



Differences of μ -opioid receptors between Lewis and F344 rats

G. Herradón, L. Morales, L.F. Alguacil*

Laboratory of Pharmacology, San Pablo CEU University, 28668 Boadilla, Madrid, Spain

Received 3 December 2002; accepted 11 March 2003

Abstract

F344 and Lewis rats show different responses to opioids in several experimental paradigms. In this study we have used the specific μ -opioid agonist DAMGO to find out if these differences could be attributed to heterogeneity of μ -opioid receptors. The density of [H^3]DAMGO binding sites was similar in the brain cortex and spinal cord of both strains, but DAMGO affinity for μ -opioid receptors was higher in F344 tissues. Moreover, a parallel study of the effects of DAMGO on electrically-evoked twitches of isolated vasa deferentia revealed that this drug was also more effective in F344 preparations. These results suggest that μ -opioid receptors of F344 rats are more sensitive to pharmacological stimulation *in vitro*, which could be related to a higher drug affinity.

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Keywords: Lewis; Fischer 344; DAMGO; μ -opioid receptor

Introduction

F344 and Lewis rats are two inbred strains that show different pharmacological responses to several kind of drugs, which has been partially attributed to a dissimilar reactivity of the hypothalamic-pituitary-adrenal (HPA) axis (see review by [Kosten and Ambrosio, 2002](#)). In the case of opioids it has been reported that Lewis rats are more sensitive to exogenous agonists, i.e. they develop more conditioned place preference and self-administration upon opioid agonist exposure ([Guitart et al., 1992](#); [Martín et al., 1999](#); [Herradón et al., 2001](#)); however, other comparative studies on opioid-induced antinociception have rendered conflictive results ([Vaccarino and Couret, 1995](#); [Woolfolk and Holtzman, 1995](#); [Morgan et al., 1999](#)). Some authors have also reported significant differences in the

* Corresponding author. Tel.: +34-91-3724729; fax: +34-91-3510475.

E-mail address: laguacil@ceu.es (L.F. Alguacil).

activity of the endogenous opioid system between these strains, which could be directly related to the observed variations in the sensitivity to exogenous opioids; thus, it has been shown that Lewis rats exhibit lower enkephalin gene expression and endogenous dynorphine levels than F344 in several rat brain areas (Nylander et al., 1995; Martín et al., 1999). Despite this fact, comparative studies between these rat strains at the opioid receptor level are scarce: to our knowledge, there is only a brief communication reporting lower density of μ -opioid receptors in some brain areas of Lewis rats (Oliva et al., 1999), but neither ligand affinity nor functional data have been provided. To further extend this knowledge, we have used the μ -opioid receptor ligand DAMGO to study possible differences between Lewis and F344 rats concerning the number and affinity of μ -opioid receptors in the brain cortex and spinal cord; furthermore, we have also studied the effect of this agonist on the electrically-evoked contractions of the isolated vas deferens of the animals as a functional bioassay that could detect possible changes of drug efficacy in vitro. A preliminary report of this work has been presented recently (Alguacil et al., 2002).

Methods

Animals and drugs

Male Lewis and F344 rats (7 weeks old; Harlan, Spain) were used. The animals had free access to water and standard diet and were maintained in a controlled environment (20–22 °C, 12 h/12 h dark/light cycle) until the day of the experiment. The assay was carried out in accordance with the Guide for the Care and Use of Laboratory Animals promulgated by the National Institutes of Health.

DAMGO ([D-Ala², N-methyl-Phe⁴, Gly⁵-ol]enkephalin) was obtained from Sigma (Spain) and [³H]DAMGO (2.81 TBq/mmol) was purchased from Amersham (UK). Morphine sulphate was purchased from Alcaliber (Spain).

[³H]DAMGO binding to brain and spinal cord membranes

Rats were sacrificed by decapitation and the whole brain and spinal cord were immediately removed to dissect the brain cortex and the segment of the spinal cord between the sixth lumbar and tenth thoracic vertebrae. The tissues were homogenized in 10 volumes of ice-cold 50 mM Tris-HCl buffer (pH = 7.4) using a polytron homogenizer, and the homogenate centrifuged at 20000 × g for 30 min. The pellet obtained was again homogenized and centrifuged at 20000 × g for 30 min. The final pellet was suspended in ice-cold buffer containing 1 mg/ml bovine serum albumin. All steps were performed at 4 °C.

