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SPECIAL REPORT

Effects of σ ligands on the nociceptin/orphanin FQ receptor co-expressed with the G-protein-activated K^+ channel in *Xenopus* oocytes

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Taking advantage of the functional coupling of the nociceptin/orphanin FQ receptor with the G-protein-activated inwardly rectifying K^+ (GIRK) channel, we investigated the effects of various σ ligands on the nociceptin/orphanin FQ receptor in *Xenopus* oocytes co-injected with the cloned nociceptin/orphanin FQ receptor and GIRK1 mRNAs. Carbetapentane and rimcazole, which induced no current response at 100 μM, reversibly suppressed the inward K^+ current responses induced by nociceptin in a concentration-dependent manner, and the IC_{50} values (μM) for these compounds were 9.0 and 12.6, respectively. (±)-N-allylnormetazocine, (+)-cyclazocine, (+)-3-(3-hydroxyphenyl)-N-(1-propyl)piperidine and 1,3-di-(2-tolyl)guanidine, at 100 μM, had no effect on the receptor. These results suggest that carbetapentane and rimcazole act as antagonists at the nociceptin/orphanin FQ receptor and may be involved in pain regulation.

Keywords: σ Ligand; carbetapentane; rimcazole; nociceptin/orphanin FQ receptor; G-protein-activated inwardly rectifying K^+ (GIRK) channel; *Xenopus* oocyte

Introduction σ Ligands comprise diverse chemical and pharmacological classes including benzomorphans, butyrophenones, guanidines, 3-phenylpiperidines, peptides and steroids, such as (+)-N-allylnormetazocine, haloperidol, 1,3-di-(2-tolyl)guanidine, (+)-3-(3-hydroxyphenyl)-N-(1-propyl)piperidine, neuropeptide Y and progesterone, respectively (Walker *et al*., 1990). σ Ligands have been shown to mediate various effects, including psychotomimetic, antipsychotic, neuroprotective, antitussive and antiepileptic ones, and to be involved in modulation of motor, immune and endocrine functions (Walker *et al*., 1990; Su, 1993). The actions of σ ligands are thought to be related to modulation of the dopaminergic system, the N-methyl-D-aspartate (NMDA) receptor-mediated response, phosphatidylinositol turnover and the activity of tonic potassium channels (Walker *et al*., 1990). Recently, we demonstrated that various σ ligands directly interact with the cloned μ-, δ- and κ-opioid receptors by use of a *Xenopus* oocyte system in which each opioid receptor and the G-protein-activated inwardly rectifying K^+ (GIRK) channel are co-expressed, suggesting that some effects of the σ ligands may be mediated by the opioid receptors (Kobayashi *et al*., 1996).

Following the cloning of three major subtypes of opioid receptor, the opioid receptor-like (ORL_1) receptor which exhibits sequence similarity to the opioid receptors was cloned (e.g. Mollereau *et al*., 1994). The endogenous agonist for the receptor was isolated from brain tissue (Meunier *et al*., 1995; Reinscheid *et al*., 1995). The agonist, a heptadecapeptide, structurally resembles dynorphin A. Whereas opioid agonists induce analgesia, the novel neuropeptide induces hyperalgesia and has been named nociceptin or orphanin FQ (OFQ) (Meunier *et al*., 1995; Reinscheid *et al*., 1995). Nociceptin action inhibits the formation of adenosine 3′:5′-cyclic monophosphate (cyclic AMP) (Mollereau *et al*., 1994), opens the GIRK channel *in vitro* and in various neurones (e.g. Ikeda *et al*., 1997) and inhibits activities of spinal dorsal horn neurones (Stanfa *et al*., 1997).

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Because of the interaction of various σ ligands with the opioid receptors and similarities in the amino acid sequences of the nociceptin/OFQ receptor and the opioid receptors, we assumed that σ ligands interact with the nociceptin/OFQ receptor. Taking advantage of the functional coupling of the nociceptin/OFQ receptor with the GIRK channel, we investigated the effects of various σ ligands on the nociceptin/OFQ receptor in *Xenopus* oocytes co-injected with the nociceptin/OFQ receptor and GIRK1 mRNAs.

Methods The specific mRNAs for the rat nociceptin/OFQ receptor and for the mouse GIRK1 channel were synthesized *in vitro* as described by Ikeda *et al*. (1997). *Xenopus* oocytes were co-injected with the nociceptin/OFQ receptor mRNA (~10 ng/oocyte) and GIRK1 mRNA (~12 ng/oocyte), and incubated at 19°C (Kobayashi *et al*., 1996). Whole-cell currents were recorded by a conventional two-micropipette voltage clamp method. The oocytes were superfused with a high-potassium solution (composition in mM: KCl 96, NaCl 2, $MgCl_2$ 1 and $CaCl_2$ 1.5). The membrane potential was held at −70 mV. The values obtained are expressed as mean±s.e.mean. Data were fitted to a standard logistic equation to compute the IC_{50} value.

σ Ligands, (±)-N-allylnormetazocine hydrochloride ((±)-SKF10047), (+)-cyclazocine, 1,3-di-(2-tolyl)guanidine (DTG), (+)-3-(3-hydroxyphenyl)-N-(1-propyl)piperidine hydrochloride ((+)-3PPP), carbetapentane citrate (CBP) and rimcazole dihydrochloride (RIM) were purchased from Research Biochemicals Inc. The endogenous agonist for the nociceptin/OFQ receptor, nociceptin, was synthesized *in vitro* (Research Genetics).

