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Attenuation of ethanol self-administration and of conditioned reinstatement of alcohol-seeking behaviour by the antiopioid peptide nociceptin/orphanin FQ in alcohol-preferring rats

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Abstract

Rationale—Nociceptin/orphanin FQ (N/OFQ), the endogenous ligand of the opioid-like orphan receptor NOP, was shown to reduce home-cage ethanol consumption, ethanol-induced conditioned place preference and stress-induced reinstatement of alcohol-seeking behaviour.

Objectives—The present study, using genetically selected Marchigian Sardinian alcohol-preferring (msP) rats, was designed to evaluate the effect of this opioid peptide on 10% ethanol and 10% sucrose self-administration, under a fixed-ratio 1 (FR 1) or a progressive-ratio (PR) schedule of reinforcement. Furthermore, using an experimental model of relapse in which rats were trained to lever press for ethanol in the presence of the discriminative stimulus of an orange odour (S⁺) and a 1-s cue light (CS⁺) or for water in the presence of anise odour (S[−]) and 1-s white noise (CS[−]), the effect of N/OFQ on cue-induced reinstatement of extinguished ethanol responding was investigated.

Results—Sub-chronic (6 days) intracerebroventricular (i.c.v.) injection of 0.5 μ g or 1.0 μ g N/OFQ per rat significantly reduced alcohol self-administration under both the FR 1 and PR schedules of reinforcement. Conversely, i.c.v. administration of 0.5, 1.0 or 4.0 μ g of the peptide per rat did not affect sucrose self-administration. In addition, i.c.v. N/OFQ (1.0–2.0 μ g per rat) significantly inhibited the reinstatement of extinguished ethanol responding under an S^+/CS^+ condition, whereas lever pressing under S^-/CS^- was not altered.

Conclusions—The present study demonstrates that the reinforcing effects of ethanol are markedly blunted by activation of the opiodergic N/OFQ receptor system. Moreover, the data provide evidence of the efficacy of N/OFQ to prevent reinstatement of ethanol-seeking behaviour elicited by environmental conditioned stimuli.

Keywords

Nociceptin; Orphanin FQ; ORL1 receptors; NOP receptors; Relapse; Alcohol self-administration; Alcohol-preferring rats

Introduction

Nociceptin (N/OFQ), also referred to as orphanin FQ, is a 17-aminoacid peptide that shows structural homology with opioid peptides, particularly with dynorphin A, but is lacking the N-terminal tyrosine necessary for activation of traditional opioid receptors (Meunier et al. 1995; Reinscheid et al. 1995, 1998). The N/OFQ peptide binds with high affinity the opioid receptor-like 1 (ORL1) receptor, recently included in the opioid receptor family and renamed NOP, whereas it does not activate the classical opioid receptors (μ , κ , and δ). At the intracellular level, however, the activation of membrane NOP receptors exerts actions similar to those induced by activation of the other opioid receptors, namely, inhibition of cAMP production, closure of voltage-sensitive Ca^{++} channels and enhancement of an outward K^+ conductance (Meunier et al. 1995; Reinscheid et al. 1995, 1998). Nevertheless, naloxone, a non-selective opioid antagonist, does not block N/OFQ intracellular events (Henderson and McKnight 1997; Darland et al. 1998), confirming that the pharmacological actions of this peptide are not mediated by the classic opioid receptors.

Neuroanatomical and immunohistochemical studies (Darland et al. 1998; Mollereau and Mouledous 2000) have shown a wide distribution of N/OFQ and its receptor in various corticomesolimbic structures, including the amygdala, the bed nucleus of the stria terminalis, the nucleus accumbens (Nacc) and various fronto-cortical areas involved in the regulation of the motivational effect of drugs of abuse (Koob et al. 1998; Wise 1998; Everitt and Wolf 2002).

