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Short communication

Tonic inhibition by orphanin FQ/nociceptin of noradrenaline neurotransmission in the amygdala

Yukie Kawahara[a,*], Mayke B. Hesselink[b], Guus van Scharrenburg[b], Ben H.C. Westerink[c]

[a] Department of Pharmacology, Kurume University School of Medicine, Asahi-machi 67, Kurume 830-0011, Japan
[b] Solvay Pharmaceuticals Research Laboratories, Weesp 1380 DA, The Netherlands
[c] Department of Biomonitoring and Sensoring, University Center for Pharmacy of Groningen, Groningen 9713AV, The Netherlands

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Abstract

The present microdialysis study investigated whether nociceptin/orphanin FQ exerts a tonic inhibition of the release of noradrenaline in the basolateral nucleus of the amygdala in awake rats. The non-peptide competitive nociceptin/orphanin FQ (N/OFQ) peptide receptor antagonist J-113397 (20 mg/kg i.p.) induced an increase in the release of noradrenaline to about 150–200%. The increase was strongly suppressed by local infusion of an endogenous N/OFQ peptide receptor agonist, nociceptin/orphanin FQ (1 μM) via retrograde microdialysis, into the basolateral nucleus of the amygdala. Local infusion of nociceptin/orphanin FQ (1 μM) itself reduced noradrenaline release in the basolateral nucleus of the amygdala to about 70% of basal levels. These results indicate that a large part of basal release of noradrenaline in the basolateral nucleus of the amygdala is under tonic inhibitory control by endogenous nociceptin/orphanin FQ through the N/OFQ peptide receptors localized within the basolateral nucleus of the amygdala.

Keywords: J-113397; Microdialysis; Orphanin FQ; Nociceptin; Amygdala; (Rat)

1. Introduction

The nociceptin/orphanin FQ (N/OFQ) peptide receptor was discovered in 1994. A year later the endogenous ligand nociceptin/orphanin FQ was identified by two different groups almost simultaneously (Meunier et al., 1995; Reinscheid et al., 1995).

Activation of the central N/OFQ peptide receptors produces a unique range of responses that differ from the properties of classical opioid agonists (review, Darland et al., 1998; Jenck et al., 2000). N/OFQ peptide receptor lacking mice displayed increased anxiety, disruption of stress adaptation, better memory and learning ability (Reinscheid and Civelli, 2002; Noda et al., 2000). These studies indicate the presence of a physiological tone by N/OFQ peptide receptor system in anxiety, stress adaptation, learning and memory.

The amygdala complex is an important structure in the processing of aversive stimuli, integration of appropriate responses to stress, and memory consolidation (LeDoux, 2000; Tanaka et al., 1991). Many of these functions relate to the role of the N/OFQ peptide receptor in the brain. Receptor binding studies indicated that the amygdala complex contains relatively dense concentration of N/OFQ peptide receptors (Florin et al., 2000). One of the neurotransmitters within the amygdala that might be regulated by N/OFQ peptide receptors is noradrenaline. We therefore determined the effect of N/OFQ peptide receptor ligands on noradrenaline neurotransmission in this brain structure. To that end a microdialysis probe was directed to the basolateral nucleus, an area known to be particularly involved in aversive emotions such as anxiety and fear, and in stress-related hypoalgesia (Helmstetter et al., 1995).

2. Materials and methods

2.1. Animals and chemical assays

Male albino rats of a Wistar-derived strain (285–320 g) were maintained under isoflurane anesthesia (2%) in 30% of

* Corresponding author. Tel.: +81-942-31-7545; fax: +81-942-31-7696.
E-mail address: yukikawa@med.kurume-u.ac.jp (Y. Kawahara).

