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Activation of brain NOP receptors attenuates acute and protracted alcohol withdrawal symptoms in the rat

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Abstract

BACKGROUND—Alcohol withdrawal, refers to a cluster of symptoms that may occur from suddenly ceasing the use of alcohol after chronic or prolonged ingestion. These symptoms make alcohol abstinence difficult and increase the risk of relapse in recovering alcoholics. In previous studies, we demonstrated that treatment with N/OFQ significantly reduces alcohol consumption and attenuates alcohol-seeking behaviour induced by environmental conditioning factors or by stress in rats. In the present study we evaluated whether activation of brain NOP receptors may also attenuate alcohol withdrawal signs in rats.

METHODS—For this purpose animals were subjected to a 6 day chronic alcohol intoxication (by intragastric administration) and at 8, 10 and 12 hours following cessation of alcohol exposure they were treated intracerebroventricularly (ICV) with N/OFQ (0.0, 1.0 and 3.0 μ g/rat). Somatic withdrawal signs were scored after ICV treatment. In a subsequent experiment, to evaluate N/OFQ effects on alcohol withdrawal-induced anxiety another group of rats was subjected to ethanol intoxication and after one week was tested for anxiety behavior in the elevated plus maze (EPM). In the last experiment an additional group of rats was tested for anxiety elicited by acute ethanol intoxication (hangover anxiety). For this purpose, animals received an acute dose (3.0 g/kg) of 20% alcohol and 12-h later were tested in the EPM following ICV N/OFQ (0.0, 1.0 and 2.0 μ g/rat).

RESULTS—Results showed that N/OFQ significantly reduced the expression of somatic withdrawal signs and reversed anxiety-like behaviors associated with both chronic and acute alcohol intoxication. N/OFQ did not affect anxiety scores in nondependent animals.

CONCLUSIONS—The present findings suggest that the N/OFQ-NOP receptor system may represent a promising target for the development of new treatments to ameliorate alcohol withdrawal symptoms.

Keywords

Nociceptin; Orphanin FQ; Alcoholism; Withdrawal; Anxiety

INTRODUCTION

Alcohol withdrawal refers to a cluster of symptoms that occurs from suddenly ceasing alcohol use after chronic or prolonged ingestion. More than 70% of individuals presenting for alcohol detoxification, show significant alcohol withdrawal symptoms (Myrick and Anton, 1998). The most common signs include tremor, anxiety, insomnia, agitation, hypervigilance, irritability, and sometimes seizures (Hunter et al., 1974; Saitz, 1998). The emergence of these withdrawal symptoms makes attempts to abstain from alcohol difficult and increases the risk of relapse in recovering alcoholics (Anton, 1999; Spanagel, 2003).

Neural hyperexcitability following abrupt cessation of alcohol use is a core feature of the ethanol withdrawal syndrome (Becker, 2000; Saitz, 1998; Victor, 1970). This withdrawal-related hyperexcitability has been linked to neuroadaptive changes in various brain systems by chronic alcohol use (Dodd et al., 2000; Hoffman, 2003; Krystal et al., 2003; Kumar et al., 2004; Sanna et al., 2003), with the GABAergic and the glutamatergic systems playing a pivotal role (Bradford 1995; Burnham 1989; Collingridge and Singer, 1990). Chronic alcohol use induces an upregulation of NMDA receptor numbers and function (Bao et al., 2001; Chandler et al., 1999; Follesa and Ticku, 1996; Trevisan et al., 1994) accompanied by a simultaneous decrease of GABA_A receptor activity (Kumar et al. 2004). Once ethanol intake is abruptly discontinued, imbalance between glutamate excitatory and GABA inhibitory activity leads to a neural hyperexcitability state causing the appearance of somatic alcohol withdrawal signs (De Witte, 2004).

