation, the NOP system is not indirectly activated by the κ-opioid receptor stimulation, but instead this system is able by itself to directly regulate the acute κ-withdrawal response. Specifically, our results clearly highlight first the existence of an endogenous tone of the NOP system in GPI, and second that it behaves as a functional anti-opioid system. We also found that, the NOP receptor system is involved in the regulation of the CCk-8-induced contracture intensity, only when in the presence of the κ-opioid receptor stimulation. This effect seems to be regulated by an activation threshold mechanism. In conclusion, the NOP system could act as neuromodulatory system, whose action is strictly related to the modulation of both excitatory and inhibitory neurotransmitters released in GPI enteric nervous system.

1. Introduction

Opium is one of the oldest herbal medicines, that has being used as an analgesic, a sedative and anti-diarrheal drug for thousands of years [24]. Independently of their plant, mammalian or synthetic origin, opioids are neuroactive substances, their actions being mediated by the principal μ-, κ- and δ-opioid receptors [35].

The κ-opioid receptor is widely expressed in the central nervous system and peripheral tissues such as the gastrointestinal tract, where they are confined to the myenteric plexus [23,60]. Substantial evidence has shown that activation of the κ-opioid receptor by agonists and endogenous opioid peptides *in vivo* may produce a strong analgesic effect that is free from the abuse potential and the adverse side effects of the μ-opioid receptor agonists, such as morphine [70]. In addition, activation of the κ-opioid receptor has also been shown to exert an inverse effect on morphine-induced adverse actions, such as tolerance, reward, and impairment of learning and memory. Therefore, the κ-opioid receptor has received much attention in the effort to develop alternative analgesics to μ-opioid receptor agonists and agents for the treatment of drug addiction. However, the activation of the κ-opioid receptor also produces several undesirable side effects such as dysphoria, water diuresis, salivation, emesis, and sedation in non-human primates, which may limit the clinical utility of κ-opioid receptor agonists for pain and drug abuse treatment [4,70,71].

The κ-opioid receptor is a G protein coupled receptor (GPCR) and its stimulation leads to inhibition of adenylyl cyclase [1,31,51], regulation of calcium currents acting mainly on the inhibition of N-type calcium channel and regulation of neurotransmitters release [61]. Furthermore, the κ-opioid receptor agonists have been shown to stimulate inwardly rectifying potassium channels that contribute to the maintenance of the resting potential of neurons [20,36].

In 1994, a novel member of the opioid receptor family was cloned from mice and human and later also from a multitude of other species. The nociceptin receptor (NOP), also known as opioid

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receptor like-1 (ORL-1), shares considerable sequence homology (>60%) with the traditional opioid receptors [43]. Transcripts of the NOP receptor are widely distributed in the central and peripheral nervous systems and the presence of NOP receptors in the gastrointestinal tract was demonstrated by molecular methods and by immunohistochemistry [5,42,49].

The NOP receptor stimulation shows the same pharmacological characteristics that are seen following activation or inhibition of μ, δ and κ-opioid receptors. Similar to opioid receptor activation, stimulation of NOP receptors leads to the inhibition of adenylyl cyclase [6,52], activation of inwardly rectifying potassium channels [39] and inhibition of voltage-activated calcium currents [11,27].

Orphanin FQ (OFQ/N), also known as nociceptin, was suggested to be the endogenous agonist of the NOP receptor [6], and together with the endogenous opioid peptides enkephalins, endorphins and dynorphins, shares a neuromudulatory effect in both the central and peripheral nervous systems as well as in the enteric nervous system [8,9,13,50,56,57,65]. OFQ/N structurally resembles dynorphin A, both of them being heptadecapeptides, and sharing six homologous amino acids. Sequence homologies are observed at the level of the receptors as well as among the precursor polypeptides assign OFQ/N as a member of the opioid peptides. Despite these similarities, opioid ligands are usually unable to activate the native or recombinant NOP receptors, and with few exceptions, the NOP receptor has no common ligands with opioid receptors [43].

The mechanisms underlying the acute opioid withdrawal response in GPI have been explored using different experimental approaches. The isolated GPI is the most versatile and highly employed preparation for the study of both opioid dependence and tolerance mechanisms [40,41,45,64]. In particular, studies on the acute κ-opioid withdrawal responses have been carried out in GPI isolated preparations, which exhibit a typical withdrawal response (dependence) when challenged with opioid receptor antagonists (*e.g.* naloxone), after a brief exposure (5 min) to the κ-opioid receptor agonists [38,45,64]. Generally, in GPI, the intensity of the naloxone (NL)-withdrawal response is controlled by complex interactions among different neuronal systems (mainly inhibitory systems) that can be indirectly activated by the opioid receptor stimulation [40]. Basically, the stimulation of the opioid system is able to promote both an inter-systemic (μ and κ-opioid receptors) and an intra-systemic (A_1-adenosine and CB_1 receptors) indirect functional relationship that, when operating, results in an inhibitory control of the NL-withdrawal response [10,45,53,54,64].

