Orphanin FQ/nociceptin suppresses motor activity through an action along the mesoaccumbens axis in rats

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Objective: Intracerebroventricular administration of orphanin FQ/nociceptin (OFQ/N), the endogenous agonist ligand of the opioid receptor-like (ORL-1) receptor, decreases extracellular levels of dopamine and suppresses motor activity. The presence of the ORL-1 receptor on mesoaccumbal and nigrostriatal dopaminergic neurons raises the possibility that an action along these pathways may be one means by which OFQ/N produces motor suppression. Thus, the present study used local administration of OFQ/N into the ventral tegmental area (VTA), the substantia nigra, the nucleus accumbens and the striatum to determine the contribution of cell-body regions and terminal fields of the dopaminergic neurons to the motor-suppressant effect of OFQ/N. **Methods:** Rats were implanted bilaterally with guide cannulae into one of the brain regions and tested 4 days later. First, the effect of a single dose of OFQ/N (30 μg/0.5 μL per side) on motor activity was determined after direct injection into the VTA, substantia nigra, nucleus accumbens or striatum. Rats were habituated to activity chambers for 1 hour and then injected with either artificial cerebrospinal fluid or OFQ/N into one of the brain regions, and motor activity was recorded for a further 1 hour. Next, the dose–response effect of intra-VTA or intranigral OFQ/N (3 μg or 30 μg/0.5 μL per side) on motor activity was examined. Finally, the effect of intra-VTA OFQ/N (3 μg or 30 μg/0.5 μL per side) on motor activity was determined in the presence of J-113397, an ORL-1 receptor antagonist. **Results:** OFQ/N suppressed motor activity when injected into the VTA and to a lesser extent after direct injection into the nucleus accumbens. However, OFQ/N failed to attenuate motor activity significantly after injection into the substantia nigra or the striatum. Subsequent dose–response studies showed that OFQ/N suppressed motor activity even at a 10-fold-lower dose after intrategmental but not intranigral administration. The motor-suppressant action of intra-VTA OFQ/N was attenuated by J-113397 (1.5 μg/0.5 μL per side) administered into the VTA 10 minutes before administration of OFQ/N. **Conclusion:** Our results indicate that OFQ/N suppresses motor activity through activation of the ORL-1 receptor primarily through an action in the VTA.

Objectif : L'administration intracérébroventriculaire d'orphanine FQ/nociceptine (OFQ/N), ligand agoniste endogène du récepteur ORL-1 (de type récepteur aux opioïdes), diminue les niveaux extracellulaires de dopamine et supprime l'activité motrice. La présence du récepteur ORL-1 sur les neurones du système dopaminergique méso-accumbens et nigrostrié soulève la possibilité qu'une action sur ces cycles représente une façon pour l'OFQ/N de supprimer l'activité motrice. Aussi, la présente étude a utilisé l'administration locale d'OFQ/N dans l'aire tegmentale ventrale (ATV), la substance noire, le noyau accum-

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bens et le striatum pour déterminer si les régions du corps cellulaire et les zones terminales des neurones dopaminergiques contribuaient à l'effet suppresseur de l'OFQ/N sur l'activité motrice. **Méthodes :** On a implanté bilatéralement chez des rats un cathéter-guide dans une des régions cérébrales puis procédé à des essais quatre jours plus tard. On a d'abord déterminé l'effet d'une dose unique d'OFQ/N (30 µg/0,5 µL par côté) sur l'activité motrice après injection directe dans l'ATV, la substance noire, le noyau accumbens ou le striatum. Après avoir acclimaté les rats à des chambres d'activité pendant une heure, on leur a injecté soit un liquide cérébrospinal artificiel, soit de l'OFQ/N dans l'une des régions cérébrales, puis on a enregistré l'activité motrice pendant une autre heure. On a ensuite examiné l'effet dose-réaction de l'OFQ/N à l'intérieur de l'aire tegmentale ou de la substance noire (3 µg ou 30 µg/0,5 µL par côté) sur l'activité motrice. Enfin, on a déterminé, en présence de J-113397, un antagoniste du récepteur ORL-1, l'effet de l'OFQ/N à l'intérieur de l'ATV (3 µg ou 30 µg/0,5 µL par côté) sur l'activité motrice. **Résultats :** L'OFQ/N a supprimé l'activité motrice après injection dans l'ATV et, dans une moindre mesure, après injection directe dans le noyau accumbens. Toutefois, l'OFQ/N n'a pas réduit substantiellement l'activité motrice après injection dans la substance noire ou dans le striatum. Des études subséquentes de la dose-réaction ont révélé que l'OFQ/N supprimait l'activité motrice même après administration d'une dose 10 fois moins forte dans l'aire tegmentale mais non dans la substance noire. La suppression d'activité motrice induite par l'OFQ/N à l'intérieur de l'ATV a été atténuée par J-113397 (1,5 µg/0,5 µL par côté) administré dans l'ATV dix minutes avant l'administration d'OFQ/N. **Conclusion :** Nos résultats indiquent que l'OFQ/N supprime l'activité motrice par activation du récepteur ORL-1, en grande partie par un effet dans l'ATV.

