ELSEVIER

European Journal of Pharmacology 449 (2002) 99–103

www.elsevier.com/locate/ejphar

UCM707, a potent and selective inhibitor of endocannabinoid uptake, potentiates hypokinetic and antinociceptive effects of anandamide

Eva de Lago [a], Javier Fernández-Ruiz [a,*], Silvia Ortega-Gutiérrez [b], Alma Viso [b], María Luz López-Rodríguez [b], José A. Ramos [a]

[a] *Departamento de Bioquímica y Biología Molecular, Facultad de Medicina, Universidad Complutense, 28040-Madrid, Spain*
[b] *Departamento de Química Orgánica, Facultad de Ciencias Químicas, Universidad Complutense, 28040-Madrid, Spain*

Received 24 January 2002; received in revised form 17 June 2002; accepted 21 June 2002

Abstract

To date, UCM707, *N*-(3-furylmethyl)eicosa-5,8,11,14-tetraenamide, has the highest potency and selectivity in vitro as inhibitor of the endocannabinoid transporter, which might make this compound useful in potentiating endocannabinoid transmission, with minimal side-effects, in the treatment of several disorders. However, there is no information about how UCM707 behaves in vivo as regards certain classic effects of endocannabinoids, such as hypomotility and antinociception. In the present work, we tested in rats the dose–response effects of UCM707 in the open-field and hot-plate tests, and, in particular, we analyzed whether this compound enhanced the hypokinetic and/or the antinociceptive actions of anandamide at a subeffective dose, using these two in vivo assays. UCM707, administered alone, had no effect on ambulatory, exploratory and stereotypic activities, time spent in inactivity and sensitivity to noxious heat, with only some small responses at the highest dose used. UCM707, administered at a dose that did not produce any effects by itself or these were very small, was, however, able to significantly potentiate the action of a dose of anandamide that did not produce any effects when it was administered alone. So, the combination of both compounds produced greater decreases in exploratory activity and, particularly in ambulation, increased the time spent in inactivity and the latency to respond to a painful stimulus. In summary, UCM707, as suggested by its in vitro properties, seems also to behave in vivo as a selective and potent inhibitor of the endocannabinoid transporter, showing negligible direct effects on the receptors for endocannabinoids but potentiating the action of these endogenous compounds. This compound is, thus, a promising tool, used alone or in combination with endocannabinoids, for the treatment of a variety of disorders.

Keywords: Endocannabinoid; Uptake inhibition; UCM707

1. Introduction

Cannabinoids are a class of compounds which include, among others, the psychoactive ingredients of *Cannabis sativa* derivatives, a drug of abuse used from ancient times for recreational purposes. However, these compounds are now being considered as potentially useful therapeutic molecules. Likely, this is the consequence of the recent description of an endogenous cannabinoid signaling system (Mechoulam et al., 1994; Howlett, 1995; Pertwee, 1997; Di Marzo et al., 1998), which would contain the molecular targets for the action of plant-derived cannabinoids. This endogenous system plays a modulatory role in a variety of physiological processes, mainly in the brain (Di Marzo et al., 1998; Walker et al., 1999; Romero et al., in press), but also in the immune (Kaminski, 1998; Parolaro, 1999) and cardiovascular (Wagner et al., 1998; Harris et al., 1999; Hillard, 2000) systems. It consists of two types of GTP-binding protein-coupled receptors, named cannabinoid CB_1 (preferentially located in the brain) and CB_2 (present in the immune system) (Pertwee, 1997), and their corresponding endogenous ligands. The endogenous ligands are mainly derivatives of polyunsaturated fatty acids, such as the ethanolamide of arachidonic acid, termed "anandamide" (Devane et al., 1992; Hanuš et al., 1993), and 2-arachidonoylglycerol (Mechoulam et al., 1995; Sugiura et al., 1995). Recent and exhaustive studies have characterized the mechanisms of synthesis, release, uptake and metabolism of

* Corresponding author. Tel.: +34-91-3941450; fax: +34-91-3941691.
E-mail address: jjfr@med.ucm.es (J. Fernández-Ruiz).