Summarizing, stimulation of the μ-opioid system with selective μ-agonists indirectly activates the κ-opioid system (inter-systemic interaction), the A_1-adenosine and the CB_1 systems (intra-systemic interaction), which in turn inhibit the μ-opioid withdrawal response. Conversely, the stimulation of the κ-opioid system with selective κ-agonists induces the same functional relationships among neuronal systems: an inter-systemic interaction with the μ-opioid receptor and an intra-systemic interaction with both A_1-adenosine and CB_1 receptors [40,41,45,53,54,64]. Moreover, the withdrawal responses in GPI preparations briefly exposed to opioid agonists shows strong self blockade, a characteristic sign of tolerance development. Consequently, GPI is also a suitable tissue for the study of opioid tolerance mechanisms [2,7,14].

In this context, an important well known anti-opioid peptide, named cholecystokinin octapeptide sulphate (CCk-8), has been extensively investigated in GPI isolated preparations [55,63]. In GPI, both CCk_A and CCk_B receptors are expressed and the peptide shows some pharmacological properties when administered to the tissue. CCk-8 is able to induce a contractile response consisting of two components: a rapid phasic response, which appears to be preferentially mediated by CCk_B receptors [15], and a slower tonic response, which appears to be mediated by CCk_A receptors [12,15]. Both responses are neuronally mediated; the phasic response depends almost exclusively on the release of acetylcholine (ACh) from cholinergic neurons, while the tonic response apparently also depends on the release of substance P [12,37]. Finally, the opioid system pre-stimulation is able to induce a strong inhibition of the CCk-8 contractile response in GPI [55,63].

Interestingly, the evidence that naïve GPI tissue briefly exposed to CCk-8 contracts after the addition of naloxone, shows that this peptide indirectly activates the GPI opioid system(s) [18,63]. Furthermore, CCk-8 prevents the progression of tolerance to both μ- and κ-withdrawal responses, interfering with both intra- and inter-systemic indirect activations mediated by the opioid system stimulation [41,55,63]. In GPI, the role played by the NOP receptor in both κ-opioid withdrawal as well as CCk-8 contracture responses has been poorly understood. In the present study, we have investigated whether the NOP receptors are involved in the control of κ-opioid withdrawal responses obtained in the absence or in the presence of CCk-8. Finally, we have investigated whether the NOP receptors are also involved in the control of the CCk-8-induced response obtained in the presence of κ-opioid receptor pre-stimulation.

2. Material and methods

2.1. Animal and tissue preparation

The experimental procedure has been described previously [41]. Male guinea-pigs weighing 300–400 g (Harlan, Italy) were housed in groups of four per cage with food and water available *ad libitum*, in a room with controlled temperature ($22 \pm 1\,^{\circ}C$) and under an artificial 12-h light/12-h dark cycle for at least 4 days before use. Animals were handled according to guidelines published in the European Communities Council directives (86/609/EEC), the Italian National Regulations (D.L. 116/92) and the Declaration of Helsinki and Tokyo. The experimental procedure has been approved by the local University Ethic Committee "La Sapienza", concerning the cure and use of mammals in experimental practice. On the day of the experiment, the animals were sacrificed by a blow on the head and bled. The ileum was excised and kept in a Tyrode's solution. Three to six segments, 2–3 cm long were cleaned and set up under 1 g tension in 10 ml organ bath containing Tyrode's solution, maintained at 37 °C and gassed with 95% O_2 and 5% CO_2. Changes in tension were recorded under isotonic condition by a transducer connected to a recorder (Ugo Basile, Italy) and calibrated before each experiment. The preparations were allowed to equilibrate for 30–40 min and then stimulated two or three times with ACh (10^{-7} M) to ascertain their responsiveness and to express the contractile responses as percentage of the ACh maximum response. Tissue preparations were generally used for several consecutive tests. After each test, preparations were allowed to rest for 25 min and washed three times between tests with Tyrode's solution. Each experimental test was performed on tissue preparations coming from at least four animals.

2.2. Chemicals

Acetylcholine chloride (2-(acetyloxy)-*N*,*N*,*N*-trimethylethanaminium chloride) (ACh), atropine (endo-(±)-α-(hydroxymethyl) benzeneacetic acid 8-methyl-8-azabicyclo[3.2.1]oct-3-yl ester), cholecystokinin octapeptide sulphate (CCk-8), orphanin Q/nociceptin (OFQ/N) and tetrodotoxin (TTX) were purchased from Sigma Chemical Co. (St. Louis, MO, USA); naloxone hydrochloride ((5α)-4,5-epoxy-3,14-dihydro-17-(2-propenyl)morphinan-6-one hydrochloride) (NL), was purchased from SIFAC (Milan, Italy); (−)-U-50,488H (*trans*-(−)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide hydrochloride) (U50),