ELSEVIER

European Journal of Pharmacology 442 (2002) 237–239

www.elsevier.com/locate/ejphar

Short communication

Effect of opioid and cannabinoid receptor antagonism on orphanin FQ-induced hyperphagia in rats

Thomas A. Pietras, Neil E. Rowland *

Department of Psychology, University of Florida, P.O. Box 112250, Gainesville, FL 32611-2250, USA

Received 12 March 2002; accepted 22 March 2002

Abstract

Feeding induced in rats by cerebroventricular (i.c.v.) injection of orphanin FQ was potently and dose-dependently reversed by peripheral injection of either the opioid antagonist naloxone or the cannabinoid CB_1 receptor antagonist SR 141716{*N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophelyl)-4-methyl-3-pyrazole-carboxamine}. The combination of these two agents inhibited food intake in a manner suggestive of additivity or supra-additivity.

Keywords: Orphanin FQ; Endocannabinoid; SR 141716; Naloxone; Opioid; Hyperphagia; Anorexia; Drug additivity

1. Introduction

The heptadecapeptide orphanin FQ or nociceptin (Reinscheid et al., 1995) induces substantial food intake with short latency in non-deprived rats after either i.c.v. or brain intraparenchymal injection (Olszewski et al., 2000; Polidori et al., 2000; Pomonis et al., 1996; Stratford et al., 1997). Orphanin FQ is the endogenous agonist of a G-protein coupled receptor (ORL1) that has structural similarity to the opioid receptors (viz. μ, κ, and δ), but is functionally distinct from them because it does not bind ligands for these receptors (Mogil and Pasternak, 2001). As may be expected from this profile, eating induced by orphanin FQ can be blocked by a putative antagonist of the ORL1 receptor, but is also blocked by the non-selective opioid receptor antagonist naloxone (Polidori et al., 2000; Pomonis et al., 1996). This suggests an opioid involvement in orphanin FQ's action.

Two recent studies have shown that the combination of naloxone and SR141716 {*N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophelyl)-4-methyl-3-pyrazole-carboxamine}, a cannabinoid CB_1 receptor antagonist (Rinaldi-Carmona et al., 1994), has synergistic inhibitory effects on food intake in rats after deprivation (Kirkham and Williams, 2001) and in a 'dessert' protocol (Rowland et al., 2001). This result is of significance because many other combinations of anorectic agents seem to have additive but not synergistic actions (e.g. Roth and Rowland, 1999). The purpose of the present study is to determine whether SR141716 inhibits orphanin FQ-induced feeding and if it has a synergistic action with naloxone.

2. Materials and methods

2.1. Animals, housing and surgery

Either female retired breeder or adult male Sprague–Dawley rats from Harlan Labs (Indianapolis, IN), weighing 270–330 g, were housed individually in stainless steel mesh cages (21 × 17 × 17 cm) suspended over absorbent paper pan liners. The vivarium was illuminated by fluorescent lights from 0600 to 1800 h and was maintained at 22 ± 2 °C. Unless stated, food pellets (5001 Lab Chow, PMI International) and tap water was available at all times. Procedures were in accordance with local and federal principles for animal care and use. These studies were performed using several batches of rats; within a batch, rats of one sex were used. There were no apparent sex differences or substantial differences in drug effects between batches, which have therefore been combined for purposes of presentation.

Each rat was implanted with an indwelling 23-gauge stainless steel cannula aimed stereotaxically at its right

* Corresponding author. Tel.: +1-352-392-0601 ext. 287; fax: +1-352-392-7985.

E-mail address: nrowland@ufl.edu (N.E. Rowland).

0014-2999/02/$ - see front matter
PII: S0014-2999(02)01551-0

lateral cerebral ventricle. Surgical anesthesia was a mixture of ketamine and xylazine (50 + 7 mg/kg, i.p.). The cannula was anchored to the skull using jeweler's screws and dental acrylic and was occluded with a metal pin. Rats were given postoperative analgesic (ketorolac, 2 mg/kg, s.c.) and were kept warm until fully alert. They were then returned to their home cages and allowed 7–12 days of recovery. During this time, they were adapted to frequent handling.

2.2. Drugs

SR141716 was generously provided by Sanofi-Synthelabo Recherche (Montpellier, France). All other agents were purchased: orphanin FQ (Bachem, King of Prussia, PA), naloxone HCl (Sigma, St. Louis, MO), propylene glycol (Fisher Scientific, Orlando, FL), and surgical reagents (ketamine, xylazine, ketorolac tromethamine, Henry Schein, Melville, NY).

2.3. General procedure

Experiments were performed at 1 week intervals using non-deprived rats at a time of day (~ 1300 h) that they do not normally eat. To ensure there was no recent meal, food was removed from the cage about 90 min before treatments. Rats received designated pre-injections (s.c. or i.p.) and then i.c.v. injection of orphanin FQ or its vehicle (4 μl distilled water). For i.c.v. injection, the rats were held gently in a towel and an injector needle (11 mm) inserted into the cannula and injection made from a microsyringe over about 10 s. The rats were returned immediately to their home cage with pre-weighed chow pellets and their food intake, corrected for spillage collected on paper under the cage, was recorded after 1 h.

