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Short communication

Orphanin FQ, a novel neuropeptide with anti-stress-like activity

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Abstract

Potential anxiolytic-like properties of intracerebroventricular (i.c.v.) infusion of orphanin FQ (OFQ), a recently discovered neuropeptide, were investigated in the mouse defense test battery, a well-validated anxiolytic screening test. In this model, Swiss mice are directly confronted with a natural threat (a rat) as well as situations associated with this threat. Primary measures taken during and after rat confrontation were flight, risk assessment, defensive attack and escape attempts. Unlike the anxiolytic drug diazepam (3–10 μg/5 μl, i.c.v.), which affected all defensive responses, OFQ (0.3–3 nM/5 μl) only clearly reduced defensive upright postures and biting reactions. Subjects displayed these latter defensive behaviors upon forced contact with the threat stimulus, a situation which is considered to be highly stressful. These results suggest that the OFQ system may not be primarily involved in anxiety-related responses including cognitive aspects (i.e., risk assessment), while it may play a role in the adaptative responses to unavoidable or extreme stress stimuli.

Keywords: Anxiety; Defense behavior; Diazepam; Orphanin FQ; Stress

The heptadecapeptide orphanin FQ (nociceptin, OFQ) is a recently discovered neuropeptide that exhibits structural homology with the opioid peptides and binds to an opioid-like G protein-coupled receptor (called ORL_1) [13,15]. However, despite its close similarity to opioid receptors, this receptor does not bind any of the previously identified opioid peptides or ligands, and OFQ does not activate opioid receptors [15]. As a result, OFQ was classified separately from the opioid system. OFQ is found in the central nervous system (CNS) [16]. ORL_1 receptors are widely distributed in the rat CNS, with high levels found in the cortex, olfactory nucleus, amygdala, claustrum and endopiriform nucleus [1]. The neuroanatomical distribution of OFQ has prompted speculation about its functional role and several studies investigated the behavioral action of this neuropeptide in animal models. Intracerebroventricular (i.c.v.) injection of OFQ was shown to affect locomotion, stimulating activity at low doses (0.05 nM/mouse) and decreasing it at high doses (> 3 nM/mouse) (for review, see Ref. [5]). Using the Morris water maze test, OFQ injected into the CA3 region of the hippocampus at a high dose (10 nM/0.5 μl) was found to impair spatial learning [17]. Furthermore, OFQ reversed opioid-mediated stress-induced analgesia produced by the i.c.v. injection procedure [14]. Because of this latter effect and the distribution of OFQ in brain regions known to module stress and anxiety responses (e.g., amygdala), the effects of this neuropeptide were also investigated in animal models of anxiety. These experiments showed that i.c.v. infusion of OFQ produced anxiolytic-like activity in the elevated plus maze (0.03–0.3 nM/5 μl) and the light/dark (0.3–1 nM/5 μl) tests [12]. Interestingly, the magnitude of the anxiolytic-like effects of OFQ was generally similar to that produced by the classical anti-anxiety agent diazepam, but unlike this latter, the neuropeptide did not produce behavioral suppression at higher doses.

The aim of the present study was to examine further the effects of OFQ on emotional behaviors in an experimental procedure designed for screening anxiety-modulating agents in mice, namely the mouse defense test battery (MDTB) [9]. The MDTB elicits and measures reactions to both present and anticipated threat (i.e a rat). In this well-validated anxiolytic screening test, Swiss mice show an extremely precise delineation of defensive behaviors including flight, risk assessment, defensive threat/attack and escape attempts, with each behavior controlled by specifiable characteristics of the threat stimulus and situation (for reviews, see Refs. [3,7]). Effects were directly

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compared to those of the prototypical anxiolytic diazepam, which was used throughout as a positive control.

Animals: Subjects were naive male Swiss mice aged 10 weeks at the time of testing. They were obtained from Iffa-Credo (L'Arbresle, France). Prior to experimental testing, they were housed singly in standard cages (mice: $30 \times 20 \times 14$ cm; rats: $44 \times 30 \times 20$ cm) containing a constant supply of food pellets and water. All animals were maintained under standard laboratory conditions (22–24°C; relative humidity: 55–65%) and kept on a 12-h light/dark cycle with light onset at 0600 h. Male Long Evans rats (400–500 g) (killed by CO_2 inhalation) were used as stimulus.