Additionally, in laboratory animals, a role of the corticotropin-releasing factor system (CRF) in mediating negative emotional states during protracted withdrawal from chronic as well as acute alcohol intoxication has been established. For example, withdrawal from chronic ethanol exposure profoundly elevates extracellular CRF levels in extrahypothalamic sites (Merlo-Pich et al., 1995; Richter and Weiss, 1999; Weiss et al., 2001). This hypersecretion of CRF results in aversive and anxiety-like behavioral manifestations reversible by functional antagonism of CRF transmission (Huang et al., 2010; Rassnick et al., 1993; Valdez et al., 2002). CRF receptor antagonism also prevents the expression of anxiety-like responses following acute administration of an intoxicating dose of ethanol (Gehlert et al., 2007).

Another player relevant for the alcohol withdrawal syndrome could be the Nociceptin/orphanin FQ (N/OFQ) system. N/OFQ is a 17-amino acid peptide that shows structural homology with opioid peptides, particularly dynorphin A (Meunier et al., 1995; Reinscheid et al., 1995). N/OFQ binds with high affinity to the opioid receptor-like 1, now included in the opioid receptor family and renamed NOP receptor, but does not activate the classical μ , κ , and δ opioid receptors. Converging evidence from electrophysiological and neurochemical studies suggests that stimulation of NOP receptors by N/OFQ produces a significant presynaptic inhibition of glutamate transmission in several brain regions including the hippocampus and amygdala (Marti et al., 2002; Meis and Pape, 2001; Tallent et al., 2001). Moreover, N/OFQ inhibits stress-induced ethanol-seeking and exerts general anti-stress effects by acting as a functional antagonist of extrahypothalamic CRF transmission (Ciccocioppo et al., 2002; Ciccocioppo et al., 2003; Martin-Fardon et al., 2000). Finally, elevated plus maze (EPM) experiments showed that administration of N/OFQ into the lateral ventricle or the bed nucleus of the stria terminalis prevents the anxiogenic effect of intracerebroventricular (ICV) CRF injections (Vitale et al 2006; Rodi et al., 2008).

Based on these findings, we hypothesize that activation of NOP receptors by N/OFQ should:

- reduce the expression of somatic withdrawal signs that, to a large extent, are dependent

upon glutamatergic hyperactivity; ii) blunt heightened anxiety elicited by chronic ethanol administration as well as after an intoxicating dose of alcohol, as they are regulated by extrahypothalamic CRF transmission and are prevented by antagonism of this system (Gehlert et al., 2007; Rassnick et al., 1993).

MATERIALS AND METHODS

Subjects

Male Wistar rats (Charles River, Calco, Italy) weighing 300–350 g at the beginning of the experiments were used. Animals were housed in single cages in a room on a 12 hr light/dark cycle (lights off at 8:00 A.M.) at constant temperature (20–22°C) and humidity (45–55°), with *ad libitum* food (4RF; Mucedola, Settimo Milanese, Italy) and water. Rats were handled once a day for 5 min during the first week after arrival. All procedures were conducted in adherence to the *European Community Council Directive for Care and Use of Laboratory Animals* and the *NIH Guide for the Care and Use of Laboratory Animals*.

Intracranial surgery

For intracranial surgery, animals were anesthetized by intramuscular injection of 100–150 µl of a solution containing tiletamine chlorohydrate (58.17 mg/ml) and zolazepam chlorohydrate (57.5 mg/ml). For intracerebroventricular (ICV) administrations, a guide cannula aimed at the left lateral ventricle was stereotaxically implanted and cemented to the skull (with respect to bregma: AP – 0.8; ML + 1.8 and DV – 2 from the skull surface (Paxinos and Watson, 1998).

Drugs

Nociceptin/orphanin FQ (Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asp-Glu) was synthesized at the Department of Pharmacological Sciences and Biotechnology Centre (University of Ferrara, Ferrara, Italy), and was a generous gift of Dr. R. Guerrini. N/OFQ was dissolved in sterile isotonic saline and injected ICV in a volume of 1.0 µl by means of a stainless-steel injector 2.5 mm longer than the guide cannula, so that its tip protruded into the ventricle. To verify the cannula placement, 1 µl of black India ink was injected ICV immediately before the rat was sacrificed, under anaesthesia, and ink diffusion into the ventricles was histologically evaluated.