In recent studies using genetically selected Marchigian Sardinian alcohol-preferring rats (msP), it has been demonstrated that chronic intracerebroventricular (i.c.v.) N/OFQ injections significantly reduced home-cage ethanol intake in the two bottle choice and ethanol-induced conditioned place-preference paradigms (Ciccocioppo et al. 1999, 2002b). In addition, N/OFQ has been shown to inhibit stress-induced reinstatement of alcohol-seeking behaviour in rats trained to self-administer ethanol (Martin-Fardon et al. 2000). Lastly, several lines of evidence suggest that although it does not bind to the opioid receptors, N/OFQ is able to function as an “antiopioid” peptide that inhibits the rewarding properties of morphine. For instance, it has been shown that pretreatment with N/OFQ inhibits morphine-induced conditioned place preference (Murphy et al. 1999; Ciccocioppo et al. 2000), whereas microdialysis studies demonstrated that central administration of this peptide can block morphine-induced dopamine (DA) release in the NAcc of freely moving rats (Di Giannuario et al. 1999).

To further investigate the involvement of the N/OFQ in the control of alcohol abuse, in the present work, the effect of the peptide on ethanol consumption under operant conditions was studied. For this purpose, using msP rats, the peptide was tested on ethanol-self-administration under both fixed ratio 1 (FR 1) and progressive ratio (PR) contingences. Moreover, using an animal model of relapse, the ability of N/OFQ to prevent reinstatement of ethanol-seeking behaviour elicited by environmental conditioning factors was investigated. Lastly, in order to evaluate the selectivity of the effects of the peptide, its ability to affect 10% sucrose self-administration under FR 1 and PR schedules of reinforcement was studied.

Materials and methods

Animals

Male genetically selected Marchigian Sardinian alcohol-preferring rats were employed. They were bred in the Department of Pharmacological Sciences and Experimental Medicine of the University of Camerino (Marche, Italy) for 38 generations from Sardinian alcohol-preferring rats (sP) of the 13th generation, provided by the Department of Neurosciences of the University of Cagliari (Fadda et al. 1990; Gessa et al. 1991). These animals are referred to as Marchigian sP (msP) rats. At the beginning of the experiments, their body weight ranged between 200 g and 250 g. They were kept in a room with a reverse 12-h/12-h light/dark cycle (lights off at 0930 hours), temperature of 20–22°C and humidity of 45–55%. All animals were handled once daily for 5 min for 1 week before the beginning of the experiments. All procedures were conducted in adherence with the European Community Council Directive for Care and Use of Laboratory Animals. During the experiments, rats were offered free access to tap water and food pellets (4RF18, Mucedola, Settimo Milanese, Italy) except during the first 3 days of training to establish operant responding (see below).

Intracranial surgery

For intracranial surgery, each msP rat was anaesthetized by i.m. injection of 100–150 μ l of a solution containing tiletamine cloridrate (58.17 mg/ml) and zolazepam cloridrate (57.5 mg/ml). A guide cannula for i.c.v. injections aimed at the left lateral cerebroventricle was stereotactically implanted and cemented to the skull. The following co-ordinates, taken from the atlas of Paxinos and Watson (1986), were used: antero-posterior = 0.8 mm behind the bregma, lateral = 1.8 mm from the sagittal suture, ventral = 2 mm from the surface of the skull.

Self-administration apparatus

The self-administration stations consisted of operant conditioning chambers (Med Associate, Inc) enclosed in sound-attenuating, ventilated environmental cubicles. Each chamber was equipped with a drinking reservoir (volume capacity: 0.2 ml) positioned 4 cm above the grid floor in the centre of the front panel of the chamber, and two retractable levers located 3 cm (one to the right and the other to the left) of the drinking receptacle. An infusion pump was activated by responses on the right, or active, lever, while responses on the left, or inactive, lever were recorded but did not result in activation of the pump. Activation of the pump resulted in a delivery of 0.1 ml fluid (either ethanol, sucrose or saccharin). During the infusion of ethanol or sucrose (10% w/v), a house light located on the front panel was turned on for 1.0 s (which corresponded to the duration of the syringe-pump activation). Lever presses during this period were counted but did not lead to further infusions. An IBM-compatible computer controlled the delivery of fluids, presentation of visual stimuli and recording of the behavioural data.

Drug injections

Nociceptin (Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asp-Glu) was a generous gift of Dr. R. Guerrini, Department of Pharmaceutical Sciences of the University of Ferrara, Italy. It was dissolved in sterile isotonic saline and injected i.c.v. in a volume of 1 μ l per rat 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, immediately before the rat was sacrificed, 1 μ l of black India ink was injected i.c.v., and ink diffusion into the ventricles was evaluated using a histological method.

