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Prejunctional modulation by nociceptin of nerve-mediated inotropic responses in guinea-pig left atrium

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Abstract

The superimposition of a train of electrical stimuli (15 Hz, 1 ms, 60 V for 2.5 s) to the electrically driven (3 Hz) isolated left atria from reserpine-pretreated guinea pigs in the presence of atropine (1 μM) produces a delayed positive inotropic response due to the antidromic activation of capsaicin-sensitive primary afferents and the release of the sensory neuropeptide, calcitonin gene-related peptide (CGRP). The novel opioid peptide nociceptin, inhibited (E_{max} 88% inhibition at 1 μM) in a concentration-dependent manner (10 nM–1 μM) (EC_{50} 33 nM) the delayed positive inotropic response induced by train electrical field stimulation, without affecting the positive inotropic response produced by exogenous CGRP (10 nM) or capsaicin (30 nM). The inhibitory effect of nociceptin on the delayed positive inotropic response induced by train electrical field stimulation was not antagonized by the opioid receptor antagonists naloxone, naltrindole and nor-binaltorphimine (1 μM each) nor was it modified by a cocktail of peptidase inhibitors (bestatin, captopril and thiorphan, 1 μM each). A significant inhibition by nociceptin (1 μM) was also observed toward the sympathetic positive inotropic response produced by EFS at 5 Hz in the presence of atropine (1 μM) and after in vitro capsaicin desensitization and toward the parasympathetic negative inotropic response produced by EFS at 10 Hz in atria from reserpine-pretreated guinea pigs and after in vitro capsaicin desensitization. We conclude that nociceptin exerts a prejunctional inhibitory effect on evoked release of CGRP from capsaicin-sensitive sensory nerve terminals in guinea-pig left atria. The effect of nociceptin occurs independently from the activation of μ-, δ- or κ-opioid receptors. Nociceptin, at appropriate frequency of stimulation, appears to exert a general inhibitory neuromodulation on transmitters release in guinea-pig left atria. © 1997 Elsevier Science B.V.

Keywords: Nociceptin; Capsaicin; Sensory nerve; Efferent function; Atrium; (Guinea pig)

1. Introduction

Nociceptin (Meunier et al., 1995), also termed orphanin FQ (Reinscheid et al., 1995), is a novel heptadecapeptide which binds with high affinity and is considered to be the endogenous ligand for the G-protein-coupled orphan receptor ORL_1 (Mollereau et al., 1994; Wang et al., 1994). ORL_1 gene transcripts are present in the mouse and rat central nervous system as well as in some peripheral organs such as the intestine, vas deferens and spleen (Wang et al., 1994). ORL_1 possesses a high degree of homology with μ-, δ- and κ-opioid receptors, which may forward a similarity of actions between nociceptin and opioid peptides. This expectation does not match with the reported hyperalgesic and functional anti-opioid action of nociceptin, exerted centrally (Reinscheid et al., 1995; Mogil et al., 1996). On the other hand, both nociceptin and opioid receptor agonists share a prejunctional site of action in inhibiting evoked transmitter release in the peripheral nervous system (Giuliani and Maggi, 1996; Berzetei-Gurske et al., 1996; Calò et al., 1996; Nicholson et al., 1996): the action of nociceptin at this level is unaffected by opioid receptor blockers, indicating an independent mechanism of action, putatively the activation of the ORL_1 receptor. This neuromodulatory effect also includes the inhibition of evoked tachykinin release from the peripheral endings of sensory nerve terminals (Giuliani and Maggi, 1996).

The aim of the present study was to assess whether nociceptin may affect the release of another major sensory neuropeptide, calcitonin gene-related peptide (CGRP), from the peripheral endings of sensory nerves. With this aim we have investigated the effect of nociceptin in the guinea-pig isolated left atria a preparation in which, under appropriate experimental conditions, the release of CGRP from sen-

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sory nerves can be conveniently evoked in a reproducible manner for studying the pharmacological modulation of this response (Maggi et al., 1988; Giuliani et al., 1989a,b, 1990). In the electrically-driven guinea-pig left atria it is also possible to selectively evoke noradrenaline-mediated sympathetic positive inotropic responses or acetylcholine-mediated parasympathetic negative inotropic responses by selecting appropriate pharmacological pretreatments (Goto et al., 1987): therefore, experiments were designed to compare the activity of nociceptin toward changes in atrial inotropism produced by acetylcholine, noradrenaline and CGRP release from parasympathetic, sympathetic and sensory nerves, respectively.