Introduction

Orphanin FQ/nociceptin (OFQ/N), the endogenous agonist ligand of the opioid receptor–like (ORL-1) receptor,[1,2] is a 17-amino-acid peptide that shows structural similarities to the traditional endogenous opioid peptides, and to dynorphin A (1-17) in particular.[1] Likewise, the ORL-1 receptor displays a high degree of homology to the classical (mu, delta and kappa) opioid receptors.[3–6] Moreover, the ORL-1 receptor, in a similar fashion to the classical opioid receptors, is coupled to the same effector systems. Thus, activation of the ORL-1 receptor leads to inhibition of the enzyme adenylyl cyclase[1,2] and calcium channel conductance.[7,8] Furthermore, activation of inwardly rectifying potassium channels is associated with stimulation of the ORL-1 receptor.[9–11]

OFQ/N decreases dopaminergic neurotransmission across the mesoaccumbens axis in a counter-opioid manner.[12] OFQ/N, for example, decreases extracellular dopamine (DA) in the nucleus accumbens after either intracerebroventricular[12,13] or intra–ventral tegmental area (intra-VTA) administration.[14] Moreover, intracerebroventricular OFQ/N administration attenuates the increase in extracellular DA induced by morphine in the ventral striatum without having a significant effect on DA levels in the dorsal striatum.[15] Thus, it seems reasonable to hypothesize that OFQ/N preferentially regulates the activity of the mesoaccumbens versus the nigrostriatal dopaminergic axis. Interestingly, when injected into the striatum, OFQ/N apparently increases extracellular DA in this structure.[16]

The behavioural action of OFQ/N on motor activity is consistent with the ability of the drug to decrease extracellular DA along the mesoaccumbens axis. OFQ/N suppresses motor activity after intracerebroventricular administration in mice[2] and rats.[13,18,19] However, the site of action of OFQ/N is not fully characterized. Both the mesoaccumbens and nigrostriatal pathways are implicated in the modulation of motor activity. Importantly, the ORL-1 receptor is expressed on the DA cells within the VTA and the substantia nigra.[20,21] Thus, an action along the mesoaccumbens or the nigrostriatal axis, or both, is one possible means for the motor-suppressant effect of OFQ/N. The present study, therefore, used direct injection of OFQ/N into the cell-body regions and terminal fields of the 2 major dopaminergic pathways to determine the contribution of these sites to the motor-suppressant effect of OFQ/N.

1-[(3R,4R)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1,3-dihydro-2H-benzimidazol-2-one (J-113397) is characterized as a selective and potent nonpeptidic ORL-1 receptor antagonist.[22] Radioligand binding assays using mouse brain homogenates revealed that J-113397 acts as a competitive antagonist at the ORL-1 receptor, with at least 350-fold selectivity for the ORL-1 receptor over the classical opioid receptors.[23] Therefore, the effect of intra-VTA OFQ/N on motor activity was also tested in the presence of J-113397 to confirm that the action of OFQ/N is mediated via activation of the ORL-1 receptor.

Methods

Male Sprague–Dawley rats, weighing 180–200 g, obtained from Harlan (San Diego, Calif.) were housed 2–3 per cage with free access to food and water. All experiments were conducted during the light phase of a 12-hour light/dark cycle in accordance with the *National Institute Council Guide for the Care and Use of Animals in Research* and approved by the UCLA Institutional Animal Care and Use Committee.