Alcohol self-administration training procedures

Animals were trained to self-administer 10% (w/v) ethanol in 30-min daily sessions under a FR 1 schedule of reinforcement where each response resulted in delivery of 0.1 ml fluid, as previously described (Weiss et al. 1993). During the first 3 days of training, the rats were placed under a restriction schedule limiting water availability to 2 h per day in order to facilitate acquisition of operant responding maintained by a liquid reinforcer. During this time, responses at the lever were reinforced by delivery of a 0.2% (w/v) saccharin solution into the drinking receptacle on a FR 1 schedule, throughout daily 30-min sessions. During all subsequent training and testing, water was freely available in the home cages. After successful acquisition of saccharin-reinforced responding, rats were trained to self-administer ethanol using a modification of the “sucrose-fading procedure” (Samson 1986), which employed saccharin instead of sucrose (Weiss et al. 1993). During the first 2 days of training, responses at the lever were reinforced by a 0.2% saccharin solution containing 5.0% (w/v) ethanol. Beginning on day 3, the concentration of ethanol was gradually increased from 5.0% to 8.0% and finally 10% (w/v), while the concentration of saccharin was correspondingly decreased to 0%. From the first day, rats began to press for 10% ethanol, the house light located on the front panel was turned on for 1.0 s.

Sucrose self-administration training procedures

For sucrose self-administration, the training procedures were identical to that described for alcohol self-administration, except that lever pressing during the first 3 days of water deprivation was reinforced by water delivery and, after successful acquisition of operant responding, animals received immediately 10% (w/v) of sucrose. From the first day, rats began to press for 10% sucrose, the house light located on the front panel was turned on for 1.0 s.

Ethanol PR

In this experimental paradigm, the breaking point (BP) of ethanol was evaluated under a PR schedule of reinforcement. For this purpose, animals were first trained to self-administer 10% alcohol under a FR 1 schedule of reinforcement. Following acquisition of a stable baseline of responding with 10% ethanol, animals were tested under the PR condition where the response requirement (i.e., the number of lever responses or “ratio” required to receive one dose of 0.1 ml of 10% ethanol) was increased in the following manner: for each of the first 5 ethanol deliveries, the ratio was increased by 1; for all the following deliveries the ratio was increased by 2. Each ethanol-reinforced response resulted in a 1.0 s illumination of the “house light” while sessions were terminated when more than 30 min had elapsed since the last reinforced response. Baseline PR was established for two consecutive days, and the third day drug testing begun. Between experiments, the baseline under PR schedule of reinforcement was re-established for two consecutive days.

Sucrose PR

For the determination of the BP for sucrose, an experimental procedure identical to that used for ethanol was used except that every ratio completed was reinforced by the delivery of 0.1 ml of 10% sucrose.

Cues-induced reinstatement of alcohol-seeking behaviour

This experimental procedure consisted of three phases.

Conditioning phase—The purpose of the conditioning phase was to train rats to discriminate the availability of ethanol (reward) versus water (non-reward). Conditioning sessions began immediately after termination of the saccharin-fading procedure and were composed of ten ethanol and ten water 30-min daily sessions, during which discriminative stimuli (S^D) predictive of ethanol versus water availability were presented. The S^D for ethanol consisted of the odour of an orange extract (S^+), whereas water availability was signalled by an anise extract (S^-). The olfactory stimuli were produced by depositing five to six drops of the respective extract into the bedding of the operant chamber immediately before extension of the levers and session initiation, and remained present throughout the 30-min sessions. At the end of each session, the bedding of the chamber was changed, and bedding trays were thoroughly cleaned. In addition, each lever press resulting in delivery of ethanol was paired with illumination of the chamber's house light for 1.0 s (CS^+), while lever presses resulting in water delivery were followed by a 1.0-s white noise (CS^-). During the 1.0-s presentation of these contingent cues, responses at the active lever were recorded but not reinforced by ethanol or water infusions (time out). During the first 3 days of this phase, the rats were given ethanol sessions only. Subsequently, ethanol and water sessions were conducted in random order.