2. Methods

2.1. General

Male albino guinea pigs (Charles River, Calco) weighing 350–400 g received i.p. reserpine (5 mg/kg) and were sacrificed for the experiment 48–96 h later. The whole heart was rapidly removed and placed in Tyrode solution of the following composition in mM: NaCl 137, KCl 2.68, $CaCl_2$ $2H_2O$ 1.8, $MgCl_2$ 1.05, NaH_2PO_4 0.42, $NaHCO_3$ 11.9, glucose 5.5. The right and left atria were dissected and mounted in an organ bath containing 5 ml of Tyrode solution at 37°C and were oxygenated with a mixture of 95%O_2 and 5%CO_2. The atria were connected under a resting tension of 5 mN to an isometric force transducer and their mechanical activity was recorded on a Basile 7050 Unirecord.

2.2. Right atria

Under these conditions, the right atrium is spontaneously beating. After 60 min equilibration period, the effect of nociceptin on frequency and amplitude of spontaneous contractions of the right atria were evaluated in preparations obtained from either vehicle- or reserpine-pretreated guinea pigs.

2.3. Left atria

The left atria were suspended between two vertical platinum wire electrodes for field stimulation placed at the top and the bottom of the organ bath and connected to a GRASS S88 stimulator. After 15 min equilibration period, the atria were electrically driven at a frequency of 3 Hz (0.5 ms pulse width, maximal voltage) to induce a 'resting' level of inotropism. After a 2 h equilibration period train of electrical stimuli at frequency of 15 Hz for 2.5 s (1 ms pulse width, 60 V) were delivered at 15 min intervals by means of a second stimulator: the cycles of train electrical field stimulation superimposed to the basal stimulation at 3 Hz produce an immediate myogenic positive inotropic response followed by a delayed positive inotropic response which is mediated by CGRP release produced by antidromic activation of capsaicin-sensitive sensory nerve terminals (Goto et al., 1987; Saito et al., 1987; Maggi et al., 1988; Giuliani et al., 1989a,b, 1990). The delayed positive inotropic response was expressed as % increase of the basal contractile activity and the effect of nociceptin as % inhibition of the control delayed positive inotropic response to train electrical field stimulation.

The effect of nociceptin on the train electrical field stimulation-evoked delayed positive inotropic response was assessed after 5 min contact time: a non-cumulative concentration–response curve to nociceptin was constructed by administering the increasing concentrations of the peptide at 15 min intervals with interevening washouts. Preliminary experiments had shown that the inhibitory effect of 1 μM nociceptin on the delayed positive inotropic response evoked by train electrical field stimulation was fully reversible by washout. In some experiments, the effect of nociceptin was studied in the presence of a cocktail of opioid receptor antagonists (naloxone, naltrindole and nor-binaltorphimine, 1 μM each, 10 min before) or in the presence of a cocktail of peptidase inhibitors (bestatin, captopril and thiorphan, 1 μM each, 10 min before).

In separate experiments, the effect of nociceptin was investigated on the positive inotropic effect induced by capsaicin (30 nM) or by exogenous calcitonin gene-related peptide (10 nM).

In the course of the above experiments, it was noted that nociceptin produces a modest but consistent depressant effect on the resting inotropic activity of left atria electrically driven at 3 Hz: experiments were performed in capsaicin-pretreated left atria to verify the hypothesis that this effect may depend upon inhibition of calcitonin gene-related peptide released from sensory nerves by the 'resting' driving frequency of 3 Hz.

By selecting appropriate pharmacological pretreatment, selective changes in atrial inotropism can be elicited by inducing mediators release from sympathetic (noradrenaline-mediated), parasympathetic (acetylcholine-mediated) and sensory nerves (Saito et al., 1986). Therefore, in separate experiments, we studied the effect of 1 μM nociceptin on the inotropic responses induced by train electrical field stimulation at 5–15 Hz and 10–15 Hz for sympathetic and parasympathetic responses, respectively (other parameters as above), in the presence of atropine (1 μM) and after in vitro capsaicin desensitization (10 μM for 10 min) (noradrenergic positive inotropic response) or in atria from reserpine-pretreated (5 mg/kg i.p.) animals and after in vitro capsaicin desensitization (cholinergic negative inotropic response). The effect of nociceptin on the sympathetic inotropic response was calculated by measuring the area of the positive inotropic response. The effect of propranolol (1 μM) was used in each preparation as an internal check to establish the intensity of the sympathetic response.