Rats were anesthetized with halothane in a mixture (1:1) of oxygen and nitrous oxide, placed on a stereotactic frame and implanted bilaterally with 22-gauge guide cannulae (inside diameter 0.39 mm, outside diameter 0.71 mm, length 5.0 mm; Plastics One, Roanoke, Va.). The coordinates were set according to the atlas of Paxinos and Watson,[17] with the bregma as the point of reference. The guide cannulae were aimed at a cell-body region or terminal field of the dopaminergic neurons and secured to the skull by dental cement and 2 metallic screws. The following coordinates were used: VTA (AP –4.8, ML ±2.7, DV –8.6 at a 10° angle), substantia nigra (AP –5.2, ML ±2.6, DV –7.6), nucleus accumbens (AP +1.6, ML ±1.6, DV –7.8) and the striatum (AP +0.3, ML ±2.6, DV –7.0). The rats were allowed at least 4 days to recover from the surgery.

A total of 167 rats were used. Distance travelled (cm), used as a measure of motor activity, was recorded during the 2-hour testing period using a Videomex-V motor activity apparatus (Columbus Instruments, Columbus, Ohio). Initially, the effect of a single dose of OFQ/N (30 µg/0.5 µL per side) on motor activity was determined after injection into the cell-body regions or terminal fields of the dopaminergic neurons. Rats were habituated to testing chambers (34-cm diameter × 30 cm high, made of grey plastic) for 1 hour and then temporarily removed from the testing chambers and injected with artificial cerebrospinal fluid (aCSF) or OFQ/N into the VTA (n = 8–12 rats/group), substantia nigra (n = 8 rats/group), nucleus accumbens (n = 5 rats/group) or striatum (n = 7 rats/group). The rats were then immediately returned to the testing chambers and motor activity was recorded at 15-minute intervals for a further 1 hour.

Subsequently, the dose–response effect of OFQ/N (3 µg/0.5µL or 30 µg/0.5µL per side) on motor activity was determined after direct injection of the peptide into the cell-body regions of the 2 dopaminergic pathways. Rats were habituated to the testing chambers for 1 hour, injected with aCSF or OFQ/N into the VTA (n = 12–14 rats/dose) or substantia nigra (n = 7–11 rats/dose), and motor activity was recorded for a further 1 hour.

Finally, to confirm whether the action of OFQ/N is mediated through activation of the ORL-1 receptor, the effect of OFQ/N on motor activity was studied in the presence of J-113397, an ORL-1 receptor antagonist.[22] Thirty-eight rats (n = 5–7 rats/group) were habituated to the testing chambers for 1 hour and then injected with either dimethyl sulfoxide (20% in aCSF) or J-113397 (1.5 µg/0.5µL per side) directly into the VTA. Ten minutes later, rats were treated with aCSF or OFQ/N (3 µg/0.5µL or 30 µg/0.5µL per side) administered into the VTA, and motor activity was measured for an additional 1 hour.

At the end of each experiment, rats were anesthetized with pentobarbital (50 mg/kg, intraperitoneally) and transcardially perfused with phosphate-buffered saline (PBS), followed by 50 mL of 10% phosphate-buffered formalin. The brains were removed, and 40-µm slices were sectioned using a cryostat (Leica Instruments, Germany). The slices were stained with cresyl violet and viewed under the microscope for verification of the site of injection. Based on such examination, 14 rats were discarded from the data analysis because of the improper placement of the guide cannula.

Data are expressed as means (and standard error of the mean [SEM]). Single-dose studies were analyzed using a 2-way repeated-measure analysis of variance (ANOVA). For dose–response studies, total distance travelled during the first 15 minutes or over the entire 1-hour testing session after drug treatment was analyzed using a 1-way ANOVA. The post hoc Newman–Keuls test was used to reveal significant differences between the treatment groups. A $p < 0.05$ was considered statistically significant.

OFQ/N and J-113397 were generously supplied by the National Institute on Drug Abuse (NIDA) Drug Supply Program (Research Triangle Park, NC). Drug injection was performed slowly (0.5 µL per side over a 60-s period) to avoid tissue damage and diffusion of the peptide to neighbouring brain regions. The injection was carried out using a 30-gauge needle attached to a 10-µL Hamilton syringe by PE-10 tubing.