2.3.1. Study A: dose response for orphanin FQ

Rats were divided at random into three groups of seven and received, in mixed order, either 0, 3 or 6 nmol orphanin FQ. Food intake was recorded as described above.

2.3.2. Study B: effects of naloxone and SR141716 alone and in combination

The dose inhibitory effects of naloxone, SR141716, and their combination were examined using food intake induced by 6 nmol orphanin FQ. Rats were injected i.p. with doses of SR 141716 ranging from 0 to 2.0 mg/kg dissolved in a vehicle propylene glycol and water (3: 1 v/v) or s.c. with doses of naloxone ranging from 0 to 1.0 mg/kg dissolved in isotonic saline. In both cases, these injections were given 30–54 min before orphanin FQ. In combination studies, rats received both i.p. SR141716 and s.c. naloxone (doses were chosen from the component drug dose–effect curves) followed 30–45 min later by orphanin FQ (6 nmol). Because of inter-animal variability in the food intakes to orphanin FQ alone, all intakes after drug pretreatments were transformed to percentage of these individual orphanin FQ intakes. Rats received a total of 3–5 injections, each separated by 4–7 days.

2.4. Statistics

In Study A, food intakes were treated by one-way analysis of variance (ANOVA, Sigma Stat, SPSS), with dose of anorectic drug as main factor and significance was assessed using Student–Newman–Kuels method of multiple comparisons ($P < 0.05$). In study B, intakes after drugs were compared using paired *t*-tests with intake after orphanin FQ alone, again with $P < 0.05$.

3. Results

3.1. Study A: dose response for orphanin FQ

Orphanin FQ increased food intake in a dose-related manner. Mean ± S.E.M. 1 h intakes were 1.8 ± 4 and 2.8 ± 3 g after 3 and 6 nmol orphanin FQ, respectively, both significantly higher than after vehicle treatment (1.0 ± 2 g).

3.2. Study B: effects of naloxone and SR 141716 alone and in combination

The higher dose of orphanin FQ (6 nmol) was used in Study B. Mean absolute intakes induced by this dose were 3–4 g in 1 h. Both naloxone and SR141716 potently reversed orphanin FQ-induced feeding, with ED_{50} of ~ 0.2 mg/kg for each agent (Table 1). Combination of 0.1 mg/kg of each agent reduced food intake by ~ 50%, suggesting additivity of effect. However, the combination of 0.05 mg/kg of each agent was comparably effective, suggesting a possible supra-additive effect. The dose–response functions of the single drugs and their combinations appeared to be non-linear, so we did not attempt a formal isobolographic analysis.

Table 1
Food intake of rats treated with orphanin FQ (6 nmol) and naloxone or SR 141716 either alone or in combination

Treatment	Dose (mg/kg)	1 h food intake[a]
Naloxone	0.15	83 ± 17
	0.3	24 ± 6*
	1.0	18 ± 13*
SR141716	0.2	46 ± 12*
	0.5	13 ± 6*
	1.0	18 ± 13*
	2.0	7 ± 2*
Naloxone + SR 141716	0.05 + 0.05	56 ± 18*
	0.1 + 0.1	45 ± 13*
	0.2 + 0.15	11 ± 7*

[a] Mean ± S.E.M. as % orphanin FQ alone.
* $P < 0.05$ vs. orphanin FQ (paired *t*-tests).

4. Discussion

The present study confirms a previous report that i.c.v. injection of orphanin FQ stimulates feeding in rats and that this is reversed by peripheral injection of naloxone (Pomonis et al., 1996). We now report that the CB_1 receptor antagonist, SR141716, has a potent inhibitory effect on orphanin FQ-induced feeding. Further, a combination of low doses of naloxone and SR141716 produced at least an additive effect on feeding although, because of the non-linear nature of the dose–effect functions, we cannot clearly answer the question of whether the present results indicate drug synergy (e.g. Roth and Rowland, 1999).

Compared with food intake in other protocols, that induced by orphanin FQ appears to be more potently inhibited by SR141716. For example, we reported an ED_{50} of 1.8 mg/kg SR141716 in non-deprived rats eating a palatable dessert (Rowland et al., 2001), data that are consistent with other literature cited in that report. In the present work, the ED_{50} was $\sim$ 0.2 mg/kg. This is not due to the use of less palatable chow in the present study because, in deprived rats eating chow, 1 mg/kg SR141716 produced only 17% (Kirkham and Williams, 2001) or 30% (Rowland et al., 2001) reductions of intake from established baselines. However, those baselines were higher than the intakes stimulated by N/OFQ so we cannot rule out an influence of base rates of feeding on drug efficacy measured as percentage decrease. Naloxone also was more potent in the present study (ED_{50} $\sim$ 0.2 mg/kg) than has been reported in deprived rats (e.g. a 32% reduction at 1 mg/kg; Kirkham and Williams, 2001).