Extinction of lever-pressing phase—After completion of the conditioning phase, rats were subjected to 30-min extinction sessions, for 20 consecutive days. Extinction sessions began by extension of the levers without presentation of the olfactory discriminative stimuli, while responses at the previously active lever activated the syringe pump but did not result in the delivery of either ethanol or water or the presentation of the response-contingent cues (house light or white noise). This phase was introduced to eliminate the capacity of the self-administration chamber to non-specifically motivate the animal's behaviour by leaving unaltered the ability of the cues to predict ethanol availability.

Reinstatement testing—Reinstatement tests began the day after the last extinction session and were conducted over two consecutive days. In these tests, rats were exposed to the same conditions as those during the conditioning phase, except that liquids (alcohol or water) were not made available. Sessions were initiated by extension of both levers and presentation of either the ethanol S^+ or water S^- , that remained present during the entire 30-min session. Responses at the previously active lever were followed by activation of the syringe pump motor and presentation of the CS^+ ("house light") in the S^+ condition or the CS^- ("white noise") in the S^- condition. Half of the animals were tested under the S^+/CS^+ condition on day 1 and under the S^-/CS^- condition on day 2. The other half were first tested under S^-/CS^- and then under S^+/CS^+ .

Experiment 1. Effect of subchronic i.c.v. injections of N/OFQ on alcohol self-administration under a FR 1 schedule of reinforcement:

After acquisition of a stable 10% ethanol self-administration baseline (9 days), rats ($n=24$) were separated into three groups with similar baseline levels of responding for 10% ethanol. During the last 4 days of training (pre-treatment), immediately prior to the self-administration sessions, animals were given 1 μ l of saline i.c.v. to familiarise them with the injection procedure. At this point, for

six consecutive days, the first group ($n=9$) was injected i.c.v. with isotonic saline (control), whereas the second ($n=9$) and the third ($n=9$) groups received 0.5 μg and 1.0 μg per rat of N/OFQ, respectively. Immediately after, animals were tested for 10% ethanol self-administration. At completion of drug testing (6 days), ethanol self-administration was monitored for an additional 4 days (post-treatment) during which all animals received only i.c.v. saline. The number of responses at both active and inactive levers was recorded for the entire period of the experiment.

Experiment 2. Effect of i.c.v. injections of N/OFQ on alcohol self-administration under PR schedule of reinforcement: Following acquisition of a stable baseline of responding for ethanol (15 days) under a FR 1 condition, a group of animals ($n=8$) was treated i.c.v. with N/OFQ 0.5 μg and 1.0 μg per rat or its vehicle. In a counterbalanced order (Latin square), animals received all drug doses and vehicle. An interval of 3 days was imposed between drug testing. Responding on both the active and the inactive lever was recorded for the entire period of the experiment.

Experiment 3. Effect of i.c.v. injections of N/OFQ on cue-induced reinstatement of alcohol-seeking behaviour: After completion of the discrimination and extinction phases, animals were tested for the ability of N/OFQ to prevent the reinstatement of alcohol-seeking elicited by cues predictive of ethanol availability. For this purpose, during the last three extinction sessions msP rats were injected i.c.v. with 1 μl saline in order to familiarise them with the administration procedures. Animals were then separated into three groups with a similar baseline number of responses during extinction. For the reinstatement test, one group ($n=9$) of rats was injected i.c.v. with isotonic saline (control), while the other two groups ($n=7-8$) received 2.0 μg and 4.0 μg per rat of N/OFQ. Animals were placed in the self-administration chambers immediately after drug administration. In one-half of the rats, the effect of N/OFQ was tested the day after the last extinction session under the S^+/CS^+ condition and on the following day under the S^-/CS^- condition. In the other half, N/OFQ was first tested under S^-/CS^- and then under S^+/CS^+ condition. The number of responses on both the active and the inactive levers was recorded throughout the experiment.

Experiment 4. Effect of subchronic i.c.v. injections of N/OFQ on sucrose self-administration under FR 1 schedule of reinforcement: After acquisition of a stable 10% sucrose self-administration baseline (8 days), rats ($n=25$) were separated into four groups with similar baseline levels of responding for 10% sucrose. During the last 4 days of training (pre-treatment), immediately prior to the self-administration sessions, animals were given 1 μl saline i.c.v. to familiarise them with the injection procedure. At this point, for six consecutive days, the first group of animals ($n=7$) received i.c.v. isotonic saline (control), while the second ($n=6$), the third ($n=7$) and the fourth ($n=5$) groups received 0.5, 1.0 and 4.0 μg per rat of N/OFQ, respectively. Immediately after, animals were tested for 10% sucrose self-administration. At completion of drug testing (6 days), sucrose responding was monitored for another 4 days (post-treatment), during which all animals received only i.c.v. saline. The number of responses on both the active and inactive levers was recorded for the entire period of the experiment.

