ELSEVIER

European Journal of Pharmacology 328 (1997) 99–102

ejp

Short communication

Cyproterone acetate displaces opiate binding in mouse brain

Mercedes Gutiérrez, Luis Menéndez, Mariano Ruiz-Gayo, Agustín Hidalgo, Ana Baamonde *

Laboratorio de Farmacología, Departamento de Medicina, Facultad de Medicina, C / Julián Clavería s / n, 33071 Oviedo, Spain

Received 27 March 1997; accepted 4 April 1997

Abstract

Drugs acting on androgen receptors modify opioid transmission in the central nervous system. To investigate a direct interaction, we studied whether the binding of [^{3}H]diprenorphine to mouse brain membranes was modified by cyproterone acetate (progesterone derivative with antiandrogen activity), flutamide (non-steroidal antiandrogen), 5α-dihydrotestosterone and progesterone. Only cyproterone acetate inhibited [^{3}H]diprenorphine binding ($IC_{50} = (1.62 \pm 0.33) \times 10^{-6}$ M) without modifying its association rate. These results suggest that cyproterone acetate binds to opiate receptors independently of its classical androgenic intracellular receptor effect.

Keywords: Opioid; Steroid; Cyproterone acetate; Binding

1. Introduction

Interactions between opioids and steroids in the central nervous system (CNS) have been described. It is well known that opioids play an important role in regulating gonadotropin secretion, and consequently, the level of sexual steroids (Pang et al., 1977; Veldhuis et al., 1984). The converse is also true, sex steroids modify opioid transmission in the CNS. Changes in opiate receptor number have been described after chronic steroidal treatments (Zhou and Hammer, 1995), gonadectomy (Piva et al., 1995), or even during the estrous cycle (Hammer, 1990). In both animals and humans (Ruda, 1993; Unruh, 1996), gender differences in pain sensitivity have been reported. Many studies in animals have been carried out under different hormonal treatments, and generally, the presence of androgens is related to lower pain responsiveness (Candido et al., 1992) or greater analgesic responses to morphine (Kepler et al., 1991) or stress (Baamonde et al., 1989; Kavaliers and Galea, 1995). In addition, some anabolic steroids have been shown to have abuse liability and dependence potential (Lukas, 1993) and may show a similar withdrawal syndrome to opioids. Although most of these interactions could be attributed to the long-term effects of steroids mediated through transcriptional mechanisms, a direct interaction of androgens and opioids at the membrane level cannot be discarded. To our knowledge, two previous reports have studied the interactions between sexual steroids and opiate binding in the CNS of the rat (LaBella et al., 1978; Schwarz and Pohl, 1994). Despite their extensive study (150 compounds assayed), LaBella et al. (1978) described that only 17α-hydroxy-estrogens, but no other estrogens, gestagens or androgens, were able to displace the binding of [^{3}H]naloxone in rat brain. Schwarz and Pohl (1994) also reported that only estrogens, but not gestagens, androgens or other steroids modified this binding. In both cases, the phenolic nature of the A steroidal ring seemed important for competition to occur. Thus, from these previous experiments a direct interaction between androgenic drugs and opioids has not been reported. However, except for danazol (Schwarz and Pohl, 1994), only natural androgens but not synthetic antiandrogens (that have a higher affinity for the androgen receptor) were tested. Consequently, our aim was to further investigate a possible direct interaction of androgenic drugs in the opiate receptor by assaying synthetic antiandrogens. For this purpose, we chose cyproterone acetate (a synthetic progesterone derivative with antiandrogen activity of wide clinical use), flutamide (a synthetic non-steroidal antiandrogen) and, for comparison, the natural androgen 5α-dihydrotestosterone, and the natural progestagen, progesterone. The effect of these drugs on the binding of the non-specific opiate antagonist, [^{3}H]diprenorphine, to crude membrane fractions of mice brains was studied.

* Corresponding author. Tel.: (34-8) 510-3551; Fax: (34-8) 523-2255.