Results Application of (±)-SKF10047, (+)-cyclazocine, DTG, (+)-3PPP, CBP or RIM, at 100 μM, induced no current response in the oocytes co-injected with the nociceptin/OFQ receptor and GIRK1 mRNAs ($n=3$; data not shown), although application of nociceptin (200 nM) induced inward K^+ currents (93.2±6.7 nA; $n=7$). These results indicate that none of the σ ligands tested act as agonists at the receptor.

To investigate the antagonism of σ ligands for the nociceptin/OFQ receptor, we applied each σ ligand together with

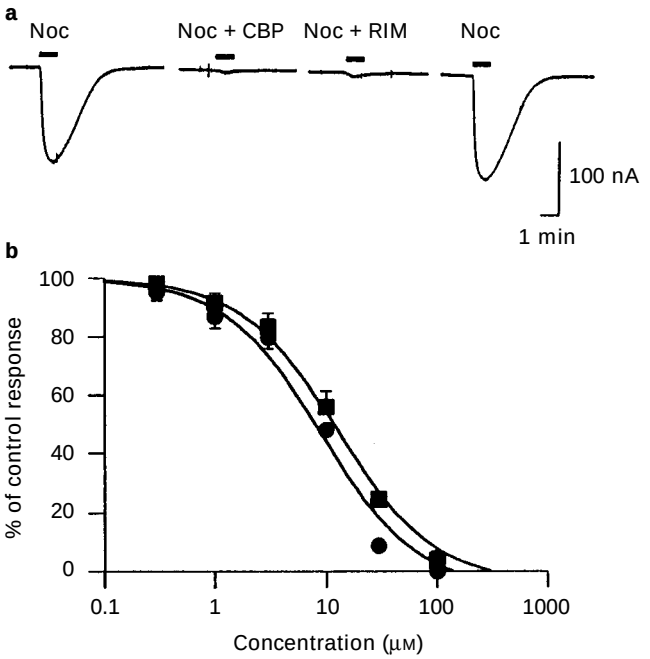

Figure 1 Inhibition, by σ ligands, of nociceptin-induced current responses in oocytes co-injected with the nociceptin/OFQ receptor and GIRK1 mRNAs. (a) Current responses to 300 nM nociceptin (Noc), 300 nM Noc plus 100 μM carbetapentane (CBP), 300 nM Noc plus 100 μM rimcazole (RIM) and 300 nM Noc in the same oocyte. Bars show the duration of application. The time intervals between the applications were approximately 10 min. (b) Concentration-dependent inhibition of the current responses by carbetapentane (●) and rimcazole (■). Each point represents the mean % of the control responses obtained from 5 oocytes; vertical lines show s.e.mean.

nociceptin to the co-injected oocytes. Nociceptin was used at a concentration of 10 fold the EC_{50} value at which the current response was near the full response (Ikeda *et al*., 1997). The control current response induced by nociceptin (300 nM) was 119.2 ± 11.3 nA ($n = 10$). The current responses to nociceptin were reversibly suppressed by CBP or RIM (Figure 1a). As shown in Figure 1b, these σ ligands concentration-dependently suppressed the current responses to nociceptin. The IC_{50} values (μM) for CBP and RIM are 9.0 ± 0.8 and 12.6 ± 2.2, and the Hill coefficient values for these compounds are 1.62 ± 0.17 and 1.33 ± 0.13, respectively. The current responses to nociceptin were not affected by (±)-SKF10047, (+)-cyclazocine, DTG or (+)-3PPP (100 μM, $n = 3$; data not shown). These results indicate that CBP and RIM act as antagonists at the nociceptin/OFQ receptor.

Discussion To identify ligands that interact with the nociceptin/OFQ receptor, we used the *Xenopus* oocyte system in which the nociceptin/OFQ receptor and GIRK1 channel were co-expressed. Although several radiolabelled σ ligands failed to bind to membranes prepared from COS-7 cells transfected with nociceptin/OFQ receptor cDNA (e.g. Mollereau *et al*., 1994), we demonstrate here that CBP and RIM, among the various σ ligands tested, act as antagonists at the nociceptin/OFQ receptor. It has been shown that CBP, which is a centrally acting non-opioid antitussive agent with anticonvulsant activity, exhibits high affinity for σ receptors (Walker *et al*., 1990) and the M_1-muscarinic receptor (Hudkins & DeHaven-Hudkins, 1991) and acts as a κ-agonist and μ-antagonist (Kobayashi *et al*., 1996) and that RIM, which has antipsychotic activity without producing significant extrapyramidal side effects, exhibits a selective but relatively weak affinity for σ receptors (Davidson *et al*., 1982; Su, 1993). Since the nociceptin/OFQ receptor mRNA exists in several brain areas that are known to be involved in pain regulation and emotions (Mollereau *et al*., 1994), the σ ligands may mediate many brain functions through the receptor. Since repeated intracerebroventricular administration of an antisense oligonucleotide to nociceptin/OFQ receptor mRNA induced analgesia (Meunier *et al*., 1995), two of the σ ligands that act as nociceptin/OFQ receptor antagonists may be involved in pain regulation. RIM has also been shown to ameliorate negative schizophrenic symptoms, such as anergia, suggesting that RIM has activating effects (Davidson *et al*., 1982). Since nociceptin induces a decrease in locomotor activity (Reinscheid *et al*., 1995), the antagonism of RIM for the nociceptin/OFQ receptor may be related to the activating effects in the treatment of schizophrenia.

Further studies with CBP and RIM as nociceptin/OFQ receptor antagonists may be useful in the characterization of the functions of the receptor, although action of these ligands at other sites must be considered. Our *Xenopus* oocyte co-expression system can be used to develop potent and efficacious ligands for the nociceptin/OFQ receptor.

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