To induce dependence, ethanol was administered orally (gavage) in a liquid-diet containing 20% w/v ethanol, 6% w/v saccharin and powdered milk (Mellin 2, Mellin S.p.A). For ethanol-induced anxiety EtOH (20% v/v in physiological saline) was administered intraperitoneally in a volume of 18.75 ml/kg.

Induction of ethanol dependence

Intoxication—Rats were made dependent by repeated daily intragastric ethanol administrations (gavage) as previously described (Braconi et al., 2010). Briefly, ethanol was administered four times per day, between 8:00 am and 8:00 pm, for six consecutive days. On the first day, rats were intubated with a total of 11.0 g/kg ethanol in four fractional doses of 3.0, 3.0, 2.5, and 2.5 g/kg of ethanol administered at 4 h intervals. On days 2–6, 12 h after the last intubation on the preceding day, rats received a total of 10.0 g/kg ethanol in four fractional doses of 3.0, 2.5, 2.5, and 2.0 g/kg, again separated by 4 h intervals. Rats serving as non-dependent controls received intragastric administration of vehicle in a total volume of 22 ml/kg/day at time intervals and relative volumes identical to those in ethanol-treated rats.

Measurement of blood alcohol levels (BALs)—Tail blood (approximately 200 µl) was collected on day 5 immediately before the first daily dose of ethanol and 1 h after the second and third daily doses (Braconi et al., 2010). Samples were collected on ice and immediately centrifuged (10 min, 5000 rpm). BALs then were assayed from 5 µl plasma aliquots using an oxygen-rate alcohol analyzer (Analox Instruments, Lunenburg, MA).

Measurement of ethanol withdrawal signs—Using a withdrawal rating scale adapted from Macey et al. (1996), ethanol withdrawal signs including irritability to touch (Vocalization), Tail Rigidity, Tail Tremors and Ventro-medial Limb Retraction (VmLR) were scored on day 7 [for details see, Braconi et al. (2010)]. Each sign was assigned a score of 0–2, based on the following severity scale: 0 = no sign, 1 = moderate, 2 = severe. The sum of the four observation scores (0–8) was used as a quantitative measure of withdrawal severity. For these behavioral observations, animals were individually transferred from their home cages to a quiet observation room to avoid extraneous stimulation.

Elevated Plus Maze: To measure anxiety-like responses, the elevated plus-maze (EMP) test was used. The apparatus consisted of two black wooden open arms and two enclosed arms (40 cm high walls), arranged such that the respective arms were opposite to each other. The maze, elevated 50 cm above the floor, was cleaned with water and dried after each trial. The 5 min test procedure began when the animal was placed in the centre of the maze, facing a closed arm. The percentage of time spent in open arms and the number of open arm entries were used as measures of anxiety-like behavior, while the number of total arm entries was used as an indicator of general motor activity (Cruz et al., 1994).

Experiments

Experiment 1: Effect of ICV N/OFQ treatment on the expression of somatic alcohol withdrawal signs—To evaluate the effect of N/OFQ on alcohol withdrawal signs, animals (n=24) were divided into 3 groups at the end of the intoxication cycle (n=8/group) and received N/OFQ at doses of 0.0, 1.0 and 3.0 µg/rat (ICV) 10 min before behavioral measures of ethanol withdrawal. A fourth group of rats (n=8) was prepared in parallel and served as a control. This group received alcohol vehicle during the intoxication phase and N/OFQ vehicle injections prior to withdrawal testing. Behavioral observations were carried out in all animals at 8, 10, and 12 h after the last ethanol (or vehicle) dose.

Experiment 2: Effect of ICV N/OFQ treatment on anxiety-like behaviour following chronic ethanol intoxication—To evaluate the effect of N/OFQ on anxiety-like behavior in post-dependent rats, animals (n=47) were divided into 2 groups. As in Experiment 1, one group of rats (n=24) was made ethanol dependent while the second group (n=23) was treated with alcohol vehicle and served as a non-dependent control. The EPM test was carried out one week after completion of the ethanol intoxication cycle. Prior to testing, post-dependent and non-dependent rats were subdivided into 3 groups (n=7–8) and treated ICV with N/OFQ (1.0 and 2.0 µg/rat) or its vehicle administered 10 min before the EPM test. Behavioral observations were performed by an experimenter blind to the treatment condition. As described in a previous work, to attenuate the potential hypolocomotor effect of N/OFQ, the peptide (2.0 µg/rat) was ICV injected to all rats 24 hour before the EPM test (Vitale et al 2006). After the injection the animals were returned in their home cages.