2.4. Statistical analysis

Each value in text and figures is mean ± S.E.M. Statistical analysis was performed by means of the Student's *t*-test for paired or unpaired data, when appropriate. Regression analysis was performed by means of the least squares method: EC_{50} and 95% confidence limits (c.l.) were calculated accordingly.

2.5. Drugs

The drugs used were: atropine hydrochloride and reserpine (Serva, Heidelberg), capsaicin, naloxone hydrochloride, propranolol hydrochloride, thiorphan and captopril (Sigma, St. Louis, MO, USA), nociceptin (Tocris Cookson, Bristol, UK), human calcitonin gene-related peptide (CGRP), human CGRP (8-37) and bestatin (Peninsula, St. Helens, UK), nor-binaltorphimine dihydrochloride, naltrindole hydrochloride (Research Biochemicals International, Natick, MA, USA).

3. Results

3.1. Guinea-pig left atria: Positive inotropic response to sensory nerve stimulation

The superimposition of a train of electrical stimuli (15 Hz for 2.5 s) onto the resting inotropism induced by electrically driving (at 3 Hz) the left atria from reserpine-pretreated guinea pigs in the presence of atropine (1 μM), produced an immediate, myogenic positive inotropic response during application of the train followed by a delayed positive inotropic response which reaches its maximum within 2 min from the application of the stimulus (cf. Maggi et al., 1988; Giuliani et al., 1989a,b). As shown previously, the delayed positive inotropic response is produced by an electrical field stimulation-induced CGRP release from sensory nerves.

The amplitude of control delayed positive inotropic response averaged 67 ± 8% increase ($n = 8$) over the resting inotropism and was reproducible if elicited at 15–20 min intervals. Previous studies have established that the delayed positive inotropic response is produced through the release of CGRP from peripheral endings of capsaicin-sensitive primary afferent nerves (Goto et al., 1987; Saito et al., 1987; Maggi et al., 1988; Giuliani et al., 1989a,b, 1990).

Nociceptin (10 nM–1 μM, 5 min before, $n = 6–8$) produced a concentration-dependent inhibition of the delayed positive inotropic response in response to train electrical field stimulation: the maximal effect of nociceptin averaged 88 ± 3% inhibition at 1 μM (Figs. 1 and 2) and was fully reversible after 15 min from washout of the peptide. The EC_{50} of nociceptin was 33 nM (14–172 are 95% c.l.).

The inhibition of delayed positive inotropic response induced by nociceptin was unchanged by previous administration of a mixture of opioid receptor antagonists naloxone, naltrindole and nor-binaltorphimine (1 μM each, 10 min before nociceptin) to block the μ-, δ- and κ- receptors (Fig. 2).

The inhibitory effect produced by a submaximally effective concentration of nociceptin (30 nM) on train elec-