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oxygen and 70% of nitrous oxide with local application of lidocaine (10%) for the probe implantation procedure. Probe were implanted in the basolateral nucleus of the amygdala (exposed length 1.0 mm). Coordinates of the implantation were as follows: A/P − 2.8 mm, L/M 5.0 mm, V/D 8.8 mm from bregma and dura. Microdialysis experiments and chemical assays were performed as previously described (Kawahara et al., 2001). The microdialysis probe was perfused with a Ringer's solution at a flow rate of 2.0 μl/min. The experiments were approved by the Animal Care Committee of the Faculty of Mathematics and Natural Science of the University of Groningen.

2.2. Drugs

1-[(3*R*,4*R*)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1,3-dihydro-*2*H*-benzimidazol-2-one (J-113397) was synthesized and provided by Solvay Pharmaceuticals (Weesp, Netherlands). Nociceptin/orphanin FQ was obtained from Bachem, Switzerland. J-113397 was suspended in 1% methylcellulose saline.

Nociceptin/orphanin FQ was dissolved in Ringer's solution.

2.3. Data analysis

All values given are expressed as percent of control. The average concentration of three stable baseline samples was set at 100%. Statistic analysis (Statview; SAS Institute, Cary, NC, USA, 1998) was performed using one-way analysis of variance with repeated measures and Dunnett's multiple comparison test for post hoc determination of significant differences between baseline and after drug administration. Two-way analysis of variance and Scheffe's multiple comparison test for post hoc determination were used for comparison between experimental groups. The area under curve (AUC) was presented as the ratio of total amount of noradrenaline recorded after to before a treatment. The level of significance was set at $P < 0.05$.

3. Results

The mean basal value for noradrenaline in the basolateral nucleus of the amygdala was 0.75 ± 0.19 fmol/min (mean ± S.E.M., $n = 17$).

First, the effect of local infusion of nociceptin/orphanin FQ on extracellular noradrenaline in the basolateral nucleus of the amygdala was established. In vitro studies have shown that inhibits the electrically evoked noradrenaline release in rat cortical slices with an EC50% of about 10 nM (Rominger et al., 2002). Since the recovery of nociceptin/orphanin FQ across the dialysis membrane, based on its chemical structure, was estimated to be around 1% (Murphy and Maidment, 1999), nociceptin/orphanin FQ was infused in a concentration of 1 μM. Fig. 1 shows that local application of nociceptin/orphanin FQ induced a small but statistically significant decrease of extracellular noradrenaline levels to about 70% of controls (Fig. 1A, $n = 4$). The AUC of the nociceptin/orphanin FQ infusion experiment was statistically significantly different from controls (Fig. 1B, $n = 5$).

To investigate the existence of a possible tonic inhibition by nociceptin/orphanin FQ releasing neurons on noradrenaline release in the basolateral nucleus of the amygdala, we administered the N/OFQ peptide receptor antagonist J-113397. J-113397 (20 mg/kg i.p.) increased the release of noradrenaline to about 200% of basal levels (Fig. 2A, $n = 4$). The increase was statistically significantly different during a period of 60 min. Apart from the initial rise (at $t = 15$ min), the effect of J-113397 was significantly suppressed when nociceptin/orphanin FQ (1 μM) was infused continuously through the microdialysis probe (Fig. 2A, $n = 5$). During the infusion of nociceptin/orphanin FQ, extracellular noradrenaline levels again decreased to about 70% of controls. The AUCs of the J-113397 group were statistically significantly