Results

Rats showed exploratory behaviours upon initial placement in the testing chambers that declined significantly

over the 1-hour habituation period. Analysis of data collected during this period using a repeated-measure ANOVA showed no significant difference between the rats treated with aCSF or OFQ/N in the subsequent hour (Fig. 1, Fig. 2, Fig. 3).

Effects of intra-VTA OFQ/N administration on motor activity

Fig. 1A illustrates the time course of the motor-suppressant effect of a single dose of OFQ/N (30 μg) after intra-VTA administration. The action of OFQ/N was maximal during the first 15-minute session and appeared to wear off thereafter. A 2-way repeated-measure ANOVA showed a significant effect of treatment ($F_{1,18} = 17.76$, $p < 0.05$), a significant effect of time ($F_{3,54} = 7.18$, $p < 0.05$) and a significant interaction between treatment and time ($F_{3,54} = 13.34$, $p < 0.05$), indicating that direct injection of OFQ/N into the VTA suppressed motor activity as compared with aCSF ($p < 0.05$). The dose–response relation of OFQ/N showed that the peptide produced motor suppression even at a dose that was 10 times lower (Fig. 1B, Fig. 1C). One-way ANOVA of the distance travelled during the first 15-minute session ($F_{2,37} = 10.73$, $p < 0.05$; Fig. 1B) or the entire 1-hour testing period ($F_{2,37} = 5.75$, $p < 0.05$; Fig. 1C) revealed that OFQ/N, as compared with aCSF, significantly attenuated motor activity at both doses (3 μg and 30 μg) ($p < 0.05$).

Effects of intranigral OFQ/N administration on motor activity

The time course of the action of OFQ/N on motor activity after direct injection into the substantia nigra is shown in Fig. 2A. Unlike intra-VTA OFQ/N administration, injection of OFQ/N (30 μg) into the substantia nigra produced a short-lasting inhibition of motor activity. Despite the fact that OFQ/N reduced motor activity during the first 15-minute testing session (Fig. 2A), a 2-way repeated-measure ANOVA revealed no significant effect of treatment ($F_{1,14} = 1.11$, $p > 0.05$) or interaction between treatment and time ($F_{3,42} = 3.20$, $p = 0.06$). Analysis of the dose–response relation of OFQ/N (0, 3 or 30 μg) during the first 15-minute testing session revealed a trend toward attenuation of motor activity ($F_{2,26} = 2.58$, $p = 0.09$) (Fig. 2B). However, analysis of the total distance travelled during the entire 1-hour testing period (Fig. 2C) revealed no significant motor suppression after OFQ/N administration ($F_{2,26} = 1.04$, $p > 0.05$), suggesting that injection of OFQ/N into this brain region produced motor suppression that was limited to the first 15-minute session.