0014-2999/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0014-2999(02)01996-9

endocannabinoids (Di Marzo et al., 1998; Giuffrida et al., 2001), which occur by mechanisms in part similar to those for other neurotransmitters, although their lipid structure implies some differences with respect to classic amino acid, amine and peptide transmitters (Howlett, 1995; Di Marzo et al., 1998).

Special attention has been paid to the mechanism of endocannabinoid uptake, which is part of the process of termination of the biological activity of these endogenous compounds (Giuffrida et al., 2001). This attention was due to the therapeutic possibilities offered by compounds, such as *N*-(4-hydroxyphenyl)-arachidonamide (AM404; Khanolkar et al., 1996; Beltramo et al., 1997) or *N*-(4-hydroxy-2-methylphenyl)-arachidonamide (VDM11; De Petrocellis et al., 2000), that block this process. These compounds, called indirect agonists, act by potentiating the action of endogenous ligands and, hence, they may be used in diseases where an increase in endocannabinoid transmission has been postulated to be of therapeutic value (Pertwee, 2000; Giuffrida et al., 2001). The use of these compounds makes it possible to minimize the unwanted effects produced by the direct activation of cannabinoid CB_1 receptors with classic cannabinoids, through the control of endocannabinoid levels in a concentration range that avoids psychoactive side effects (Felder and Glass, 1998). However, some of these compounds, such as AM404, may also behave as agonists of vanilloid receptors (Zygmunt et al., 2000) and exhibit hypokinetic effects by itself (González et al., 1999; Giuffrida et al., 2000). We have recently designed and synthesized a series of arachidonic acid derivatives (López-Rodríguez et al., 2001) that exhibit a high potency and selectivity in vitro as inhibitors of the endocannabinoid transporter, with negligible affinity for cannabinoid, CB_1 and CB_2, and vanilloid VR1 receptors. One of these compounds, *N*-(3-furylmethyl)eicosa-5,8,11,14-tetraenamide, so-called UCM707, is the most potent and selective endocannabinoid transporter inhibitor described to date (López-Rodríguez et al., 2001). Its characteristics as transporter inhibitor and its affinity for the receptors that bind endocannabinoids, in comparison with those of the other endocannabinoid transporter inhibitors, have been recently reviewed (López-Rodríguez et al., 2002). However, the available information on this compound was obtained in vitro and there are no data about how UCM707 behaves in vivo regarding some classic effects of endocannabinoids, such as hypomotility and antinociception. The objectives of the present work were: (i) to test in rats the dose–response motor and analgesic effects of UCM707, using the open-field and the hot-plate tests, respectively, and (ii) to examine if this compound is able to enhance the hypokinetic and/or the antinociceptive actions of a subeffective dose of anandamide in these two in vivo assays.

2. Materials and methods

2.1. Animals and treatments and sampling

Male Wistar rats were housed 2 weeks before the onset of the experiments in a room with controlled photoperiod (0800–2000 light) and temperature (23 ± 1 °C). They had free access to standard food and water. Animals were used at about 2 months of age (250–350 g weight) in all experiments, which were always conducted according to European and local rules on the care of and research with experimental animals. In a first experiment, rats were injected i.p. with three different doses (0.1, 1.0 and 10 mg/kg) of UCM707, synthesized as we have previously reported (López-Rodríguez et al., 2001), or with vehicle (Tween 80-saline, 1:16). Ten minutes later, animals were assessed in the open-field test or in the hot-plate test. In a second experiment, rats were divided into four groups and subjected to the following i.p. injections: (i) vehicle (Tween 80-saline, 1:16), (ii) a dose of UCM707, selected from the doses that did not produce any effects in the above experiment (0.5 mg/kg for the open-field test and 1 mg/kg for the hot-plate test), (iii) a subeffective dose of anandamide (0.3 mg/kg for the open-field test and 2 mg/kg for the hot-plate test), and (iv) the

Table 1
Ambulatory, exploratory and stereotypic activities, and time spent in inactivity, measured in the open-field test, and antinociception, measured in the hot-plate test, in adult male rats given an acute i.p. injection of UCM707 (three different doses) or vehicle (Tween 80-saline). See details in the text