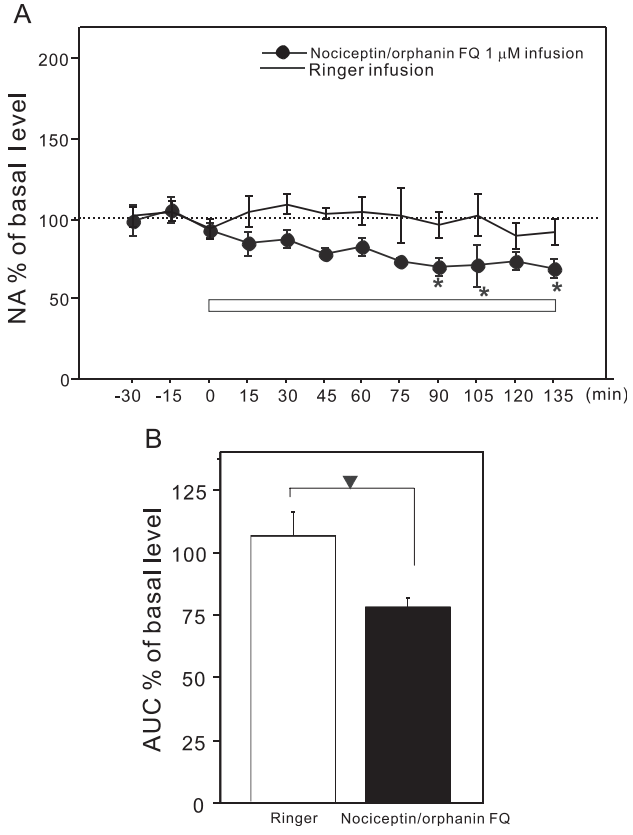

Fig. 1. (A) Effect of nociceptin/orphanin FQ (1 μM) (closed bar, $n = 4$) and Ringer's solution infusion into the basolateral nucleus of the amygdala ($n = 5$) on extracellular noradrenaline in the basolateral nucleus of the amygdala. Data are expressed as a percentage (mean ± S.E.M.) of basal levels. *$P < 0.05$ vs. basal level. $P < 0.05$ as indicated. (B) The comparison of ratio of AUC between two experimental groups. Data are expressed as a percentage (mean ± S.E.M.) of AUC of basal levels. ▼$P < 0.05$ as indicated.

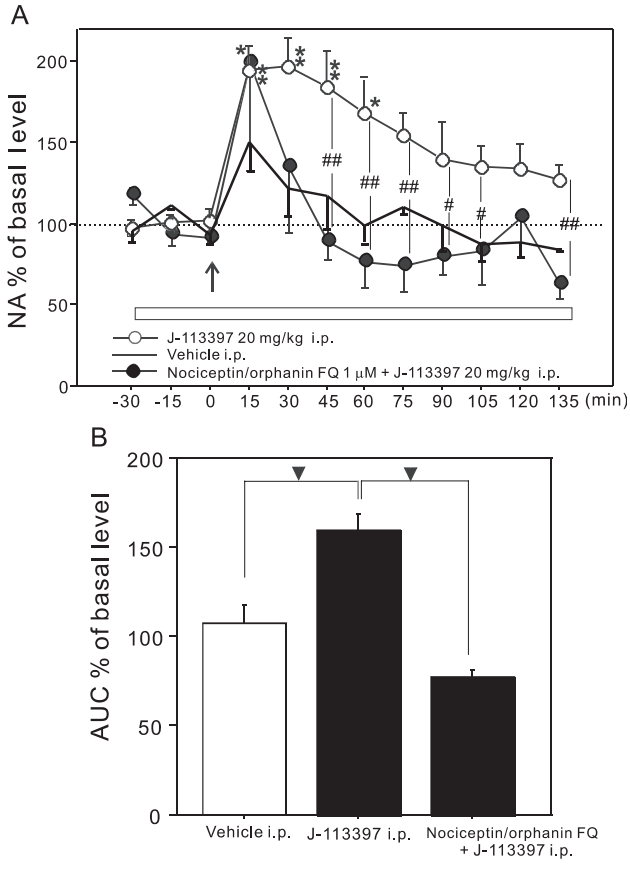

Fig. 2. (A) Effect of J-113397 (20 mg/kg i.p., arrow, $n=4$), vehicle (0.5 ml i.p., arrow, $n=4$) and local infusion of nociceptin/orphanin FQ (1 μM, closed bar) into the basolateral nucleus of the amygdala followed by J-113397 (20 mg/kg i.p., arrow, $n=5$), 30 min after initiation of nociceptin/orphanin FQ infusion, on extracellular noradrenaline in the basolateral nucleus of the amygdala. Data are expressed as a percentage (mean ± S.E.M.) of basal levels. $*P<0.05$, $**P<0.01$ vs. basal level. $\#P<0.05$, $\#\#P<0.01$ as indicated. (B) The comparison of ratio of AUC between three experimental groups. Data are expressed as a percentage (mean ± S.E.M.) of AUC of basal levels. ▼$P<0.05$ as indicated.

different from the J-113397 + nociceptin/orphanin FQ group as well as the vehicle group (Fig. 2B).

