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Biphasic regulation of the acute μ-withdrawal and CCk-8 contracture responses by the ORL-1 system in guinea pig ileum

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ABSTRACT

The cloning of the opioid-receptor-like receptor (ORL-1) and the identification of the orphan-inFQ/nociceptin (OFQ/N) as its endogenous agonist has revealed a new G-protein-coupled receptor signalling system. The structural and functional homology of ORL-1 to the opioid receptor systems has posed a number of challenges in the understanding the often competing physiological responses elicited by these G-protein-coupled receptors.

We had previously shown that in guinea pig ileum (GPI), the acute μ-withdrawal response is under the inhibitory control of several systems. Specifically, we found that the exposure to a μ-opioid receptor agonist activates indirectly the κ-opioid, the A_1-adenosine and the cannabinoid CB_1 systems, that in turn inhibit the withdrawal response. The indirect activation of these systems is prevented by the peptide cholecystokinin-8 (CCk-8).

In the present study, we have investigated whether the ORL-1 system is also involved in the regulation of the acute μ-withdrawal response.

Interestingly, we found that in GPI preparation, the ORL-1 system is not indirectly activated by the μ-opioid receptor stimulation, but instead the system is able by itself to directly regulate the acute μ-withdrawal response.

Moreover, we have demonstrated that the ORL-1 system behaves both as anti-opioid or opioid-like system based on the level of activation. The same behaviour has also been observed in presence of CCk-8. Furthermore, in GPI, the existence of an endogenous tone of the ORL-1 system has been demonstrated. We concluded that the ORL-1 system acts as a neuromodulatory system, whose action is strictly related to the modulation of excitatory neurotrasmitters released in GPI enteric nervous system.

1. Introduction

Enteric neurons can contain several neurotransmitters and neuromodulatory substances. Endogenous opioid peptides, including those ones derived from preproenkephalin, proopiomelanocortin, and prodynorphin are among the plethora of substances detected in enteric neurons by chemical or immunohistochemical techniques [1]. Gastrointestinal tissues have played an important historical role in the pharmacological characterization of opioid peptides [2–11]. In this context, after initial discovery of OFQ/N and the identification of previously cloned orphan opioid-like receptor (ORL-1) as its cognate receptor [12,13], it was therefore logical to investigate the biology of the OFQ/N-ORL-1 system in the guinea pig ileum tract, in which the system is expressed [14–16].

Isolated preparations, particularly guinea pig ileum (GPI), have been widely employed for assessing the acute effects of opioids and as a model for studying the interactions of opioids with other neuronal systems [17–19]. Opioid tolerance and physical dependence can be induced at the level of GPI myenteric plexus by exposing *in vitro* segments of ileum from naïve animals to opioids [19]. The withdrawal response of the tissue from the state of opioid physical dependence is revealed by the addition of naloxone (NL) or other selective antagonists. This response is observed few minutes after the exposure to the opioids [6,20–22], thus indicating that the cellular mechanisms underlying the acute dependence in the myenteric plexus are similar to those ones observed in experimental animals and man [23–25]. In GPI, the acute withdrawal response is under the inhibitory control of several neuronal systems. Exposure to a

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μ-agonist indirectly activates the κ-opioid, the adenosine A_1 and the cannabinoid CB_1 systems [9–11,18]. As observed *in vivo*, these indirectly activated systems inhibit the withdrawal responses induced by the μ-opioid receptor stimulation [26–31].

In vivo studies have suggested the existence of a physiological interaction between CCk-8 and opioids [32–35], as well as the evidence that CCk-8 may act as an endogenous inhibitor of opioid response [36,37]. In the isolated GPI preparation, CCk-8 induces tissue contraction through the release of ACh and substance P, preferentially by the CCk_A receptor stimulation [38]. The opioids not only suppress GPI spontaneous contraction, but also block the stimulatory effect of this peptide [9,10,18,22,39,40]. Finally, CCk-8 counteracts the inhibitory effect of indirectly activated neuronal systems by μ-opioid receptor stimulation (κ-opioid, adenosine A_1 and cannabinoid CB_1 systems), and plays by itself a modulatory effect on the control of opioid withdrawal response [6,9–11,22,40]. The role played by the ORL-1 receptor in the opioid withdrawal syndrome induced by μ-opioid receptor stimulation, in GPI isolated preparation, remains poorly understood.