Parameters	+ Vehicle	+ UCM707			Statistics
		0.1 mg/kg	1 mg/kg	10 mg/kg	
Ambulatory activity (number of sector crossings)	49.8 ± 4.3	50.7 ± 9.2	71.5 ± 4.3	38.2 ± 6.2	$F(3,23) = 2.708$, $p = 0.073$
Time spent in inactivity (s)	23.2 ± 7.6	36.7 ± 9.6	22.0 ± 13.4	76.5 ± 11.6[a]	$F(3,23) = 5.236$, $p < 0.05$
Exploratory activity (number of hole entries)	8.1 ± 2.1	2.6 ± 0.7	6.8 ± 1.6	5.2 ± 0.9	$F(3,23) = 3.061$, $p = 0.06$
Stereotypic activity (grooming + shaking + rearing)	6.2 ± 2.2	2.1 ± 1.0	6.5 ± 1.6	5.2 ± 1.3	$F(3,23) = 2.565$, $p = 0.083$
Antinociception (latency to respond to a noxious stimulus)	15.7 ± 0.9	15.7 ± 1.0	17.3 ± 1.3	20.9 ± 2.6	$F(3,24) = 2.314$, $p = 0.101$

Values are means ± S.E.M. for more than 6–7 determinations per group. Data were assessed by one-way analysis of variance followed by the Student–Newman–Keuls test ([a]$P < 0.05$).

Table 2
Ambulatory, exploratory and stereotypic activities, and time spent in inactivity, measured in the open-field test, and antinociception, measured in the hot-plate test, in adult male rats given an acute injection of anandamide, UCM707, or both. See details in the text

Parameters	+ Vehicle	+ Anandamide[1]	+ UCM707[2]	+ Both[1,2]	Statistics
Ambulatory activity (number of sector crossings)	39.7 ± 10.1	22.2 ± 7.1	45.5 ± 13.4	12.8 ± 6.1[b]	$F(3,21) = 2.80$, $p < 0.05$
Time spent in inactivity (s)	29.8 ± 7.6	30.3 ± 5.1	25.0 ± 10.5	80.2 ± 30.9[a]	$F(3,22) = 4.27$, $p < 0.05$
Exploratory activity (number of hole entries)	6.3 ± 2.8	5.0 ± 1.4	8.3 ± 2.2	2.0 ± 0.7	$F(3,21) = 1.04$, ns
Stereotypic activity (grooming + shaking + rearing)	1.3 ± 0.8	3.0 ± 0.9	2.6 ± 0.6	1.8 ± 0.4	$F(3,22) = 1.595$, ns
Antinociception (latency to respond to a noxious stimulus)	12.3 ± 1.3	15.0 ± 3.1	18.3 ± 2.2[a]	26.2 ± 1.9[c]	$F(3,23) = 7.3$, $p < 0.005$

Values are means ± S.E.M. for 5–6 determinations per group. Data were assessed by one-way analysis of variance followed by the Student–Newman–Keuls test ([a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.005$).
[1] 0.3 mg/kg used in the open-field test analyses or 2 mg/kg used in the hot-plate experiments.
[2] 0.5 mg/kg used in the open-field test analyses or 1 mg/kg used in the hot-plate experiments.

combination of both, UCM707 and anandamide, administered at the same time. Ten minutes later, animals were also assessed in the open-field test or in the hot-plate test.

2.2. Open-field test

Motor behavior was analyzed in an open-field test, whose characteristics have been previously described (González et al., 1999). Ten minutes after drug administration, the animals were placed in the open-field and their behavior was recorded for a period of 10 min, although only the last 5 min were scored (the first 5 min served as the period of habituation to the novel environment, which reduced the influence of emotional aspects). The following parameters were scored: (i) ambulation: number of sector crossings; (ii) exploratory activity: number of head entries into the square holes; (iii) frequency of stereotypic behaviors (rearing, self-grooming and shaking); and (iv) time spent in inactivity.

2.3. Hot-plate analysis

For the analysis of antinociception, we used the hot-plate procedure described by Girard et al. (2001). Rats were placed individually on a hot-plate maintained at 52 °C, and the latency to exhibit the first sign of pain (i.e., licking the hind paws or jumping) was measured for each rat. Animals not responding were removed after 30 s (cut-off time to avoid tissue damage).

2.4. Statistics

Data were assessed by the one-way analysis of variance followed by the Student–Newman–Keuls test.