The binding assay was performed by incubating membrane fractions (100 μ l of suspended tissue) with different concentrations (0.3–10 nM) of [³H]DAMGO in a total volume of 1000 μ l. Incubation was carried out for 60 min at 25 °C because [³H]DAMGO achieves steady-state in these conditions. The reaction was terminated by rapid filtration (Cell Harvester, Brandel Co., Gaithersburg, MD, U.S.A.) through Whatman GF/B glass fiber filters presoaked in 1 mg/ml bovine serum albumin for 60 min. Tissue-bound radioactivity was extracted from the filters overnight in scintillation fluid and then counted in a liquid scintillation counter. Nonspecific binding was

Table 1

Bmax and K_d values for the binding of [H^3]DAMGO to membrane fractions of brain cortex and spinal cord of F344 and Lewis rats (mean \pm S.E.M)

	Brain cortex		Spinal cord	
	F344	Lewis	F344	Lewis
K_d (nM)	0.45 \pm 0.05*	1.31 \pm 0.19	1.39 \pm 0.09*	2.87 \pm 0.46
Bmax (fmol/mg tissue)	2.83 \pm 0.40	3.10 \pm 0.33	1.15 \pm 0.21	1.75 \pm 0.41
n	8	9	7	7

* $p < 0.05$ vs. Lewis.

determined in presence of 10 μ M morphine sulphate. All assays were conducted in triplicate for brain cortex and in duplicate for spinal cord. The experiments were analysed by using the LIGAND program.

Effect of DAMGO on the electrically-evoked twitches of isolated vas deferens

The isolated rat vas deferens has been used as a functional bioassay for μ -opioid receptor function. The inhibitory effect of DAMGO on electrically-evoked twitches is observed at high concentrations (because of the receptor reserve of the preparation) and seems to be exclusively mediated by μ -opioid receptors, since the population of δ -opioid receptors is only marginal in this tissue (Smith and Rance, 1983; Sheehan et al., 1988) and therefore the effect of DAMGO is completely prevented by β -funaltrexamine in our experimental conditions (Herradón et al., 2001).

Immediately after sacrifice, vasa deferentia were removed and suspended under 1 g resting tension in a 10 ml organ bath containing Krebs solution at 35 °C and airted with a mixture of 95% O₂–5% CO₂, which maintained the pH at approximately 7.4. Electrically-evoked twitches were obtained by passing pulses (0.5 ms duration, 0.2 Hz at supramaximal voltage) between ring electrodes positioned above and below the tissue. After 30–45 min of equilibration, concentration-response curves were constructed by

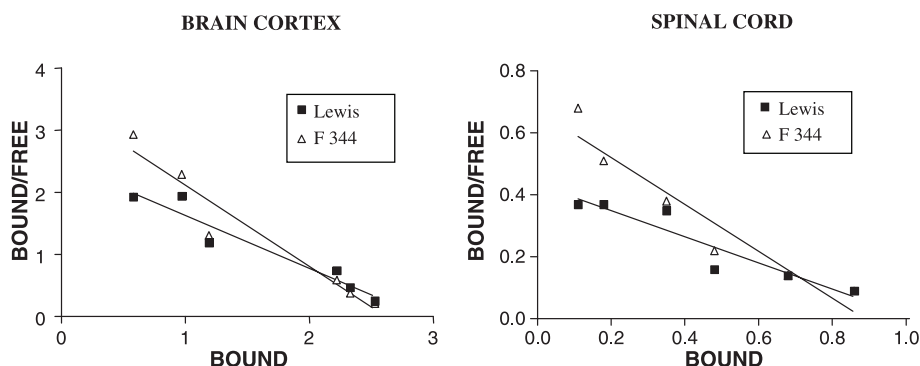


Fig. 1. Representative Scatchard plots of an assay of μ specific [H^3]DAMGO binding to membrane fractions of the brain cortex and spinal cord from one F344 and one Lewis rat.