MDTB: The test was conducted in an oval runway, 0.40 m wide, 0.30 m high, and 4.4 m in total length, consisting of two 2-m straight segments joined by two 0.4-m curved segments and separated by a median wall ($2.0 \times 0.30 \times 0.06$). The apparatus was elevated to a height of 0.80 m from the floor to enable the experimenter to easily hold the rat, while minimizing the mouse's visual contact with him. All parts of the apparatus were made of black Plexiglas. The floor was marked every 20 cm to facilitate distance measurement. Activity was recorded with video cameras mounted above the apparatus. The room illumination was provided by one red neon tube fixed on the ceiling and two desk lamps with red bulbs placed on two tables (elevated to a height of 1 m) located 1 m away from the runway. The light intensity in the runway was 7 lx. Experiments were performed under red light between 0930 and 1500 h. The experimenter was unaware of the drug treatment.

(a) Effects on spontaneous locomotor activity: the pre-test: A subject was placed into the runway for a 3-min familiarization period during which line crossings were recorded. *(b) Effects on flight responses: the rat avoidance test*: Immediately after the 3-min familiarization period, the hand-held dead rat was introduced into the runway and brought up to the subject at a speed of approximately 0.5 m/s. Approach was terminated when contact with the subject was made or the subject ran away from the approaching rat. If the subject fled, avoidance distance (the distance from the rat to the subject at the point of flight) was recorded. This was repeated five times. Mean avoidance distance (cm) was calculated for each subject. *(c) Effects on risk assessment: the chase test*: The hand-held rat was brought up to the subject at a speed of approximately 2.0 m/s. During the chase, the number of stops and orientations (subject stops, then orients the head toward the rat) were recorded. *(d) The straight alley test*: After the chase was completed, the runway was then converted to a straight alley by closing a door at one end. During 30 s, the hand-held rat remained at a constant distance of 40 cm from the subject and immobility time was recorded. *(e) Effects on defensive threat / attack responses: the forced contact test*: Finally, the experimenter brought the rat up to contact the subject. For each such contact, upright postures and bites by the subjects were noted. This was repeated three times. The results were expressed as mean number of bites. *(f) Effects on contextual defense: the post-test*: Immediately after the forced contact test, the rat was removed and the door opened. Escape attempts including wall rears, wall climbs, and jump escapes were recorded during a 3-min session. See Ref. [10] for additional details on this test battery. Data were analysed by one-way ANOVA. Subsequent comparisons between treatment groups and control were carried out using Dunnett's *t*-test

Drugs: Synthetic OFQ was purchased from RBI (Natick, USA) and diazepam was synthesized by the Chemistry Department, Synthélabo Recherche. Both drugs were freshly prepared in physiological saline for local i.c.v. delivery as previously described [6]. While OFQ was dissolved, diazepam was lightly suspended. A 26-gauge stainless steel cannula, 3 mm in length, was inserted into the ventricle at the intersection of midline and a line parallel to the anterior tip of the ear. Drugs or saline were infused over a 5-s period in a 5-μl volume. The accuracy of this procedure was examined by visualization of dye in the ventricular system. Doses of diazepam and OFQ were chosen on the basis of previous results in behavioral experiments [4,12].

(a) Effects on spontaneous locomotor activity: the pre-test: Table 1 shows that prior confrontation with the rat, diazepam ($F_{3,47} = 5.2$, $P < 0.01$) but not OFQ significantly decreased the number of line crossings. *(b) Effects on flight responses: the rat avoidance test*: Table 2 shows that the avoidance distance was significantly modified by diazepam ($F_{3,44} = 4.2$, $P < 0.05$) and by OFQ ($F_{4,44} = 4.8$, $P < 0.01$). Post-hoc analysis indicated that diazepam (3 and 10 μg) and OFQ (3 nM) significantly reduced avoidance distance. *(c) Effects on risk assessment: chase test*: Table 2 shows that diazepam (3 and 10 μg/kg), but not OFQ significantly decreased the number of stops ($F_{3,47} = 19$, $P < 0.001$) and orientations ($F_{3,47} = 3.4$, $P < 0.05$). *(d) The straight alley test*: Table 1 shows that neither drug significantly modified immobility time. *(e) Effects on de-*

Table 1
Measures of locomotor activity in the runway cage before (line-crossings) and during (immobility) confrontation with a rat. Drugs were administered i.c.v. 15 min before the beginning of the test. Data represent mean ± S.E.M. $^*P < 0.05$ (Dunnett's *t*-test). $n = 11–12$