3. Results

Table 1 shows the dose–response effects of UCM707 in the open-field test, as well as its effects on the latency to respond to a painful stimulus measured in the hot-plate test. In general, UCM707, administered alone, had no effects on ambulatory, exploratory and stereotypic activities, time spent in inactivity and sensitivity to noxious heat. Only the administration of the highest dose of UCM707 elicited some small responses that were not statistically significant, except for the increase in the time spent in inactivity in the open-field test (see Table 1).

Table 2 shows the ability of UCM707 to potentiate the effects of anandamide administered at a subeffective dose. Thus, UCM707, administered at 0.5 mg/kg, a dose that is within the range of doses that did not produce any effects in the dose–response curves, was, however, able to significantly potentiate the hypokinetic action of a dose of anandamide that did not produce any effects when administered alone. So, the combination of both compounds produced greater decreases in exploratory activity (only as a trend), particularly in ambulation, and increased the time spent in inactivity. No changes were observed in stereotypic activity. The same findings were found in the hot-plate test, where UCM707, administered at a dose of 1 mg/kg, produced by itself a small, but statistically significant, increase in the latency to respond to a noxious stimulus, an increase that was strongly enhanced when the animals were coadministered anandamide, at a dose of 2 mg/kg, a dose which was not effective when administered alone.

4. Discussion

The main finding of the present study is that UCM707, a compound that behaves in vitro as a potent and selective inhibitor of the endocannabinoid transporter (López-Rodríguez et al., 2001), seems also to act with similar potency and selectivity in vivo in tests of motor behavior and analgesia. For example, UCM707, used alone, did not produce any relevant effects over a large range of doses, thus indicating a lack of direct activation of the receptors for endocannabinoids, but when used at doses at which it had no detectable

effects by itself, UCM707 was able to potentiate the action of a subeffective dose of anandamide, thus supporting the notion of a selective effect on the endocannabinoid transporter. In addition, these observations presumably indicate that UCM707 is able to cross the blood–brain barrier and to act directly in the brain, at the regions that control motor behavior and nociception. This seems likely considering the chemical similarities of UCM707 and other endocannabinoid analogs or related compounds that easily cross the blood–brain barrier. However, our data do not exclude that the effect of UCM707 might be produced peripherally, by increasing the pharmacokinetics or bioavailability of anandamide, as is also thought to be the case for AM404, which has been reported to elevate the circulating levels of anandamide (Giuffrida et al., 2000).

Our data also indicate that the in vivo action of UCM707 differs from that of inhibitors such as AM404 (Beltramo et al., 1997). One such difference is that UCM707 did not produce any effect by itself in a range of doses of 0.1 to 10 mg/kg, whereas AM404 does (González et al., 1999; Giuffrida et al., 2000). Only with the highest dose of UCM707 we did observe small, and usually non-statistically significant, effects on hypomotility, and also on antinociception, but these effects were significantly smaller than those produced by AM404 (González et al., 1999; Giuffrida et al., 2000). We believe that, in concordance with its in vitro profile (López-Rodríguez et al., 2001), these tendencies observed with the highest dose of UCM707 were caused by the potentiation of a basal endocannabinoid tonus through its blocking effect on the endocannabinoid transporter, rather than by direct activation by UCM707 of the different receptors for endocannabinoids. This is in contrast to AM404, since the marked hypomotility elicited by this compound when administered alone (González et al., 1999; Giuffrida et al., 2000) is probably related to its ability to directly activate vanilloid VR1 receptors (Zygmunt et al., 2000). This is supported by recent data that show that the hypokinetic effect of AM404 is blocked by capsazepine, a vanilloid VR1 receptor antagonist, but not by SR141716A, a cannabinoid CB_1 receptor antagonist (Lastres-Becker, Ramos, Di Marzo and Fernández-Ruiz, unpublished results), thus supporting a preference of AM404 for the endovanilloid system. By contrast, the effects of UCM707 were comparable to those produced by VDM11, another inhibitor of endocannabinoid uptake that, like UCM707, also shows negligible activity at the cannabinoid CB_1 and vanilloid VR1 receptors (De Petrocellis et al., 2000).