Experiment 3: Effect of ICV N/OFQ on anxiety-like behaviour following acute ethanol intoxication—As previously described by Gehlert et al. (2007), alcohol-induced anxiety was measured by treating rats (n=23) IP with 3.0 g/kg of 20% alcohol and testing them 12 hrs later on the EPM. Rats (n=7–8/group) were treated with N/OFQ (1.0 and 2.0 µg/

kg) or its vehicle 10 min prior to the EPM test. Another control group (n=7) received alcohol vehicle and 12 hours later, prior to the EPM test, were injected with N/OFQ vehicle. Also in this case to attenuate the hypolocomotor action of N/OFQ, all rats including the control group received one ICV peptide (2.0 µg/rat) injection 24 hour before the EPM test.

Statistical analysis—Initially a total withdrawal score was obtained for each animal based on the cumulative scores of the four different withdrawal signs (Vocalization, VmLR, Tail Rigidity, Tail Tremors) at the 3 different time-points monitored (8 h, 10 h and 12 h). The effects of N/OFQ on the total withdrawal score was analyzed by the non-parametric Friedman test, that was also used to analyze the overall effect of N/OFQ on individual withdrawal signs. Where appropriate, the effect of N/OFQ at different time-points was also assessed by individual comparison among individual means using the non-parametric Mann-Whitney *U* Test.

EPM behavior was evaluated using a between subjects two-way (Experiment 2) or one-way ANOVA (Experiment 3), followed by Newman-Keuls *post-hoc* tests.

RESULTS

Experiment 1: Effect of ICV N/OFQ treatment on the expression of somatic alcohol withdrawal signs

On day 5 BALs measured immediately before the first alcohol daily injection, 1 h after the second and the third daily doses were 0.20 ± 0.22 , 182.0 ± 0.57 and 236.0 ± 0.28 mg%, respectively. This indicates that during alcohol administration BALs were maintained at intoxication levels, while returning to low levels between intoxication days (Braconi et al., 2010).

Mann-Whitney *U* tests were used to compare the withdrawal scores of N/OFQ vehicle (0.0µg/rat) treated rats to non intoxicated controls at different time points. These analyses revealed overall significant behavioral effects of ethanol withdrawal ($p < 0.001$). The Friedman test was used to evaluate the effect of N/OFQ on total abstinence scores. These tests (Fig. 1) yielded a significant overall effect of N/OFQ on total withdrawal scores ($p < 0.05$). As shown in Fig. 2, analysis of individual withdrawal signs revealed a significant overall effect of N/OFQ on vocalization ($p < 0.05$), tail rigidity ($p < 0.05$), tail tremors ($p < 0.05$) and in VmLR ($p < 0.05$). Subsequent Mann-Whitney tests showed that at both N/OFQ doses (1.0 and 3.0 µg) the peptide significantly reduced vocalization following 8 ($p < 0.05$; $p < 0.01$, respectively) and 10 hours ($p < 0.01$) -from alcohol intoxication procedure (Fig. 2A). Tail rigidity was significantly reduced at 10 and 12 hours ($p < 0.05$ and $p < 0.01$), respectively for both doses tested (Fig. 2B). Tail tremors were significantly ($p < 0.05$) lowered only at the highest N/OFQ dose at the 12 hour time point (Fig. 2C). N/OFQ (3.0 µg) significantly increased VmLR responses ($p < 0.05$) but only at 12 hours post withdrawal (Fig. 2D).