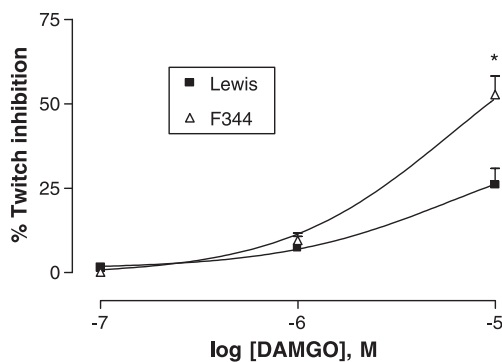


Fig. 2. Effects of DAMGO on the electrically stimulated rat vas deferens. Points represent means \pm S.E.M from at least 6 determinations. * $p < 0.05$ vs. Lewis.

adding cumulative concentrations of DAMGO to the bath. The experiments were analysed by using the MacLab program.

Statistical analysis

Statistical analysis was performed by Student t-test. A value of $p < 0.05$ was considered significant.

Results

The density of [H^3]DAMGO binding sites in the spinal cord and brain cortex (B_{max}) was not found to be statistically different in Lewis and F344 rats; however, the affinity of the radioligand for μ -opioid receptors was higher in F344 rats as indicated by the K_d obtained in both tissues (Table 1). Representative Scatchard plots are presented in Fig. 1. The concomitant comparative evaluation of DAMGO efficacy in the isolated vas deferens bioassay showed that the pharmacological effect of this drug was higher in tissues obtained from F344 rats (Fig. 2).

Discussion

The density of μ -opioid receptors in the whole brain cortex and spinal cord of Lewis and F344 rats seems to be quantitatively similar, since our binding study did not show significant differences in B_{max} values between strains. However, these results do not exclude possible differences in certain brain areas that could be of biological significance. Thus, Oliva et al. (1999) have presented autoradiographic data (not yet published in full) showing lower receptor density in the ventromedial hypothalamic nucleus, amygdala and dorsomedial and anteroventral thalamic nuclei of Lewis rats. While these last studies seem to be limited to the evaluation of receptor number, we have used multiple concentrations of the radioligand to further study receptor affinity in both strains. Our results clearly show that DAMGO exhibits a higher affinity for μ -opioid receptors in F344 rats, a finding that

could be related to possible differences in the structure or conformation of μ -opioid receptors and predicts functional differences in receptor activation between strains *in vitro*. The parallel study of DAMGO efficacy in the isolated vas deferens confirms this since the drug exhibited a higher effect in the tissues taken from F344 rats.

The combined results of the binding study and the functional bioassay performed are enough to affirm that μ -opioid receptors are functionally different in F344 and Lewis rats; however, the possible correlation between receptor affinity and activation requires further confirmation by studying both variables in the same tissue. An increased sensitivity of F344 receptors matches with some *in vivo* comparative pharmacological studies showing higher antinociceptive effects of exogenous opioids in F344 rats (Vaccarino and Couret, 1995; Morgan et al., 1999), but not with others showing just the opposite; thus, Lewis rats are more sensitive to morphine in experimental models of addiction (Suzuki et al., 1988, 1992; Guitart et al., 1992; Ambrosio et al., 1995; Martín et al., 1999), feeding (Gosnell and Krahn, 1993) electroencephalographic activity (Mayo-Michelson and Young, 1993) and even in some antinociception studies (Woolfolk and Holtzman, 1995). These apparent discrepancies could be related to the existence of additional strain differences in the biological mechanisms involved in each of these responses (i.e., HPA axis activity as previously mentioned) and/or differences in drug pharmacokinetics, thus leading to more diverse and complex results *in vivo* in comparison with much simple *in vitro* systems.

It is interesting to compare the observed effects of DAMGO with the endogenous opioid tone, since this drug showed more affinity and potency in F344 rats which in turn have been reported to have a globally reduced endogenous opioid activity (Sternberg et al., 1989a,b; Nylander et al., 1995; Martín et al., 1999). It is tempting to suggest that this balanced state could reflect a compensatory adaptation in order to preserve opioid activity within certain physiological limits. Obviously, this hypothesis requires direct confirmation.

Conclusion

Our results show that μ -opioid receptor stimulation is more effective in F344 than in Lewis rats *in vitro*. This could be at least partially attributable to an enhanced ligand affinity as suggested by DAMGO binding data, but other mechanisms such as different receptor coupling to G proteins could be also involved since it has been reported that F344 rats show higher levels of $G_{i\alpha}$ and G_{β} in the brain (Guitart et al., 1993).

Acknowledgements

This work was supported by grants from the Comunidad de Madrid (08.0/0004/97-02) and Laboratorios Dr. Esteve, Spain. The authors also thank Dr Mariano Ruiz-Gayo for scientific advice.

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