Despite the apparent lack of hypokinetic and antinociceptive effects of UCM707, when administered alone, and consistent with results obtained with the highest dose used in the dose–response studies, our inhibitor showed the ability to potentiate the action of anandamide when it was administered in combination with UCM707. So, even though we used doses of anandamide and UCM707 that were individually subeffective, the administration of both compounds together produced a clear and marked hypokinetic and antinociceptive effect. This supports again that, through the blockade of endocannabinoid uptake by cells located in the vicinity of the synapse, UCM707 has a protective effect on exogenous anandamide, resulting in an increase in the magnitude, and possibly duration, of the effect of this endocannabinoid after binding to its different receptors. This potentiating ability of UCM707 was particularly strong for inactivity in the open-field test and for latency to respond to a thermal stimulus in the hot-plate test, which means that this compound could be used to potentiate endocannabinoid transmission in certain disorders, particularly in those where increased activity of this system has been postulated to be of therapeutic value (for recent reviews, see Pertwee, 2000; Romero et al., in press). This therapeutic strategy has been used for other neurotransmitters (i.e., serotonin uptake inhibitors for the treatment of depression) and has the advantage that it minimizes the unwanted effects produced by direct activation of cannabinoid CB_1 receptors with classic cannabinoids, by keeping endocannabinoid levels in a concentration range that avoids psychoactive side effects (Felder and Glass, 1998).

In summary, UCM707, as suggested by its in vitro properties, seems also to behave as a selective and potent inhibitor of the endocannabinoid transporter, showing negligible effects on the receptors for endocannabinoids. Thus, this compound could be a promising tool, used alone or in combination with endocannabinoids, for the treatment of a variety of disorders where an elevation of the endocannabinoid tone has been proposed to have therapeutic benefits.

Acknowledgements

This work has been supported by grants from CAM-PRI (08.5/0029/98) and from CICYT (PM99-0056) (ELF, JFR and JAR), and MCT (BQU2001-1459) (SOG, AV and MLLR). Eva de Lago and Silvia Ortega-Gutiérrez are a predoctoral fellows of the Spanish Ministry of Education, respectively.

References

Beltramo, M., Stella, N., Calignano, A., Lin, S.Y., Makriyannis, A., Piomelli, D., 1997. Functional role of high-affinity anandamide transport, as revealed by selective inhibition. Science 277, 1094–1097.

De Petrocellis, L., Bisogno, T., Davis, J.B., Pertwee, R.G., Di Marzo, V., 2000. Overlap between the ligand recognition properties of the anandamide transporter and the VR1 vanilloid receptor: inhibitors of anandamide uptake with negligible capsaicin-like activity. FEBS Lett. 483, 52–56.

Devane, W.A., Hanuš, L., Breuer, A., Pertwee, R.G., Stevenson, L.A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A., Mechoulam, R., 1992. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258, 1946–1949.

Di Marzo, V., Melck, D., Bisogno, T., De Petrocellis, L., 1998. Endocannabinoids: endogenous cannabinoid receptor ligands with neuromodulatory action. Trends Neurosci. 21, 521–528.

Felder, C.C., Glass, M., 1998. Cannabinoid receptors and their endogenous agonists. Ann. Rev. Pharmacol. Toxicol. 38, 179–200.

Girard, P., Pansart, Y., Coppe, M.-C., Gillardin, J.-M., 2001. Nefopam reduces thermal hypersensitivity in acute and postoperative pain models in the rat. Pharmacol. Res. 44, 541–545.

Giuffrida, A., Rodríguez de Fonseca, F., Nava, F., Loubet-Lescoulie, P., Piomelli, D., 2000. Elevated circulating levels of anandamide after administration of the transport inhibitor, AM404. Eur. J. Pharmacol. 408, 161–168.

Giuffrida, A., Beltramo, M., Piomelli, D., 2001. Mechanisms of endocannabinoid inactivation: biochemistry and pharmacology. J. Pharmacol. Exp. Ther. 298, 7–14.

González, S., Romero, J., de Miguel, R., Lastres-Becker, I., Villanúa, M.A., Makriyannis, A., Ramos, J.A., Fernández-Ruiz, J.J., 1999. Extrapyramidal and neuroendocrine effects of AM404, an inhibitor of the carrier-mediated transport of anandamide. Life Sci. 65, 327–336.