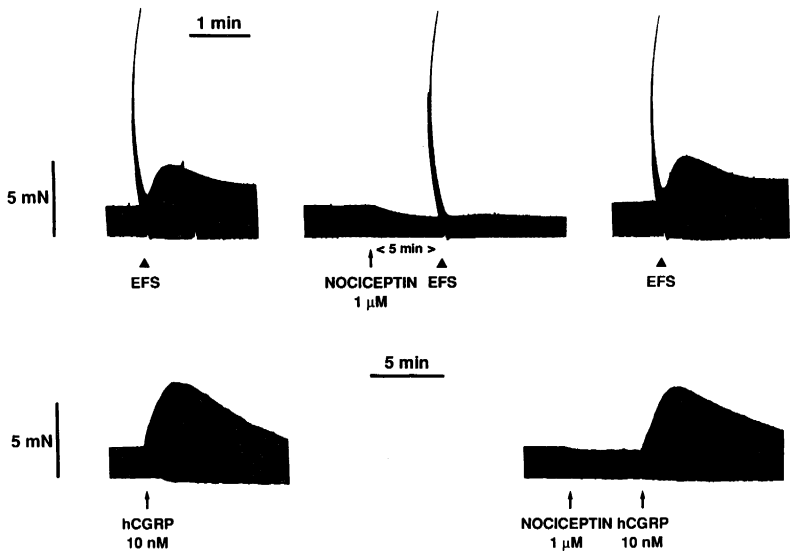

Fig. 1. Tracings showing the inhibitory effect of nociceptin (1 μM) on the delayed positive inotropic response produced by train electrical field stimulation (15 Hz, 1 ms, 60 V for 2.5 s at the triangles) in the guinea-pig left atrium (upper panel). Note that nociceptin depressed the resting contractile activity. In the third tracing is shown the recovery of the delayed positive inotropic response 15 min after washout. Nociceptin inhibited the inotropic response to electrical field stimulation without affecting that to exogenously administered human calcitonin gene-related peptide (10 nM, lower panel) indicating a prejunctional site of action on calcitonin gene-related peptide-containing sensory nerves. In the upper panel the chart speed after nociceptin administration, till train electrical field stimulation, was slowed to have a more compressed tracing.

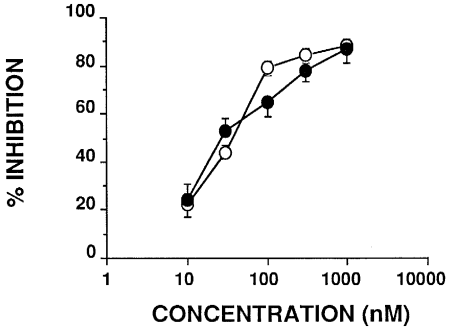

Fig. 2. Concentration-dependent inhibition by nociceptin of the delayed positive inotropic response produced by train electrical field stimulation of the guinea-pig isolated left atria in control (○) and in the presence of a mixture of opioid antagonists (●, naloxone, naltrindole and nor-binaltorphimine, 1 μM each). Each value is mean ± S.E.M. of 5–6 experiments.

trical field stimulation-evoked delayed positive inotropic response was not significantly affected by a cocktail of peptidase inhibitors (bestatin, captopril and thiorphan, 1 μM each, 10 min before): the inhibitory effect of nociceptin averaged 51 ± 4 and 48 ± 5% ($n = 4$ each) in the absence or the presence of peptidase inhibitors, respectively (Fig. 3a).

Neither the cocktail of opioid receptor antagonists nor the cocktail of peptidase inhibitors did affect the train electrical field stimulation-evoked delayed positive inotropic response.

Nociceptin (1 μM, 5 min before) did not affect the positive inotropic response to exogenous human CGRP (10 nM) (Figs. 1 and 3b) nor that induced by capsaicin (30 nM) (Fig. 3b). The effect of CGRP averaged 57 ± 8 and 54 ± 10% ($n = 5$) of the maximal inotropic response to CGRP (100 nM) in the absence and presence of nociceptin, respectively. The inotropic effect observed in response to capsaicin (30 nM) averaged 41 ± 5 and 47 ± 6% ($n = 5$) of the maximal inotropic response to CGRP (100 nM) in the absence and presence of nociceptin, respectively.

In parallel to the above described inhibitory effect on electrical field stimulation-evoked delayed positive inotropic response, nociceptin (10 nM–1 μM) also induced a reproducible negative inotropic effect on the resting inotropism evoked by electrically driving the atria at a frequency of 3 Hz. This effect averaged 25 ± 5, 30 ± 7 and 30 ± 6% inhibition of resting inotropism at 10 nM, 0.1 and 1 μM, respectively, and peaked at about 5 min from administration of nociceptin.

The inhibitory effect of nociceptin on resting inotropism was not observed in atria subjected to in vitro capsaicin desensitization (10 μM for 10 min) ($n = 4$). It is worth being noted that furthermore the CGRP antagonist, CGRP (8-37) (1 μM) inhibited at a similar extent (−34 ± 3%, $n = 4$) the resting inotropism (data not shown).