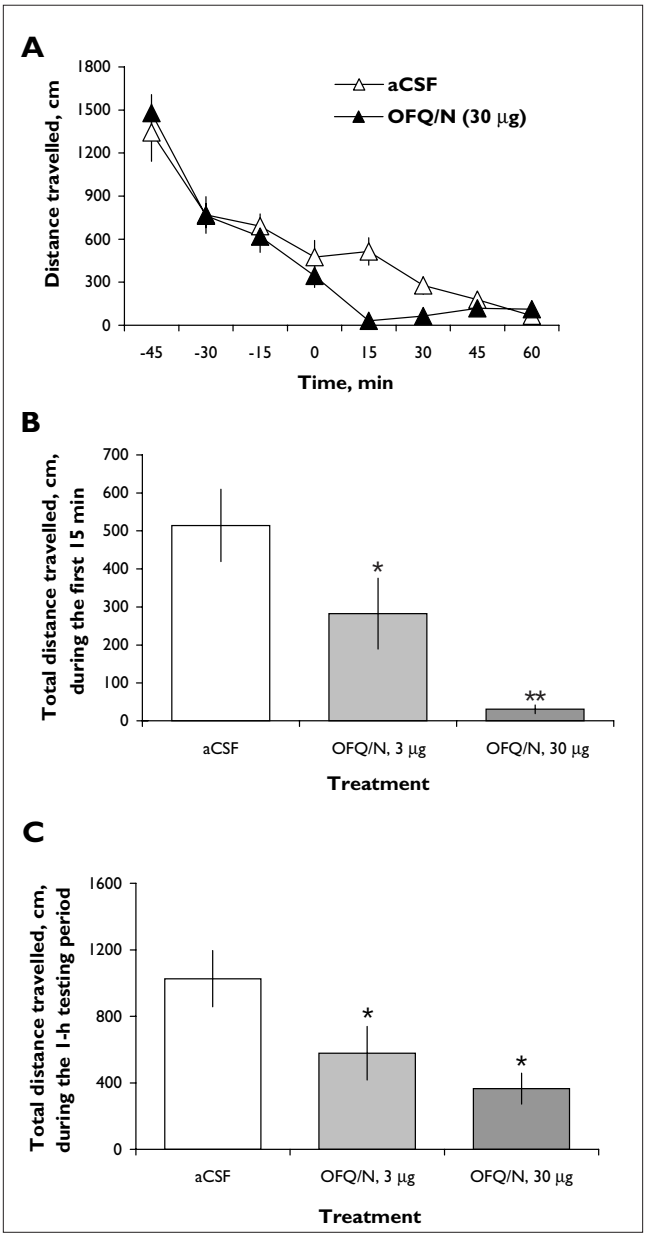

Fig. 1: Time course (A) and dose–response relation (B and C) of the actions of orphanin FQ/nociceptin (OFQ/N) on motor activity after direct injection into the ventral tegmental area (VTA). Rats were habituated to the testing chambers for 1 h and injected with either artificial cerebrospinal fluid (aCSF) or OFQ/N into the VTA at 0 min. Motor activity was measured at 15-minute intervals for a further 1 h. Values are means (and standard error of the mean [SEM]). *Significantly different from aCSF ($p < 0.05$). **Significantly different from all other groups ($p < 0.05$).

Effects of intra-accumbal OFQ/N administration on motor activity

Bilateral injection of OFQ/N (30 μg) into the nucleus accumbens also resulted in suppression of motor activity (Fig. 3A). Analysis of the data revealed a significant effect of treatment ($F_{1,8}$ = 13.48, $p < 0.05$), a significant effect of time ($F_{3,24}$ = 24.25, $p < 0.05$) and a significant interaction between treatment and time ($F_{3,24}$ = 7.36, $p < 0.05$), indicating that OFQ/N, as compared with aCSF, significantly suppressed motor activity over the 1-hour testing period. Despite the fact that OFQ/N-treated group showed less exploratory behaviour during the habituation period, there was no significant difference between the 2 groups ($p > 0.05$).

Effects of intrastriatal OFQ/N administration on motor activity

Administration of OFQ/N (30 μg) directly into the striatum failed to produce motor suppression (Fig. 3B). A 2-way repeated-measure ANOVA revealed a significant effect of time ($F_{3,36}$ = 3.11, $p < 0.05$), but no significant effect of treatment ($F_{1,12}$ = 0.68, $p > 0.05$) or interaction between time and treatment ($F_{3,36}$ = 0.59, $p > 0.05$),