Similarly to the individual drugs, the combination of naloxone and SR141716 inhibited orphanin FQ feeding (ED_{50} $\sim$ 0.1 mg/kg total drug dose) more potently that feeding in either dessert ($\sim$ 0.6 mg/kg; Rowland et al., 2001) or deprivation ($>$1 mg/kg; Kirkham and Williams, 2001) protocols. In both of those studies, the effect of the combined drugs was statistically greater than that of either alone (i.e. a synergistic action), and the present results are consistent with but do not prove that point in relation to orphanin FQ-induced feeding.

The mechanism by which these agents might act synergistically to inhibit food intake remains unclear, although an interaction between opioid and cannabinoid systems has been noted in other contexts (e.g. Ledent et al., 1999; Navarro et al., 2001; Welch and Eads, 1999). The anatomical substrate remains unclear. Thus, microinjection of orphanin FQ into the shell of the nucleus accumbens or the ventromedial hypothalamus increased food intake (Stratford et al., 1997). These regions did not, however, exhibit Fos-like immunoreactivity following cerebroventricular injection of orphanin FQ; regions activated included nucleus of the solitary tract, paraventricular hypothalamus, and central nucleus of the amydgala (Olszewski et al., 2000). Further studies will be needed to clarify the role of endogenous orphanin FQ in feedings, as well as its interaction with systems such as opioids and cannabinoids that may be involved in behaviors of excess, including unnecessary consumption of food.

References

Kirkham, T.C., Williams, C.M., 2001. Synergistic effects of opioid and cannabinoid antagonists on food intake. Psychopharmacology 153, 267–270.

Ledent, C., Valverde, O., Cossu, G., Petitet, F., Aubert, J.-F., Beslot, F., Bohme, G.A., Imperato, A., Pedrazzini, T., Roques, B.P., Vassart, G., Fratta, W., Parmentier, M., 1999. Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB_1 receptor knockout mice. Science 283, 401–404.

Mogil, J.S., Pasternak, J.W., 2001. The molecular and behavioral pharmacology of the orphanin FQ/nociceptin peptide and receptor family. Pharmacol. Rev. 53, 381–415.

Navarro, M., Carrera, M.R., Fratta, W., Valverde, O., Cossu, G., Frattore, L., Chowen, J.A., Gomez, R., del Arco, I., Villanua, M.A., Maldonado, R., Koob, G.F., de Fonseca, F.R., 2001. Functional interaction between opioid and cannabinoid receptors in drug self-administration. J. Neurosci. 21, 5344–5350.

Olszewski, P.K., Billington, C.J., Levine, A.S., 2000. Fos expression in feeding-related brain areas following the intracerebroventricular administration of orphanin FQ in rats. Brain Res. 855, 171–175.

Polidori, C., de Caro, G., Massi, M., 2000. The hyperphagic effect of nociceptin/orphanin FQ in rats. Peptides 21, 1051–1062.

Pomonis, J.D., Billington, C.J., Levine, A.S., 1996. Orphanin FQ, agonist of orphan opioid receptor ORL1, stimulates feeding in rats. NeuroReport 8, 369–371.

Reinscheid, R.K., Nothacker, H.P., Bourson, A., Ardati, A., Henningsen, R.A., Bunzow, J.R., Grandy, D.K., Langen, H., Monsuma Jr., F.J., Civelli, O., 1995. Orphanin FQ: a neuropeptide that activates an opioid-like G-protein-coupled receptor. Science 270, 792–794.

Rinaldi-Carmona, M., Barth, F., Heaulme, M., Shire, D., Calandra, B., Congy, C., Martinez, S., Maruani, J., Neliat, G., Caput, D., Ferrara, P., Soubrie, P., Breliere, J.C., Le Fur, G., 1994. SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. FEBS Lett. 350, 240–244.

Roth, J.D., Rowland, N.E., 1999. Anorectic efficacy of the fenfluramine/phentermine combination in rats: additivity or synergy? Eur. J. Pharmacol. 373, 127–134.

Rowland, N.E., Mukherjee, M., Robertson, K., 2001. Effects of the cannabinoid receptor anatgonist SR141716, alone and in combination with dexfenfluramine or naloxone, on food intake in rats. Psychopharmacology 159, 111–116.

Stratford, T.R., Holanan, M.R., Kelley, A.E., 1997. Injections of nociceptin into the nucleus accumbens shell or ventromedial hypothalamic nucleus increase food intake. NeuroReport 8, 423–426.

Welch, S.P., Eads, M., 1999. Synergistic interactions of endogenous opioids and cannabinoid systems. Brain Res. 848, 183–190.