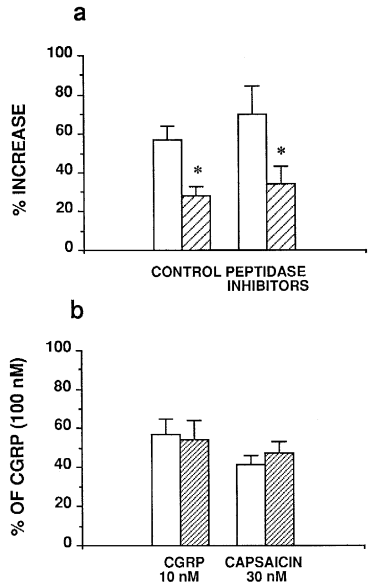

Fig. 3. (a) The effect of nociceptin (30 nM, hatched columns) on the control delayed positive inotropic response (open columns) induced by train electrical field stimulation (15 Hz, 1 ms, 60 V for 2.5 s) of the guinea-pig isolated left atria in the absence and in the presence of peptidase inhibitors (bestatin, captopril and thiorphan, 1 μM each). (b) The positive inotropic responses induced by administration of calcitonin gene-related peptide (10 nM) and capsaicin (30 nM) alone (open columns) or in the presence of nociceptin (1 μM, 5 min before, hatched columns) in the guinea-pig isolated left atria. Each value is mean ± S.E.M. of 4–5 experiments. * $P < 0.05$, significantly different from the control response.

3.2. Guinea-pig left atria: Inotropic responses to stimulation of sympathetic and parasympathetic nerves

The application of train electrical field stimulation (5–15 Hz, 1 ms pulse width, 60 V for 2.5 s) in the presence of atropine (1 μM) and after in vitro capsaicin desensitization (10 μM for 10 min) induced a positive inotropic response which peaked within 15 s from the end of stimulation: the positive inotropic response at 5 or 15 Hz was abolished by propranolol (1 μM). Nociceptin (1 μM) inhibited the sympathetic inotropic response produced by EFS at 5 Hz by 61 ± 13% ($P < 0.05$, $n = 4$) while it had no significant effect (12 ± 7% inhibition, $n = 4$) toward that evoked at 15 Hz.

The application of train electrical field stimulation (5–20 Hz, 1 ms pulse width, 60 V for 2.5 s) to guinea-pig left atria from reserpine-pretreated guinea pigs and after in vitro capsaicin desensitization (10 μM for 10 min) induced a negative inotropic response which peaked within 15–20 s from the end of stimulation and is frequency-dependent: 32 ± 5, 54 ± 7, 66 ± 7 and 79 ± 4% inhibition at 5, 10, 15 and 20 Hz, respectively. The negative inotropic response at 10 Hz, selected for inducing a submaximal response, was abolished by atropine (1 μM) and slightly but significantly reduced (21 ± 4%, $P < 0.05$, $n = 4$) in the presence of nociceptin (1 μM). At a higher frequency of stimulation

(15 Hz) the electrical field stimulation evoked cholinergic negative inotropic response, was unaffected ($7 \pm 2\%$ inhibition, $n = 4$) by 1 μM nociceptin.

3.3. Guinea-pig right atria

The activity of nociceptin was also evaluated on guinea-pig right atria from both control and reserpine pretreated guinea pigs. Spontaneously beating guinea-pig right atria from control animals developed a tension of 1.61 ± 0.15 mN at a rate of 184 ± 8 beats/min ($n = 8$). The atria from reserpine-pretreated guinea pigs in the presence of atropine (1 μM) in the bath, developed a tension of 4.3 ± 0.4 mN at a rate of 179 ± 6 beats/min ($n = 10$). Nociceptin (1 μM) had no significant effect on frequency and spontaneous contractions of guinea-pig right atria from either control or reserpine-pretreated guinea pigs during the 10 min observation period. The difference in the values of the basal tension between the control and reserpine-pretreated group was statistically significant ($P < 0.05$).

4. Discussion

The mammalian atria receive a dense capsaicin-sensitive sensory innervation (Papka et al., 1981) which can be evidenced at immunohistochemistry as a plexus of CGRP-positive fibers (Mulderry et al., 1985). It is now well established that CGRP is the main neurotransmitter of the non-adrenergic non-cholinergic (NANC) excitatory innervation to the heart (Lundberg et al., 1984). Both capsaicin and electrical field stimulation induce CGRP release from the peripheral endings of capsaicin-sensitive nerves in guinea-pig left atria: after blockade of the cholinergic and noradrenergic transmission, this determines a positive inotropic response suitable for studying the prejunctional modulation of CGRP release from sensory nerves, as established previously (Franco-Cereceda and Lundberg, 1985; Saito et al., 1987; Maggi et al., 1989; Giuliani et al., 1989a,b, 1990).