4. Discussion

Local infusion of nociceptin/orphanin FQ into the basolateral nucleus of the amygdala caused a small decrease of noradrenaline release in the basolateral nucleus of the amygdala to 70% of controls. These results indicate that not all N/OFQ peptide receptors involved in the inhibition of noradrenaline release—are activated during control conditions. The inhibition of transmitter release by nociceptin/orphanin FQ had been described for various neurotransmitters systems both in vitro and in vivo (Murphy and Maidment, 1999; Schlicker and Morari, 2000).

Next we investigated whether nociceptin/orphanin FQ exerts a tonic inhibition of the release of noradrenaline in the basolateral nucleus of the amygdala of the awake rat. For that aim we have used J-113397. This compound was recently developed as a non-peptide N/OFQ receptor antagonist (Kawamoto et al., 1999). In the dose range of 3–30 mg/kg, it was reported to reverse various nociceptin/orphanin FQ-induced effects from the cellular level to behaviour in rodents (review: Calo' et al., 2002). J-113397 as well as the peptide N/OFQ receptor antagonist [Nphe^1] NC(1–13)NH_2^3), produced antinociceptive (Di Giannuario et al., 2001) and antidepressant-like effect (Redrobe et al., 2002). However, pharmacological studies showed no effect at the level of the neocortex in vitro (Rominger et al., 2002) and in the locus coeruleus in vivo (Okawa et al., 2001).

In the present study, i.p. administration of J-113397 clearly enhanced the release of noradrenaline in the basolateral nucleus of the amygdala, indicating that the basal noradrenaline neuronal activity is under tonic inhibitory control by endogenous nociceptin/orphanin FQ. The fact that J-113397 caused an increase in the release of noradrenaline to about 200% indicates that during resting conditions a substantial part of the release of noradrenaline in the basolateral nucleus of the amygdala is tonically suppressed by endogenous nociceptin/orphanin FQ.

As J-113397 was systemically administered, its site of action is unclear. The amygdaloid complex receives multiple afferents originating from noradrenergic cell groups in the brain stem (Williams et al., 2000; Byrum and Guyenet, 1987). Since the brain stem also contains dense concentration of N/OFQ peptide receptors, the site of action of J-113397 might have been in this brain area. However, the finding that infusion by retrograde microdialysis of nociceptin/orphanin FQ into the basolateral nucleus of the amygdala blocked the effect of J-113397 suggest that the N/OFQ peptide receptors involved are located within the basolateral nucleus of the amygdala.

The initial rise in the release of noradrenaline ($t=15$ min) seen after J-113397 was larger than the effect of a vehicle injection. This initial rise was not blocked by co-infusion of nociceptin/orphanin FQ. Since the release of noradrenaline from the basolateral nucleus of the amygdala in general is very sensitive to vehicle injections (unpublished observations), probably caused by pain and emotional stimuli, other neuronal systems not related to the N/OFQ peptide receptor might be implicated here.

During stressful events and memory consolidation, an enhanced release of noradrenaline in the basolateral nucleus of the amygdala has been demonstrated (Ferry and McGaugh, 2000). The question remains whether this stimulation of the release of noradrenaline is the consequence of removal of nociceptin/orphanin FQ mediated inhibitory tone. Additional studies are required to further characterize this issue.

In conclusion, the present results indicate that noradrenaline transmission in the basolateral nucleus of the amygdala is under tonic inhibitory control by nociceptin/orphanin FQ.

Acknowledgements

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