Hanuš, L., Gopher, A., Almog, S., Mechoulam, R., 1993. Two new unsaturated fatty acid ethanolamides in brain that bind to the cannabinoid receptor. J. Med. Chem. 36, 3032–3034.

Harris, D., Kendall, D.A., Randall, M.D., 1999. Characterization of cannabinoid receptors coupled to vasorelaxation by endothelium-derived hyperpolarizing factor. Arch. Pharmacol. 359, 48–52.

Hillard, C.J., 2000. Endocannabinoids and vascular function. J. Pharmacol. Exp. Ther. 294, 27–32.

Howlett, A.C., 1995. Pharmacology of cannabinoid receptors. Ann. Rev. Pharmacol. Toxicol. 35, 607–634.

Kaminski, N.E., 1998. Regulation of the cAMP cascade, gene expression and immune function by cannabinoid receptors. J. Neuroimmunol. 83, 124–132.

Khanolkar, A.D., Abadji, V., Lin, S., Hill, W.A., Taha, G., Abouzid, K., Meng, Z., Fan, P., Makriyannis, A., 1996. Head group analogs of arachidonylethanolamide, the endogenous cannabinoid ligand. J. Med. Chem. 39, 4515–4519.

López-Rodríguez, M.L., Viso, A., Ortega-Gutiérrez, S., Lastres-Becker, I., González, S., Fernández-Ruiz, J.J., Ramos, J.A., 2001. Design, synthesis and biological evaluation of novel arachidonic acid derivatives as highly potent and selective endocannabinoid transporter inhibitors. J. Med. Chem. 44, 4505–4508.

López-Rodríguez, M.L., Viso, A., Ortega-Gutiérrez, S., Fernández-Ruiz, J.J., Ramos, J.A., 2002. Endocannabinoid transporter inhibitors. Curr. Med. Chem.-Central Nerv. Syst. Agents 2, 129–141.

Mechoulam, R., Hanuš, L., Martin, B.R., 1994. Search for endogenous ligands of the cannabinoid receptors. Biochem. Pharmacol. 48, 1537–1544.

Mechoulam, R., Ben-Shabat, S., Hanuš, L., Ligumsky, M., Kaminski, N., Schatz, A.R., Gopher, A., Almog, S., Martin, B.R., Compton, D.R., Pertwee, R.G., Griffin, G., Bayewitch, M., Barg, J., Vogel, Z., 1995. Identification of an endogenous 2-monogliceride, present in canine gut, that binds to cannabinoid receptors. Biochem. Pharmacol. 50, 83–90.

Parolaro, D., 1999. Presence and functional regulation of cannabinoid receptors in immune cells. Life Sci. 65, 637–644.

Pertwee, R.G., 1997. Pharmacology of cannabinoid CB_1 and CB_2 receptors. Pharmacol. Ther. 74, 129–180.

Pertwee, R.G., 2000. Cannabinoid receptor ligands: clinical and neuropharmacological considerations, relevant to future drug discovery and development. Expert Opin. Investig. Drugs 9, 1553–1571.

Romero, J., Lastres-Becker, I., de Miguel, R., Berrendero, F., Ramos, J.A., Fernández-Ruiz, J.J., 2002. The endogenous cannabinoid system and the basal ganglia: biochemical, pharmacological and therapeutic aspects. Pharmacol. Ther. (in press).

Sugiura, T., Kondo, S., Sukagawa, A., Nakane, S., Shinoda, A., Itoh, K., Yamashita, A., Waku, K., 1995. 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. Biochem. Biophys. Res. Commun. 215, 89–97.

Wagner, J.A., Varga, K., Kunos, G., 1998. Cardiovascular actions of cannabinoids and their generation during shock. J. Mol. Med. 76, 824–836.

Walker, J.M., Hohmann, A.G., Martin, W.J., Strangman, N.M., Huang, S.M., Tsou, K., 1999. The neurobiology of cannabinoid analgesia. Life Sci. 65, 665–673.

Zygmunt, P.M., Chuang, H., Movahed, P., Julius, D., Hogestatt, E.D., 2000. The anandamide transport inhibitor AM404 activates vanilloid receptors. Eur. J. Pharmacol. 396, 39–42.