The present data show that nociceptin exerts a prejunctional modulatory effect on the non-adrenergic non-cholinergic response to electrical field stimulation which is induced by the release of CGRP from sensory nerves in the guinea-pig left atrium. The EC_{50} of nociceptin (33 nM) in this assay compares well to the EC_{50} of 28 nM for inhibiting tachykinin release from capsaicin-sensitive afferents in the guinea-pig renal pelvis (Giuliani and Maggi, 1996). Although it was found that peptidase inhibitors determine about a four fold increase in the potency of nociceptin in the rat vas deferens (Nicholson et al., 1996), no effect of this type was detected in the guinea-pig atria. As observed in the guinea-pig renal pelvis, the prejunctional inhibitory effect of nociceptin is independent from the activation of the classical opioid receptors, in keeping with the reported poor binding affinity of nociceptin for the μ-, δ- or κ-opioid receptors (Meunier et al., 1995; Reinscheid et al., 1995). On the other hand, Stanfa et al. (1996) reported that a high dose of naloxone reversed the depressant effect of nociceptin on C-fibre-evoked responses in rat dorsal horn neurones in vivo.

Both train electrical field stimulation and capsaicin produce a positive inotropic effect ascribable to CGRP release from peripheral endings of sensory nerves. However, important differences exist in the mechanisms supporting CGRP release in this preparation: one mechanism, selectively activated by electrical field stimulation, involves the recruitment of N-type voltage-dependent Ca^{2+} channels to support CGRP release while the other one involves Ca^{2+} influx into sensory nerve terminals via the capsaicin-receptor/ion channel (Maggi, 1991, 1995 for reviews).

We observed previously that various prejunctional modulators (neuropeptide Y, galanin, opioids, GABA, etc.) which share the ability of inhibiting the release of sensory neuropeptides from capsaicin-sensitive afferent nerves, are effective on the electrical field stimulation-evoked release whilst being barely effective or even ineffective toward the capsaicin-evoked release (Maggi, 1991, 1995, for reviews). The action of nociceptin appears to be likewise restricted to the first mechanism of CGRP release, the one selectively activated by electrical field stimulation. Indeed nociceptin inhibits voltage-dependent N-type Ca^{2+} channels in a human neuroblastoma cell line (Connor et al., 1996) which could account for the effect observed in this study.

In addition to inhibiting the CGRP-mediated stimulated inotropism, nociceptin also reduced, by about 30%, the 'resting' inotropism in left atria electrically driven at a frequency of 3 Hz. This effect is not likely dependent upon a direct effect of nociceptin on cardiac cells, since it was not observed in capsaicin-pretreated atria and no effect was likewise observed in the spontaneously beating right atria. Rather, we speculate that some amount of CGRP is released when driving the left atria at 3 Hz to produce a resting level of inotropism and that nociceptin depresses this 'basal' CGRP release. This interpretation is further suggested by the observation that the CGRP receptor antagonist produced a significant depression of resting inotropism of similar intensity of that produced by nociceptin.

In previous studies, nociceptin has been shown to produce a prejunctional inhibition of noradrenaline and acetylcholine release from peripheral nerves in the rat and mouse vas deferens and guinea-pig ileum, respectively (Nicholson et al., 1996; Berzetei-Gurske et al., 1996; Calò et al., 1996). A similar mechanism appears to be operating on parasympathetic and sympathetic nerve endings in guinea-pig atria providing that a submaximal stimulus intensity is used to evoke acetylcholine or noradrenaline release. Nociceptin appears to exert a general inhibitory neuromodulation on transmitters release in guinea-pig left atria.

In conclusion the present data provide evidence that nociceptin exerts a prejunctional inhibitory effect on elec-

trical field stimulation-evoked CGRP release from capsaicin-sensitive sensory nerve terminals in guinea-pig left atrium independently from activation of μ-, δ- or κ-opioid receptors.

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