Experiment 2: Effect of ICV N/OFQ treatment on anxiety-like behaviour following chronic ethanol intoxication

For the percentage of time spent in the open arms of the EPM, ANOVA revealed a significant difference between the non-dependent and post-dependent groups ($F_{1,41}=7.15$, $p < 0.01$). ANOVA also revealed a significant effect of N/OFQ ($F_{2,41}=3.23$, $p < 0.05$) on open arm time and a significant treatment $\times$ intoxication interaction ($F_{2,41}=11.07$, $p < 0.01$). Newman Keuls *post-hoc* tests showed that post-dependent rats spent significantly less time in the open arms of the EPM than nondependent controls ($p < 0.001$). N/OFQ significantly

increased the percentage of open arm time in post-dependent rats at both 1.0 ($p<0.05$) and 3.0 μg ($p<0.001$), but was without effect in non-dependent controls (Table 1, Fig 3A).

For the percentage of entries onto open arms, ANOVA confirmed a significant difference between the non-dependent and post-dependent groups ($F_{1,41}=17.44$, $p<0.001$). Additionally, ANOVA revealed a significant N/OFQ treatment effect ($F_{2,41}=4.82$, $p<0.05$) on open arm entries and a significant treatment $\times$ intoxication (dependent vs nondependent) interaction ($F_{2,41}=10.11$, $p<0.001$). Newman Keuls *post-hoc* tests confirmed that post-dependent rats showed a significantly higher number of open arm entries than non-dependent controls ($p<0.01$). In post-dependent rats, N/OFQ significantly increased the percentage of open arm entries at both 1.0 ($p<0.01$) and 3.0 μg ($p<0.001$). The peptide did not alter open arm entries in non-dependent controls (Table 1, Fig. 3B).

For the percentage of entries onto closed arms, ANOVA revealed significant difference between the nondependent and post-dependent groups ($F_{1,41}=7.5$, $P<0.01$) a significant N/OFQ treatment effect ($F_{2,41}=6.4$, $P<0.01$) and a significant treatment $\times$ intoxication interaction ($F_{1,41}=4.3$, $P<0.05$). Newman Keuls *post-hoc* tests confirmed that in post-dependent rats the highest dose of N/OFQ reduced the percentage of entries into closed arms ($p<0.01$). No other significant effects were observed (Table 1, Fig. 3C).

Finally, the total number of entries (number of open arms + closed arms entries) was unchanged by alcohol intoxication nor was it modified by N/OFQ treatment (Table 1).

Experiment 3: Effect of ICV N/OFQ treatment on anxiety-like behaviour following acute ethanol intoxication

Withdrawal from a single large dose of alcohol elicits a marked anxiogenic-like response (Fig. 4), resulting in a significant reduction of the percentage time spent in open arms of the EPM ($F_{3,26}=5.70$ $p<0.01$). Post-hoc analysis confirmed a significant reduction in open arm time in rats injected with alcohol compared to ICV vehicle injected controls ($p<0.01$). This effect was completely reversed by treatment with N/OFQ (Table 2, Fig 4A).

The percentage of open arm entries was also reduced following alcohol injection but did not reach significance (Table 2, Fig 4B).

Lastly, ANOVA showed a significant overall difference in the percentage of entries onto closed arm $F_{3,26}=15.6$, $p<0.001$. Newman Keuls *post-hoc* tests revealed a significant reduction in closed arm entries in the group treated with alcohol and injected with N/OFQ vehicle compared to the control group that received alcohol and peptide vehicles ($P<0.01$). Treatment with 1.0 and 2.0 $\mu\text{g}/\text{rat}$ of N/OFQ significantly ($p<0.01$) increased the percentage of closed arm entries compared to ICV vehicle treated rats (Table 2, Fig. 4C).

Finally a significant reduction in the number of total arm entries was observed ($F_{3,26}=13.27$ $p<0.01$). This effect, was due to a reduction of arm entries in animals injected with alcohol and treated with N/OFQ vehicle ($p<0.01$).