Experiment 5. Effect of i.c.v. injections of N/OFQ on sucrose self-administration under a PR schedule of reinforcement: Following acquisition of a stable baseline of responding for 10% sucrose (12 days) under a FR 1 condition, a group of animals ($n=7$) was treated i.c.v. with N/OFQ 0.5, 1.0 and 4.0 μg per rat or its vehicle. In a counterbalanced order (Latin square), animals received all drug doses and vehicle. An interval of 3 days was imposed between drug testing. Operant responding at both the active and the inactive lever was recorded for the entire period of the experiment.

Statistical analysis

For the self-administration experiments under the FR 1 condition, data were analysed by means of two-way analysis of variance (ANOVA) with one within-subjects factor (time) and one between-subjects factor (treatment). For the PR experiments, the number of rewards earned were analysed by one-way ANOVA with repeated means. For the reinstatement experiment, differences among responses during the training, extinction and reinstatement phases were analysed in the vehicle-treated group by one-way within-subjects ANOVA, followed by Newman-Keuls post-hoc tests to identify differences between experimental phases and responses in the presence of the S^+/CS^+ versus S^-/CS^- . The effect of N/OFQ on reinstatement responses was analysed by two-way ANOVA, one factor within (reinstatement condition) and one factor between (treatment), followed by Newman-Keuls post-hoc tests. Statistical significance was set at $P < 0.05$.

Results

Experiment 1. Effect of subchronic i.c.v. injections of N/OFQ on alcohol self-administration under a FR 1 schedule of reinforcement

All rats acquired responding reinforced by 10% ethanol and developed stable levels of ethanol-maintained behaviour. During the initial 5-day training phase, in 30-min sessions, animals responded to the active lever from 31 to 53 times to self-administer approximately 0.6–1.1 g/kg ethanol. As shown in Fig. 1, during the following 4 days, while establishing pretreatment baseline, responding ranged between 40 and 65 corresponding to 0.8–1.3 g/kg ethanol. The stability of responding, suggests, therefore, that manipulation due to i.c.v. injections did not influence animals' operant behaviour. In a separate study, measures of blood alcohol levels (BAL) taken from another group of msP rats immediately after the operant session demonstrated that over similar range of 10% ethanol self-administration pharmacologically relevant ethanol concentrations of 40–70 mg/dl are achieved.

Subchronic i.c.v. treatment with N/OFQ, 0.5 μ g and 1.0 μ g per rat, markedly reduced responding on the active lever. The effect was significant from the first day of treatment. Compared with controls, ethanol self-administration was reduced by about 40–50%, and statistical analysis revealed a significant overall effect of treatment ($F_{2,24} = 7.30$, $P < 0.01$). Moreover, the Newman-Keuls post-hoc test showed a significant difference between controls and animals treated with both 0.5 μ g and 1.0 μ g per rat of N/OFQ ($P < 0.01$). Responses at the inactive lever were almost absent in all treatment conditions and were not affected by drug injection ($F_{2,24} = 1.95$, n.s.).

At the end of N/OFQ treatment, the msP rats progressively recovered from the effect of treatment, and ethanol self-administration returned to pre-treatment levels within 4 days.

Experiment 2. Effect of i.c.v. injections of N/OFQ on alcohol self-administration under a PR schedule of reinforcement

PR baseline was established for two consecutive days before the experiment. For each of these 2 days, values of overall responding were 10.4 ± 1.5 and 9.5 ± 1.8 . On the third day, N/OFQ or its vehicle were given prior to ethanol access and, as shown by the analysis of variance, an overall drug effect ($F_{2,14} = 3.93$, $P < 0.05$) was observed. As shown in Fig. 2A, the Newman Keuls test demonstrated a significant reduction in earned reinforcers in rats treated with 1.0 μ g per rat of N/OFQ compared with drug vehicle. This effect was confirmed by the reduction of the BP for ethanol observed following N/OFQ treatment (Fig. 2B). Responses at the inactive lever were almost absent in all treatment conditions and were not affected by drug injection ($F_{2,14} = 1.48$, n.s.).