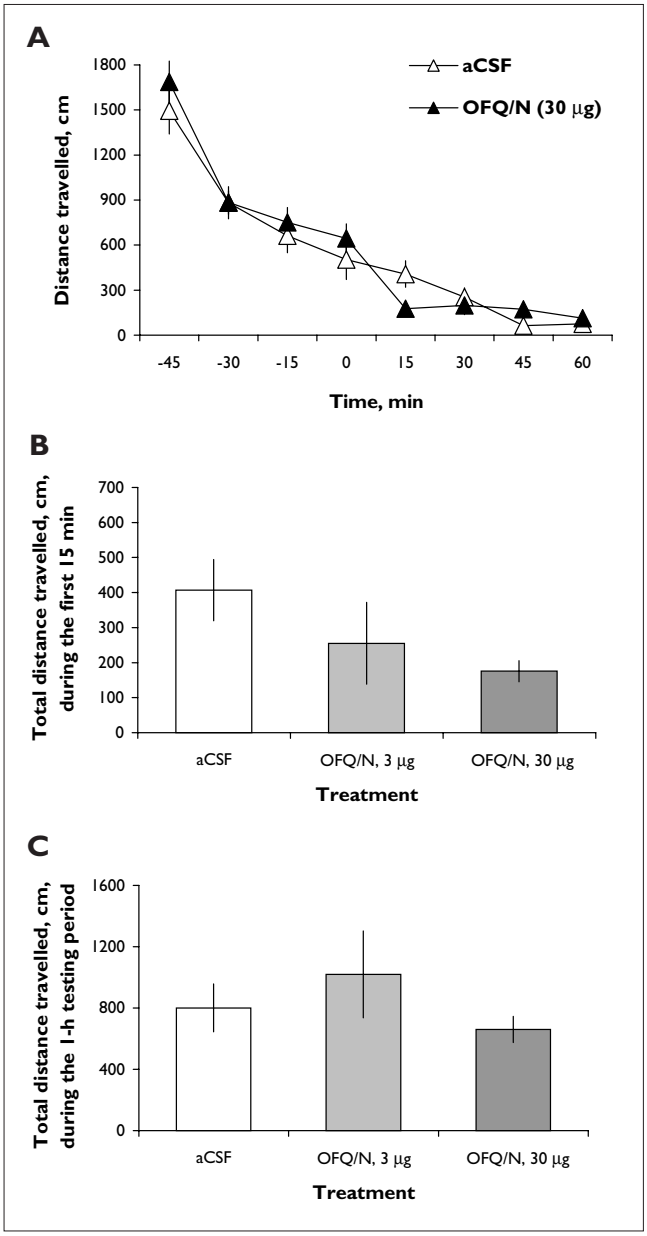

Fig. 2: Time course (A) and dose–response relation (B and C) of the actions of OFQ/N on motor activity after direct injection into the substantia nigra. Rats were habituated to the testing chambers for 1 h and injected with either aCSF or OFQ/N into the substantia nigra at 0 min. Motor activity was measured at 15-min intervals for a further 1 h. Values are means (and SEM).

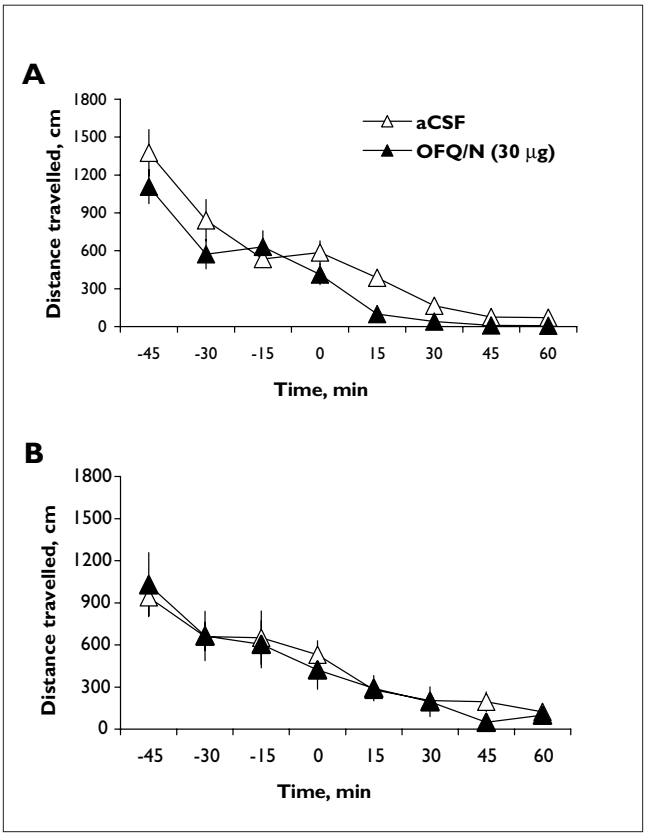

Fig. 3: Effects of OFQ/N administration on motor activity after direct injection into the nucleus accumbens (A) or the striatum (B). Rats were habituated to the testing chambers for 1 h and received an injection of either aCSF or OFQ/N into the nucleus accumbens (A) or striatum (B) at 0 min. Motor activity was measured at 15-min intervals for a further 1 h. Values are means (and SEM).

showing that OFQ/N, as compared with aCSF, did not significantly affect motor activity over the 1-hour testing period.