	Dose	Line crossings	Immobility (s)
Diazepam	0	111.3 ± 6.2	10.9 ± 0.7
(μg/5 μl)	1	107.7 ± 5.9	12 ± 0.5
	3	103.1 ± 9.1	13.3 ± 0.9
	10	77.1 ± 5.3*	12.8 ± 0.9
OFQ	0	103.3 ± 8.5	13.2 ± 0.8
(nM/5 μl)	0.1	108.9 ± 9.4	16.5 ± 1.7
	0.3	101.9 ± 11	17.8 ± 2.2
	1	107.4 ± 8.3	17.1 ± 1.4
	3	72.5 ± 8.4	17.3 ± 1.7

Table 2
Measures of anxiety in the runway cage during (avoidance distance, orientations, stops) and after (escape attempts) confrontation with a rat. Drugs were administered i.c.v. 15 min before the beginning of the test. Data represent mean ± S.E.M. * $P < 0.05$ (Dunnett's *t*-test). $n = 11–12$

	Dose	Avoidance distance (cm)	Orientations	Stops	Escape attempts
Diazepam	0	128.6 ± 6.4	5.7 ± 0.5	8.9 ± 0.2	17.3 ± 5
(μg/5 μl)	1	117.1 ± 6.9	4.6 ± 0.5	8.3 ± 0.3	12 ± 3.5
	3	102.2 ± 10.1*	4.2 ± 0.5*	7.3 ± 0.3*	9.3 ± 2.7*
	10	83.1 ± 12.5*	3.5 ± 0.5*	6.1 ± 0.3*	6.4 ± 1.9*
OFQ	0	116.5 ± 13.8	5.1 ± 0.7	7.8 ± 0.8	15.6 ± 4.5
(nM/5 μl)	0.1	134.6 ± 10.1	6.6 ± 1.1	8.7 ± 0.8	21.1 ± 6.4
	0.3	89.1 ± 8.3	5.7 ± 0.8	7.5 ± 0.7	12.5 ± 3.8
	1	113.1 ± 6.8	5.1 ± 0.8	7.5 ± 0.7	20.8 ± 6.3
	3	70.9 ± 7.9*	4.6 ± 0.8	6.4 ± 0.8	11.1 ± 3.3

fensive threat / attack responses: the forced contact test: Fig. 1 shows that diazepam and OFQ decreased significantly the number of upright postures (diazepam: $F_{3,47} = 11.5$, $P < 0.001$; OFQ: $F_{4,52} = 5.4$, $P < 0.01$) and bitings (diazepam: $F_{3,47} = 27.6$, $P < 0.001$; OFQ: $F_{4,52} = 4.8$, $P < 0.01$) at several doses. *(f) Effects on contextual defense: the post-test*: Table 2 shows that diazepam (3 and 10 μg) ($F_{3,47} = 4.8$, $P < 0.01$) significantly decreased the number of escape attempts from the runway cage following the removal of the rat.

The present results show that the neuropeptide OFQ attenuated some but not all defensive behaviors of Swiss mice confronted with a rat-stimulus, thereby confirming that this compound may modulate emotional behaviors. In the pre-test, locomotor activity was not significantly affected by OFQ, although it is worth mentioning that effects on line crossings just failed to reach statistical significance at the highest dose. The observation that OFQ at the doses tested had only limited influence on motor performance was strengthened by the data from the straight alley test, where the drug did not affect spontaneous motor activity as shown by the lack of significant effects on immobility time. Clearly, these findings have a direct bearing on the issue of the behavioral selectivity of any changes observed in defensive responding.

In the rat avoidance test, diazepam and OFQ decreased flight reactions after the rat was introduced into the runway. Importantly, diazepam reduced avoidance measure at a dose below the level required to decrease activity (3 μg). In contrast, OFQ reduced avoidance distance at a dose (3 nM) which also slightly impaired line crossings, suggesting that these effects may have been contaminated by behavioral suppression. Extensive pharmacological evaluation of the MDTB has demonstrated that anti-panic compounds specifically affect flight responses, decreasing most notably avoidance distance (for reviews, see Refs. [3,7]). The results obtained with OFQ on flight suggest that this neuropeptide system may not play a major role in the modulation of panic-related behaviors. During the chase test, diazepam, but not OFQ reduced risk assessment activities (i.e., stops and orientations), whereas both drugs clearly reduced defensive attack responses (i.e., upright postures and bitings) upon forced contact with the rat. The action of OFQ on defensive attack is not confounded by decreases in locomotor activity as positive effects occurred at a dose as low as 0.3 nM. It is also unlikely that these effects are due to decreased pain sensitivity, since OFQ did not produce analgesia unless injected at high doses (for review, see Ref. [5]).