DISCUSSION

The results show that activation of brain NOP receptors by ICV administration of N/OFQ, significantly attenuates the expression of somatic withdrawal signs in ethanol-intoxicated rats. For most of the somatic withdrawal signs, abstinence scores were significantly reduced over the entire 12 h observation period. In addition, EPM results demonstrated that N/OFQ significantly reduces the anxiogenic-like response associated with chronic ethanol intoxication and tested during protracted withdrawal, as well as following an acute

intoxicating dose of alcohol. The anxiolytic action of N/OFQ was specific and it is unlikely that it was influenced by hypolocomotor effects occasionally observed following by its administration (Vitale et al 2006). In fact, in rats subjected to acute intoxication the number of closed arm entries were decreased by alcohol injection and N/OFQ reversed this effect. In postdependent rats a significant reduction in the number of closed arm entries was observed only at the highest dose (2.0 µg/rat) of N/OFQ. While the time spent in the open arm and the number of open arm entries, two major predictors for anxiolytic-like actions were both significantly increased at a lower N/OFQ dose (1.0 µg/rat). In addition, the analysis of the total number of arm entries showed no effect of N/OFQ treatment indicating that the number of closed arm entries reduction was accompanied by a concomitant increase in the number of open arm entries. Altogether these findings show that the reduction in the number of closed arm entries at 2 µg/rat of N/OFQ is paradoxical and depends upon increased animals' propensity to stay in the open arms of the EPM (Cruz et al 1994).

Finally, in the present study the attenuation of alcohol withdrawal signs and anxiety by N/OFQ were both obtained at doses below 2 µg/rat. At this dose range it is well known that the pharmacological effects of N/OFQ are completely prevented by selective NOP receptor antagonists (Vitale et al 2006; Burmeister and Kapusta 2007).. strongly suggesting that the present behavioral effects produced by N/OFQ administration are mediated by activation of NOP receptors and not by non specific (off target) N/OFQ actions.

Previous studies demonstrated that exposure to intoxicating doses of ethanol leads to an upregulation of NMDA receptor numbers and function, with a simultaneous decrease in GABA_A receptor function (Bao et al., 2001; Chandler et al., 1999; Follesa and Ticku, 1996; Trevisan et al., 1994; for review see also Kumar et al 2004). Abrupt discontinuation of alcohol exposure perturbs the equilibrium between these two systems leading to neuronal hyperexcitability and appearance of the somatic signs of alcohol withdrawal (De Witte, 2004). This is further supported by data indicating that administration of GABAergic agonists such as clonazepam and lorazepam, or NMDA receptor antagonists such as MK-801 or CGP 39551 are both able to attenuate the severity of ethanol withdrawal symptoms (Jung et al., 2000; Liljequist, 1991; Morriset et al., 1990; Strzelec and Czarnecka, 2001; Thomas et al., 1997). N/OFQ has been shown to have a general inhibitory effect on presynaptic neuronal excitability and neurotransmitter secretion (Calo' et al., 2000), with the glutamatergic system being extremely sensitive to these effects (Meis and Pape, 2001; Nicol et al., 1996; Schlicker et al., 2000; Tallent et al., 2001). Therefore, given the pivotal role of glutamate in neuronal hyperexcitability associated with alcohol withdrawal, it seems likely that the protective effects of N/OFQ on physical signs of withdrawal are mediated by its action to blunt brain glutamatergic neurotransmission, a hypothesis that will require further direct testing.

Neuroadaptive changes in central amygdala (CeA) neurotransmission have also been linked to excessive drinking and emotional disturbances (i.e., anxiety) occurring during alcohol withdrawal, and a wealth of data shows that the heightened anxiety-like phenotype in post-dependent rats is primarily linked to aberrant CRF neurotransmission in this brain area (Koob, 2008; Koob, 2009). Indeed, electrophysiological experiments have shown that early alcohol withdrawal is associated with increased CeA GABAergic activity (Roberto et al., 2004), an effect that is mediated by activation of CRF neurotransmission and that is inhibited by bath application of N/OFQ (Cruz et al., 2009a; Cruz et al., 2009b; Roberto et al., 2006). A CRF-N/OFQ link in the CeA is also supported by data indicating that anxiety during ethanol withdrawal is associated with increased CeA CRF levels (Merlo-Pich et al., 1995; Rassnick et al., 1993), and that N/OFQ, like CRF₁ receptor antagonists, more potently reduce anxiety-like behaviors in post-dependent subjects than in controls (Martin-Fardon et al., 2010). Finally, earlier findings indicate that N/OFQ elicits anxiolytic-like actions