In the present study, we have investigated whether ORL-1 receptors are involved in the μ-opioid withdrawal response control. Furthermore, we have evaluated the ORL-1 receptor involvement on both the CCk-8-induced and NL-withdrawal contractures, when the μ-opioid receptors are previously stimulated by an agonist.

2. Methods

2.1. Animal and tissue preparation

The experimental procedure has been described previously [11]. Male guinea-pigs weighing 300–400 g (Harlan, Italy) were housed in group of four per cage with food and water available *ad libitum*, in a room with controlled temperature (22 ± 1 °C) and under an artificial 12-h light/12-h dark cycle for at least 4 days before use. 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 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. 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. The experimental procedure has been approved by the local University Ethic Committee "La Sapienza", concerning the cure and use of mammals in experimental practice.

2.2. Drugs

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), dermorphin (Derm), orphaninFQ/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 hydro chloride) (NL), was purchased from SIFAC (Milan, Italy); *nor*-binaltorphimine dihydrochloride (17,17′-(dicyclopropylmethyl)-6,6′,7,7′-6,6′-imino-7,7′-binorphinan-3,4′,14,14′-tetrol dihydrochloride) (BNI), 8-cyclopenthyl-1,3-dimethylxantine (CPT), UFP-101 (N-(piperidine1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)4-methyl-1H-pyrazole-3-carbox-amide hydrochloride) were purchased from TOCRIS (Bristol, UK). SR141716A (5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-N-1-piperidinyl-1H-pyrazole-3-carboxamide) (SR) was purchased from Cayman Chemical (Cayman Europe, Tallinn, Estonia).

2.3. Statistical analysis

The contractile responses were expressed as percentage of the maximal response to acetylcholine (ACh max). The response intensity was calculated as the mean of all tissues; those yielding a response ≤10% of Ach max were assigned as value 0. For each experimental protocol, two initial control tests were carried out; the intensity of the control test response was calculated from the first control test response when there was no statistically significant difference between the two control test responses. Statistical significance has been evaluated by one-way ANOVA for repeated measures, followed by Hold–Sidak multiple comparison test; p values lower than 0.05 were considered statistical significant. Data are presented as means ± S.E.M.

2.4. The effect of both ORL-1 receptor agonist and antagonist, OFQ/N and UFP-101, respectively, on μ-opioid withdrawal response

Before starting the experiments, the tissue preparations were preliminarily exposed to μ- and κ-opioid, A_1-adenosine and CB_1 receptor antagonists, naloxone (NL, 5.4×10^{-7} M), *nor*-binaltorphimine (BNI, 3.4×10^{-8} M), 8-cyclopentyl-1,3-dimethylxanthine (CPT, 1.2×10^{-6} M) and SR141716A (SR, 1.1×10^{-8} M), respectively (2 min exposure for each antagonist). For each antagonist we have chosen the doses at which, as we have previously reported, a complete blockade of the relative receptors has been observed [9,10,17]. In naïve guinea pig ileum preparations, the antagonists tested at these dosages, are able to elicit contractions (>10% ACh max) that disappear following a second test. It is unlike that these contractures are due to tissue stretch during dissection, because we observed that all tissue preparations from the same animal behaved in the same way (i.e. either responding or not responding to the addition of opioid, A_1-adenosine and CB_1-cannabinoid antagonists). Instead, these contractures clearly represent an indication of a high level of constitutive activity or the existence of an endogenous tone of these receptors in naïve GPI preparation. Since, both the constitutive activity and the endogenous tone of these receptors can affect the responses under investigation, the preparations that have shown a contraction (>10% ACh max) to these antagonists after a second test, were disregarded (Supplementary Material, Fig. 1).

After this preliminary procedure and three washouts, the tissues were exposed for 5 min to the selective μ-opioid agonist, dermorphin (Derm, 3.0×10^{-9} M) and then challenged with the non-selective μ-opioid receptor antagonist NL (5.4×10^{-7} M). After two consecutive tests yielding contractions to NL with reproducible intensity, both κ-opioid and A_1-adenosine receptor antagonists, BNI (3.4×10^{-8} M) and CPT (1.2×10^{-6} M), respectively, were added 2–3 min after the μ-opioid agonist. Then, after the usual three wash outs, the CB_1 receptor antagonist SR141716A (SR, 1.1×10^{-8} M) was also added. All the receptor antagonists BNI, CPT and SR were added in a total interval of 5 min after the μ-opioid receptor agonist, dermorphin. Finally, after three consecutive wash outs, the