Experiment 3. Effect of i.c.v. injections of N/OFQ on cue-induced reinstatement of alcohol-seeking behaviour

At the end of the conditioning phase, the number of ethanol-reinforced responses was significantly higher ($F_{1,46}=57.21$, $P<0.01$) compared with water-reinforced responses (Fig. 3). Lever pressing progressively decreased throughout the 20-day extinction phase (Fig. 3). For the reinstatement test, analysis of variance revealed a non-significant overall effect of treatment ($F_{2,21}=1.96$, n.s.), but a significant treatment-reinstatement condition interaction ($F_{4,42}=3.18$, $P<0.05$). Specifically, further post-hoc tests demonstrated a significant reinstatement of ethanol-seeking in the vehicle-treated group (Fig. 3) under S^+/CS^+ stimulus condition; whereas, under S^-/CS^- responding remained at extinction levels ($F_{2,16}=20.71$, $P<0.01$). Moreover, as shown in Fig. 3, pretreatment with 2.0 μ g and 4.0 μ g per rat of N/OFQ significantly ($F_{2,21}=3.73$, $P<0.05$) attenuated recovery of responding elicited by ethanol-paired cues (S^+/CS^+), whereas drug treatment did not modify responding under the S^-/CS^- condition ($F_{2,21}=0.23$, n.s.). This difference was confirmed by a Newman-Keuls test showing that under S^+/CS^+ condition revealed a significant effect of N/OFQ (4.0 μ g per rat) compared with vehicle-treated rats ($P<0.05$).

Responses at the inactive lever were almost absent throughout all experimental phases and were not affected by N/OFQ treatment ($F_{2,21}=2.34$, n.s.).

Experiment 4. Effect of subchronic i.c.v. injections of N/OFQ on sucrose self-administration under a FR 1 schedule of reinforcement

As shown by the analysis of variance, subchronic i.c.v. treatment with N/OFQ 0.5, 1.0 and 4.0 μ g per rat did not affect responding for sucrose self-administration ($F_{3,21}=0.37$, n.s.; Fig. 4). Responses at the inactive lever were almost absent in all treatment conditions and were not affected by drug injection ($F_{3,21}=0.04$, n.s.).

Experiment 5. Effect of i.c.v. injections of N/OFQ on sucrose self-administration under a PR schedule of reinforcement

As shown in Fig. 5A, N/OFQ at the doses of 0.5, 1.0 and 4.0 μ g per rat did not modify the sucrose reward earned, and statistical analysis showed absence of significant treatment effect ($F_{3,18}=0.24$, n.s.). Consistently, the BP for sucrose was not modified by drug treatment (Fig. 5B). Responses at the inactive lever were almost absent in all treatment conditions and were not affected by drug injection ($F_{3,18}=0.84$, n.s.).

Discussion

The results show that subchronic i.c.v. administration of 0.5 μ g and 1.0 μ g per rat of N/OFQ significantly reduced ethanol self-administration under a FR 1 schedule of reinforcement. Moreover, in the PR experiment a reduction in the BP for ethanol self-administration was observed following N/OFQ treatment. A limitation in the interpretation of the results of the PR experiment is that, using this schedule, even vehicle-treated animals obtained a limited number of reinforcers (10–14 ethanol doses) which unlikely lead to pharmacologically relevant BAL. However, in our animals, under PR conditions, the substitution of ethanol with water results in a 40–50% drop of lever responding (data not shown). This, therefore, argues in favour of the fact that—under this condition—the animals' behaviour is driven by the motivational value of the reinforcer (i.e., ethanol). At present we cannot exclude that factors such as the taste or the odour of ethanol may have contributed to maintain the reinforced responding. However, it is interesting to note that the peptide did not alter 10% sucrose self-administration under either a FR 1 or a PR schedule. It seems, therefore, that the effect of the peptide is selective for ethanol, whereas the reinforcing magnitude of sucrose (a natural reinforcer) is not apparently modified by drug administration. A possible explanation