following direct intra-CeA injection, an effect that is prevented by administration of the GABA-A antagonist bicuculline (Uchiyama et al 2008). Moreover, our data indicate that N/OFQ prevent anxiety-like responses associated to acute alcohol intoxication, an effect previously described following administration of CRF1 receptor antagonists (Gelbert et al 2007). Altogether these findings further support the possibility that the anxiolytic-like effects of N/OFQ depends upon its ability to blunt CRF-induced increase of GABA-A activity in the CeA (Ciccocioppo et al., 2002, 2003; Economidou et al., 2008).

It is important to note that in the present study we observed a significant anxiolytic of N/OFQ in postdependent and acutely intoxicated rats while no significant peptide effects were observed in nondependent animals. This finding contrasts with previous reports describing the anxiolytic effect of N/OFQ in rats tested under basal condition as well (Uchiyama et al 2008; Vitale et al 2006). On the other hand, our results are consistent with those published by Rodi et al (2008) where it was shown that in rats treated with CRF, N/OFQ elicits anxiolytic-like actions at doses as low as 0.5 µg/rat/ICV while in rats treated with CRF vehicle N/OFQ was effective only at ICV doses higher than 2.0µg/rat. These findings indicates that the anxiolytic effects of N/OFQ are state-dependent and are more pronounced under highly stressful condition (i.e., alcohol intoxication, stress elicited by intracranial CRF administration).

At present, one of the most frequently used pharmacological remedies for alcohol withdrawal are benzodiazepines (Mayo-Smith, 1997; Saitz et al., 1994). However, despite positive clinical outcomes are obtained with benzodiazepines, there are several limitations to the use of these medications. Benzodiazepines have several side effects including excessive sedation, memory deficits, respiratory depression, and the risk of these side effects is aggravated in patients with liver impairments, as is frequently the case in alcoholics (Mayo-Smith, 1997). In addition, benzodiazepines have abuse liability of their own, which makes their use problematic in subjects co-dependent on other substances (Mayo-Smith, 1997; Ross, 1993). It is evident that the availability of new potentially effective drugs for the treatment of alcohol withdrawal syndrome such as NOP receptor agonists is of considerable clinical importance.

Previous studies provide evidence that activation of the N/OFQ-NOP receptor system (*i*) blunts the reinforcing and motivating effects of ethanol across a range of behavioral measures, including ethanol self-administration, conditioned place preference, and conditioned reinstatement (Ciccocioppo et al., 1999, 2004; Kuzmin et al., 2003); *ii*) reverses increased anxiety-like behavior associated with neuroadaptive changes in N/OFQ function associated with a history of ethanol dependence (Martin-Fardon et al., 2010); *iii*) and prevents stress-induced reinstatement of alcohol-seeking (Martin-Fardon et al., 2000). The present study demonstrates that activation of brain NOP receptors also reduces the expression of alcohol withdrawal signs in intoxicated rats. Together, these findings suggest that agents targeting NOP receptors may provide effective novel treatments for alcoholism and especially alcohol withdrawal. This possibility should be tested in the future once small brain penetrating synthetic NOP receptor agonist will be developed and available for clinical use.

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Fig. 1.

Effect of N/OFQ administration (0.0, 1.0 and 3.0 µg; ICV) on total withdrawal scores. Total withdrawal scores were obtained for each animal by accumulating the score of the four different withdrawal signs (Vocalization, Ventromedial limb retraction, Tail Rigidity, Tremors) for the 3 different time-points monitored (8 h, 10 h and 12 h). N/OFQ was ICV injected 10 min before each observation time-point. Total withdrawal score ranged between 0 and 8. Values represent the ($\pm$ SEM) of 8 subjects per group. Statistical differences between N/OFQ Vehicle (0.0) and Control (Ctr): $##p < 0.01$. Statistical differences between Vehicle (0.0) and N/OFQ treated groups (1.0 or 3.0): $**p < 0.01$, $*p < 0.05$.