Effects of intra-VTA OFQ/N on motor activity in the presence of J-113397

The effect of intra-VTA OFQ/N administration on motor activity in the presence of J-113397, an ORL-1 receptor antagonist, is shown in Fig. 4. Once again, 1-way ANOVA revealed that intra-VTA OFQ/N administration attenuated in a dose-dependent manner total distance travelled during the entire 1-hour testing session ($F_{5,32} = 4.21$, $p < 0.05$). The ORL-1 receptor antagonist, J-113397, did not alter motor activity ($p > 0.05$; compare J-aCSF v. D-aCSF group) but significantly decreased the motor-suppressant action of OFQ/N (Fig. 4). The inhibitory effect of J-113397 was more pronounced against the low dose OFQ/N, that is, it totally abolished the motor-suppressant action of 3 μg of OFQ/N ($p > 0.05$; compare J-OFQ/N3 v. J-aCSF or D-aCSF). However, J-113397 was only able to show attenuation of the motor-suppressant action of the high dose of OFQ/N ($p > 0.05$; compare D-aCSF or J-aCSF v. J-OFQ/N 30 group).

Discussion

The main finding of the present investigation is that OFQ/N decreased motor activity after injection into the VTA and, to a lesser extent, after local injection into the nucleus accumbens. However, OFQ/N failed to produce significant motor suppression after injection into the substantia nigra or striatum. The action of intra-VTA OFQ/N on motor activity was attenuated by J-113397, an ORL-1 receptor antagonist. Overall, the present results indicate that activation of the ORL-1 receptor in the VTA and, at least in part, in the nucleus accumbens mediates the inhibitory action of OFQ/N on motor behaviours.

Opioid drugs have been shown to modulate the activity of mesolimbic and nigrostriatal dopaminergic neurons.[24,25] Activation of the mu and delta opioid receptors enhances, whereas that of the kappa opioid receptors attenuates, the activity of these pathways,[26] possibly because of their differential expression on neuronal components within the cell-body regions and terminal fields of these neurons. Whereas mu and delta opioid receptors are predominantly located on the gamma aminobutyric acid (GABA)-ergic inputs to DA cells in the VTA and substantia nigra,[27,28] kappa opioid receptors are believed to be expressed on DA cells themselves.[28] The ORL-1 receptor is distributed throughout the brain, particularly in brain regions involved in emotional and motivational behaviours.[29] It shows somewhat similar tissue distribution to that of the classical opioid receptors and, thus, its action is somewhat similar to that of dynorphin A (1-17) and other kappa opioid receptor agonists on dopaminergic neurons.[12,19]

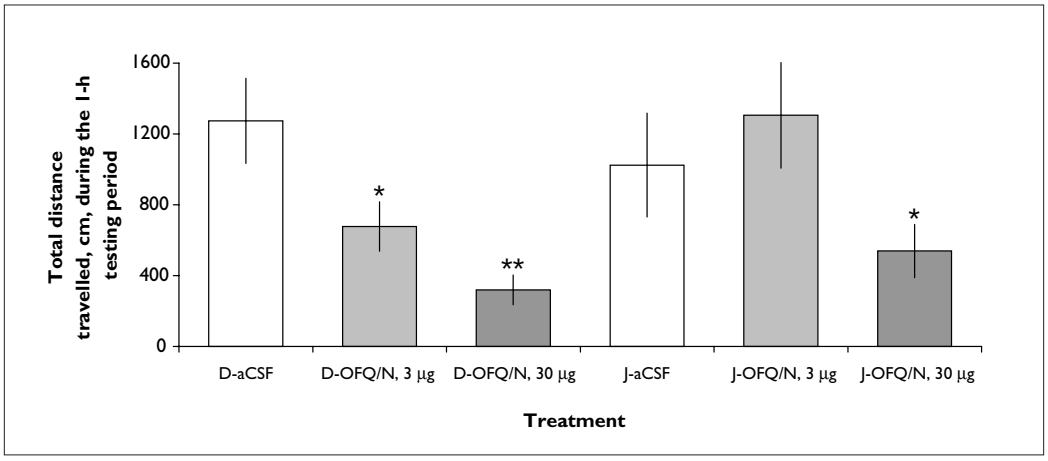

Fig. 4: The motor-suppressant action of OFQ/N was attenuated by pretreatment with J-113397, an ORL-1 receptor antagonist. Rats were habituated to the testing chambers for 1 h, injected with dimethyl sulfoxide (D) or J-113397 (J) and 10 min later received aCSF or OFQ/N (3 μg/0.5 μL or 30 μg/0.5 μL per side). Motor activity was then measured for an additional 1 h. Values are means (and SEM). *Significantly different from the respective control group ($p < 0.05$). **Significantly different from all other groups ($p < 0.05$).