for this phenomenon is that the NOP receptor system may be recruited and, therefore, may play a functional role, when the brain reward system/s is activated by potent pharmacological stimuli; whereas, it plays only a marginal role in the regulation of brain reward processes under basal conditions. This hypothesis is supported by several pieces of evidence. For example, in place conditioning studies, it has been shown that N/OFQ inhibits the rewarding effects of ethanol, morphine and cocaine, while it is devoid of motivational effects per se (Devine et al. 1996; Murphy et al. 1999; Ciccocioppo et al. 1999, 2000; Kotlinska et al. 2002). Moreover, microdialysis studies demonstrated that the activation of the NOP receptor by relatively low doses of N/OFQ potently inhibits morphine-induced mesoaccumbal DA release (Di Giannuario et al. 1999), whereas higher doses are needed to modulate basal DA activity (Murphy et al. 1996, Murphy and Maidment 1999).

Alcoholism is a chronic relapsing disorder characterised by compulsive drug-seeking behaviour and use (O'Brien et al. 1990, 1998; American Psychiatric Association 1994; O'Brien and McLellan 1996). A critical factor implicated in the relapsing nature of alcohol and other drugs of abuse as well is the conditioning of their rewarding effects with specific environmental stimuli (cues). Indeed, clinical and preclinical studies demonstrated that exposure to alcohol cues increases the urge to drink and facilitates ethanol "relapse" even after protracted periods of abstinence (McCusker and Brown 1990, 1991; Staiger and White 1991; Monti et al. 1993; Katner et al. 1999; Weiss et al. 2001; Ciccocioppo et al. 2001a, 2002a). The present study, using msP rats as an animal model of relapse, confirmed these previous findings and demonstrated that presentation of ethanol paired cues elicits a robust reinstatement of extinguished ethanol responding in this rat line. In contrast, no reinstatement was observed following presentation of cues predictive of water availability. More importantly, the present study demonstrated that treatment with 2.0–4.0 μg per rat of N/OFQ significantly reduces cue-induced resumption of responding on the previously ethanol-paired lever. This, however, indicates that higher doses of the peptide are needed to prevent cue-induced ethanol-seeking behaviour relative to ethanol intake. This suggests that different mechanisms may control these two behaviours. To confirm the selectivity of the effect of the peptide, responding at the inactive lever was unaltered by N/OFQ treatment.

Research utilising reinstatement models of relapse predominantly points to roles for DA and opioid systems in regulating the motivating effects of ethanol-associated environmental stimuli. For example, in rats, exposure to environments associated with ethanol availability increases extracellular DA levels in the NAcc (Katner et al. 1996; Gonzales and Weiss 1998), whereas blockade of either D1 or D2 receptors dose dependently reduced the cue-induced reinstatement of ethanol-seeking behaviour (Liu and Weiss 2002). In addition, a role of opioid systems in relapse has been implicated by clinical findings that the opiate antagonist naltrexone attenuates craving associated with exposure to ethanol cues (Monti et al. 1999) and by experimental evidence that naltrexone, as well as μ - and δ -selective opiate receptor antagonists, reverse conditioned reinstatement of ethanol-seeking by ethanol-associated contextual stimuli (Katner et al. 1999; Ciccocioppo et al. 2002a).

There is also evidence that N/OFQ reverses several of the actions of opiate drugs, which has given rise to the hypothesis that N/OFQ may act as a functional "anti-opioid" agent. Specifically, N/OFQ blocks the analgesic effects of morphine (Mogil et al. 1996; King et al. 1998; Mogil and Pasternak 2001) prevents the development of morphine-induced conditioned place preference (Murphy et al. 1999; Ciccocioppo et al. 2000) and, as mentioned before, inhibits morphine-induced DA release in the NAcc (Di Giannuario and Pieretti 2000). In addition, electrophysiological data has demonstrated that the N/OFQ system inhibits the firing of β -endorphin cells in the hypothalamic arcuate nucleus (Wagner et al. 1998). These arcuate neurons project, among other brain regions to the ventral tegmental area (VTA) and the NAcc, where they interact with mesolimbic DA transmission

and influence motivated behaviour (Di Chiara and North 1992; Johnson and North 1992; Devine et al. 1993a, 1993b; Herz 1997). Moreover, it has been shown that 91% of tyrosine hydroxylase-positive cells in the VTA co-express NOP receptors, and that N/OFQ can directly and indirectly (via GABA interneurons) modulate (inhibit) neural activity of VTA DA neurons (Maidment et al. 2002; Norton et al. 2002; Zheng et al. 2002).