Fig. 2.

Effect of N/OFQ (1.0 and 3.0 µg; ICV) or its vehicle (0.0), on ethanol withdrawal score of: **A)** Vocalization, **B)** Tail Rigidity, **C)** Tail Tremors and **D)** Ventromedial Limb Retraction (VmLR) measured at 8, 10 and 12 hours following the last ethanol dose. N/OFQ was administered 10 min before each observation time point. Each withdrawal sign was assigned a score of 0–2. Values represent the mean (±SEM) of 8 subjects per group. Statistical differences between N/OFQ Vehicle (0.0) and Control (Ctr): ##p<0.01; #p<0.05. Statistical differences between Vehicle (0.0) and N/OFQ treated groups (1.0 or 3.0): **p<0.01, *p<0.05.

Fig. 3. Effect of ICV treatment with N/OFQ (1.0 and 2.0 μg) or its vehicle (0.0) on postdependent rats and nondependent controls tested in the EPM test one week following withdrawal from ethanol intoxication. **A)** Percentage of time spent in open arms; **B)** Percentage of open arm entries; **C)** Percentage of closed arm entries. Control group (non intoxicated rats) received N/OFQ and alcohol vehicles. Significant differences from vehicle (0.0): *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

Fig. 4.

Effect of ICV treatment with N/OFQ (1.0 and 2.0 µg) or its vehicle (0.0) on animals treated with a single dose of 3.0 g/kg of 20% alcohol given IP 12 hrs prior to the EPM test. **A)** Percentage of time spent in the open arms; **B)** Percentage of open arm entries; **C)** Percentage of closed arm entries.. Control group (nonintoxicated rats; Ctr) received N/OFQ vehicle. Significant differences from vehicle (0.0): ** $p < 0.01$, * $p < 0.05$.

Table 1

Effect of nociceptin/orphanin FQ (N/OFQ, 0.0–2.0 µg/ICV) in the elevated plus maze test in nondependent and postdependent rats

N/OFQ (µg/rat)	Nondependent				Postdependent			
	0.0	1.0	2.0	0.0	1.0	2.0	0.0	2.0
Open arms time (sec)	71.6±2.1	54.6±2.7	57.8±4.0	19.9±2.9	50.0±11.3	66.9±11.1		
Number of open arms entries	9.6±0.5	7.9±0.5	7.9±0.6	3.0±0.4	4.6±1.0	4.6±1.1		
Number of closed arms entries	10.2±0.6	9.8±0.5	9.7±0.8	11.0±0.9	7.5±1.3	5.3±0.9		
Number of total arms entries	19.9±1.0	17.6±0.7	17.6±1.2	14.0±1.2	12.1±2.1	9.9±1.7		

Values represent the mean ±S.E.M. for 7–8 rats for each group.

Table 2

Effect of nociceptin/orphanin FQ (N/OFQ, 0.0–2.0 $\mu\text{g}/\text{ICV}$) in the elevated plus maze test in rats injected with an acute high dose (2.0 g/kg) of alcohol or vehicle (Ctr)

N/OFQ ($\mu\text{g}/\text{rat}$)	Ctr.(0.0)	0.0	1.0	2.0
Open arms time (sec)	87.0 $\pm$ 6.2	24.4 $\pm$ 5.8	55.7 $\pm$ 14.0	81.0 $\pm$ 17.4
Number of open arms entries	12.3 $\pm$ 0.9	2.9 $\pm$ 0.9	5.1 $\pm$ 1.3	8.2 $\pm$ 1.6
Number of closed arms entries	11.7 $\pm$ 1.0	2.8 $\pm$ 0.8	4.6 $\pm$ 1.2	6.3 $\pm$ 0.9
Number of total arms entries	24.0 $\pm$ 1.7	5.6 $\pm$ 1.7	9.7 $\pm$ 2.2	14.5 $\pm$ 2.5

Values represent the mean $\pm$ S.E.M. for 7–8 rats for each group.