Opioid drugs acting at the mu and delta opioid receptors increase, whereas the kappa opioid receptor agonists decrease, extracellular DA.[26,30] OFQ/N has been shown to decrease extracellular DA in the nucleus accumbens after intracerebroventricular administration[12] and therefore resembles more closely the action of kappa opioid receptor agonists on this system.[24] However, kappa opioid receptor agonists are believed to exert their effects on DA release via a direct action on DA terminals in the nucleus accumbens and striatum.[3] In contrast, our previous findings suggested a predominantly VTA site of action for OFQ/N.[13,14,19,31] Thus, the inhibitory effect of OFQ/N could be the result of direct activation of the ORL-1 receptors expressed on the dopaminergic neurons in the VTA.[20,21,32]

The present study was designed to determine where along the mesoaccumbens and nigrostriatal dopaminergic pathways OFQ/N acts to suppress motor activity. Our present behavioural data corroborate previous microdialysis data[14] by showing that OFQ/N was most potent in inhibiting locomotor activity when administered into the VTA. The observation that OFQ/N failed to produce motor suppression in the presence of J-113397, an ORL-1 receptor antagonist,[22] strongly suggests that the motor-suppressant action of OFQ/N is mediated through activation of the ORL-1 receptor. Thus, the decreased motor activity reported following intracerebroventricular OFQ/N administration[2,13,18] results, at least in part, from an action of OFQ/N on dopaminergic neurons in the VTA. Further support for this comes from our recent observation that OFQ/N attenuates the motor stimulation and the increase in extracellular DA induced by cocaine in the nucleus accumbens in rats.[13]

Given that the nigrostriatal dopaminergic neurons play a modulatory role on motor behaviour and that the ORL-1 receptor is expressed in this brain region,[20,21] our failure to observe long-lasting motor suppression after intranigral OFQ/N was somewhat surprising. Currently, it is not known what effect, if any, intranigral OFQ/N exerts on extracellular DA in the striatum, but our present data suggest that OFQ/N may have a somewhat selective action on the mesoaccumbens versus the nigrostriatal dopaminergic pathway. Consistent with this notion, we observed that direct injection of OFQ/N into the nucleus accumbens, but not the striatum, also suppressed motor activity. A previous report by Di Giannuario and Pieretti[15] further supports this notion by showing that intracerebroventricular OFQ/N administration selectively decreases morphine-induced increases in extracellular DA in the nucleus accumbens but not in the striatum.

Intra-VTA OFQ/N injection has been shown to increase extracellular GABA in this brain region, an effect associated with a decrease in extracellular DA in the nucleus accumbens.[14] Thus, the action of OFQ/N on dopaminergic as well as nondopaminergic neurons in the VTA could contribute to a greater motor suppression observed after intra-VTA OFQ/N administration. However, evidence is lacking to show that such a mechanism does not occur in the substantia nigra. An alternative explanation could, therefore, be that the density of ORL-1 receptors may be different in these brain regions. Unfortunately, earlier studies have aimed at localization rather than quantification of the ORL-1 receptor.[20,21] Because the mesoaccumbens and nigrostriatal neurons are thought to mediate different aspects of motor behaviours, it may be possible that the measure of motor activity (distance travelled) is mediated by activation of the mesoaccumbens dopaminergic neurons. This may explain why the action of OFQ/N was more pronounced after intra-VTA or intra-accumbal, as compared with intranigral or intrastriatal, administration.

In summary, OFQ/N decreased motor activity after administration into the VTA and to a lesser extent after injection into the nucleus accumbens and substantia nigra. OFQ/N, however, failed to produce any significant effect after direct injection into the striatum. Although the current data cannot rule out the involvement of additional motor brain regions and/or modulation of other neurotransmitter systems in the motor-suppressant action of OFQ/N, our results suggest that OFQ/N attenuated motor activity via activation of the ORL-1 receptor primarily along the mesoaccumbens axis. Given the fact that hyperactivity of the mesoaccumbens axis is implicated in schizophrenia[33–35] and development of drug dependency,[36,37] agonists of the ORL-1 receptor may have potential therapeutic value in this regard.

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