The exact mechanism by which N/OFQ acts in the brain to modulate ethanol intake and cue-induced reinstatement of drug-seeking behaviour is not yet clear. However, taking into consideration the important role of the DA-ergic and the opiodergic systems in the regulation of these ethanol-related behaviours, and considering the modulatory role that N/OFQ has on corticomesolimbic DA and opioid activity, it may be hypothesised that the N/OFQ system interacting with these two other systems may reduce the motivational value of alcohol as well as that of stimuli predictive of its availability.

In conclusion, the present study demonstrates that stimulation of NOP receptors by N/OFQ reduces the reinforcing effects of ethanol and prevents relapse elicited by environmental stimuli predictive of drug availability. Therefore, agents targeting NOP receptors may represent a promising treatment for alcohol-relapse prevention and abuse as an alternative to existing medications such as naltrexone. An important consideration is that naltrexone, which has been successfully employed for the treatment of alcohol craving and prevention of relapse, can produce aversive side effects that limit compliance (Kosten and Kleber 1984; Rabinowitz et al. 1997, 2002). In contrast, at least in laboratory animals, NOP receptor activation does not appear to produce aversive effects (Devine et al. 1996). Furthermore, NOP agonists exert a marked anxiolytic and anti-stress actions (Jenck et al. 1999, 2000; Martin-Fardon et al. 2000; Ciccocioppo et al. 2001b, 2002c) that may provide additional advantages over opiate antagonist treatments which, in fact, can induce anxiety (Lee and Rodgers 1990) and are ineffective in preventing stress-induced ethanol-seeking behaviour (Le et al. 1998).

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Fig. 1. Fixed ratio 1 10% ethanol self-administration in Marchigian Sardinian alcohol-preferring rats ($n=9/\text{group}$) treated i.c.v. for six consecutive days with $0.5 \mu\text{g}$ and $1.0 \mu\text{g}$ per rat of N/OFQ or its vehicle (*Veh*). * $P<0.05$ versus vehicle

Fig. 2. Performance on a progressive ratio schedule of reinforcement of rats ($n=8$) treated i.c.v. with 0.5 µg and 1.0 µg per rat of N/OFQ or its vehicle (*Veh*) in a counterbalanced order (Latin square). **A** Mean (±SEM) number of rewards earned. **B** Mean (±SEM) number of responses emitted at breaking point. * $P < 0.05$ versus vehicle

Fig. 3.

Training: responses on the last day of the discrimination training phase in the presence of distinct discriminative stimuli predictive of the availability of 10% (w/v) ethanol (S^+/CS^+) or water (S^-/CS^-). *Extinction:* extinction responses during the 20 days of this phase.

Reinstatement: responses in rats exposed to the S^+/CS^+ and S^-/CS^- conditions (in the absence of ethanol or water) and treated i.c.v. with 2.0 µg and 4.0 µg per rat of N/OFQ or its vehicle (*Veh*). Responding in the S^+/CS^+ test differed from S^-/CS^- responses ($P < 0.01$). Treatment with N/OFQ 4.0 µg per rat significantly reduced responses under S^+/CS^+ but not under S^-/CS^- conditions. Values represent the mean (±SEM) of 7–9 subjects per group. Difference from vehicle * $P < 0.05$

Fig. 4. Fixed ratio 1 10% sucrose self-administration in Marchigian Sardinian alcohol-preferring rats ($n=25$) treated i.c.v. for six consecutive days with 0.5, 1.0 and 4.0 μg per rat of N/OFQ or its vehicle (*Veh*)

Fig. 5. Performance on a progressive ratio schedule of reinforcement of rats ($n=7$) treated i.c.v. with 0.5, 1.0 and 4.0 µg per rat of N/OFQ or its vehicle (*Veh*) in a counterbalanced order (Latin square). **A** Mean (±SEM) number of rewards earned. **B** Mean (±SEM) number of responses emitted at breaking point