Risk assessment consists of various information-gathering activities which occur primarily in the context of uncertainty concerning the threat characteristics of the stimulus [2]. Because of a potential isomorphism between risk assessment activities and certain key features of gener-

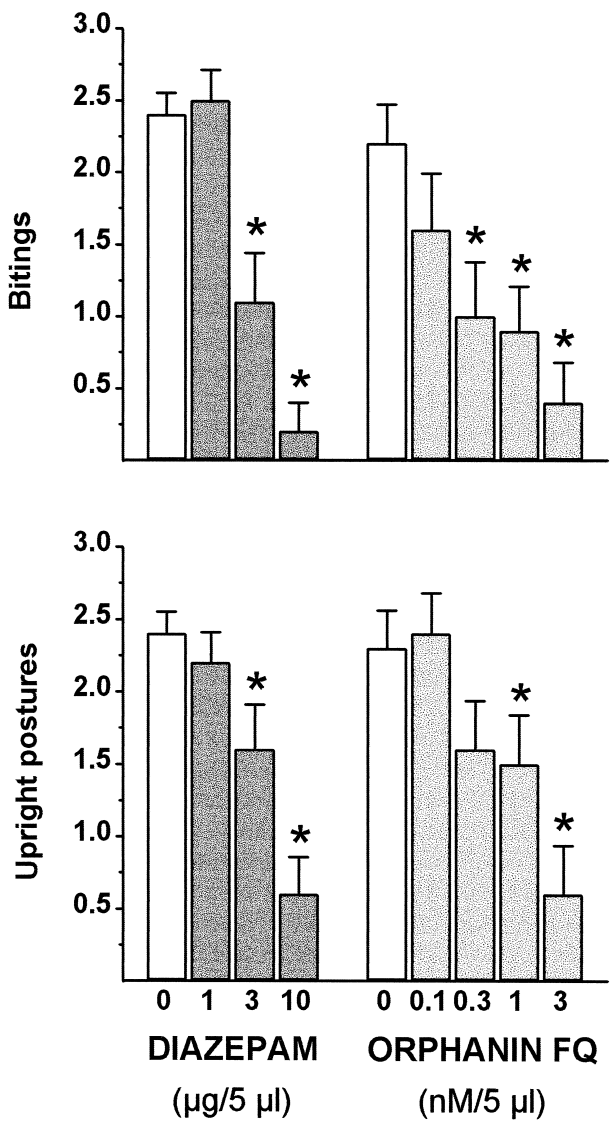

Fig. 1. Measures of anxiety in the runway cage upon forced contact with a rat. Drugs were administered i.c.v. 15 min before the beginning of the test. Data represent mean ± S.E.M. * $P < 0.05$ (Dunnett's *t*-test). $n = 11–12$.

alized anxiety disorders, it has been suggested that they may represent a pattern of responses particularly sensitive to anxiolytic drug challenge [2]. This was subsequently confirmed by extensive pharmacological investigations showing that benzodiazepines affected these responses [3]. Similarly, earlier findings from the MDTB revealed that benzodiazepines reduced defensive attack behaviors, thereby suggesting that they may also be a reliable index of anxiety [3]. However, unlike risk assessment, which includes cognitive aspects of defensive behaviors, defensive attack reflects more intense and ''affective''-orientated defenses [8]. In addition, the forced contact situation is particularly stressful for animals since they have no possibility to escape and confrontation with the threat stimulus is unavoidable. Whether this may indicate that the OFQ system may play a role in the adaptative responses to unavoidable or extreme stress stimuli remains to be established. However, this idea would be in agreement with a recent study showing that OFQ deficient mice displayed anxiolytic-like activity in the light/dark and the acoustic startle reflex tests only when animals have been exposed to social stress [11].

In conclusion, the current findings show that central administration of the heptadecapeptide OFQ is able to reduce defensive behaviors of mice exposed to a natural threatful stimulus. However, unlike the anxiolytic diazepam, OFQ displayed positive effects only on terminal defense reactions, displayed when stressful stimuli are unavoidable. This may indicate that the OFQ system is activated primarily in highly stressful situations.

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