2. Materials and methods

Swiss CD1 male mice (45–55 days old) were killed by decapitation. Brains (without cerebellum) were removed and homogenised in 17 volumes of ice-cold 50 mM Tris-HCl pH 7.4 which contained bacitracin 0.01% (subsequently referred to as buffer) using a Kinematica Polytron (20 s, set at 5). The homogenate was centrifuged at 30 000 rpm for 15 min. The pellet was resuspended in buffer, vortexed, kept for 30 min at 4°C and then centrifuged as before. The resulting pellet was resuspended in 12 volumes of buffer and gently homogenised (5 s, set at 3) to yield a final protein concentration of 2–2.5 mg/ml (Bradford, 1976). For displacement studies, 100 μl of the crude membrane suspension were incubated at 25°C for 60 min (or 100 min in a specific case) with [^{3}H]diprenorphine, $(0.25–0.4)\times10^{-9}$ M (Amersham Ibérica) in buffer with or without 100 μl naloxone (10^{-5} M final concentration) and in the presence of 100 μl of steroids, $(0.1–100)\times10^{-6}$ M final concentration, (Sigma) in a final volume of 1 ml. Certain experiments were designed to study the displacement induced on the different types of opiate receptors labelled by diprenorphine. These assays were run in the presence of 100 μl of a high concentration (10^{-6} M) of the unlabelled ligands, DAGO ([D-Ala^2,*N*-Me-Phe^4,Gly^5-ol]enkephalin), DPDPE ([D-Pen^2,D-Pen^5]enkephalin) and U-50488H (*trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl] benzeneacetamide, methanesulfonate salt) (Sigma) which remain selective at this concentration (10^{-7} M, final concentration), for μ, δ or κ receptors respectively (Goldstein, 1987). The addition of two of these ligands leaves unmasked only one type of receptor to which [^{3}H]diprenorphine can bind. In all cases, the binding reaction was terminated by rapid filtration of the mixture through Whatman GF/B filters (pre-soaked for 30 min in 0.1% bovine serum albumin incubation buffer). The filters were washed twice with 5 ml of ice-cold buffer and transferred to polyethylene counting vials. 4 ml of scintillation cocktail (Scharlau Cocktail Biogreen1), were added to each vial which was counted 24 h later by a 1211 Rackbeta (LKB) scintillation counter. For kinetic studies the method was the same except for the incubation period which ranged from 2 min to 120 min. All experiments were run in triplicate and repeated three times.

In competition studies, the IC_{50} and the slope were calculated, and, in kinetic studies, the K_{obs} (association rate constant), by using the computer program RADLIG (Elsevier Biosoft, edited by McPherson, 1994) which uses a weighted non-linear curve-fitting analysis.

3. Results

In order to ensure that the affinity value of [^{3}H]diprenorphine in our assay conditions was in the range of those reported (Sadée et al., 1982), previous saturation studies were performed. The incubation of mouse brains during 60 min with increasing concentrations of [^{3}H]diprenorphine (data not shown) yielded a K_d value of $(0.27\pm0.55)\times10^{-9}$ M, as expected. In these experimental conditions, cyproterone acetate (10^{-7}–5×10^{-5} M) completely inhibited the binding of [^{3}H]diprenorphine $(0.3–0.4)\times10^{-9}$ M (Fig. 1). The IC_{50} obtained was $(1.62\pm0.33)\times10^{-6}$ M and the slope (0.83 ± 0.08) did not deviate significantly from unity. However, neither flutamide nor 5α-dihydrotestosterone were able to modify this binding using concentrations up to 10^{-4} M. Since cyproterone acetate, unlike flutamide and 5α-dihydrotestosterone, is a progesterone derivative, a gestagen was also assayed. Concentrations of progesterone up to 10^{-4} M could not modify [^{3}H]diprenorphine binding (data not shown). To further analyse the displacement induced by cyproterone acetate, experiments were performed in which two types of opioid receptors were simultaneously masked. Cyproterone acetate was able to displace [^{3}H]diprenorphine binding in all cases. The IC_{50} obtained were $(0.66\pm0.38)\times10^{-6}$ M, $(2.67\pm1.30)\times10^{-6}$ M and $(3.02\pm1.42)\times10^{-6}$ M when μ, δ or κ were uniquely labelled by [^{3}H]diprenorphine, respectively.

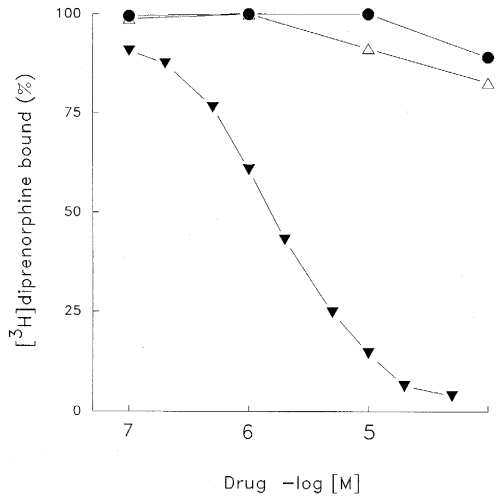

Fig. 1. Inhibition of the specific binding of [^{3}H]diprenorphine (4×10^{-10} M) to mouse brain membranes by cyproterone acetate (▼), flutamide (●) and 5α-dihydrotestosterone (△). Specific binding was calculated as the difference between the mean of the total binding and the non-specific binding (in the presence of 10^{-5} M of naloxone). Results are from one experiment run in triplicate.

To check the possibility that cyproterone acetate could modify the time to reach binding equilibrium conditions of [^{3}H]diprenorphine, kinetic analysis was performed. The association rate constant of [^{3}H]diprenorphine was unaltered by cyproterone acetate (K_{obs} values of 0.025 ± 0.005 min^{-1} and 0.019 ± 0.003 min^{-1} in the absence and in the presence of the steroid, respectively) (Fig. 2). In separate experiments, cyproterone acetate and [^{3}H]diprenorphine were incubated during 100 min, instead of 60 min. At these longer incubation times, the IC_{50} value of the dis-

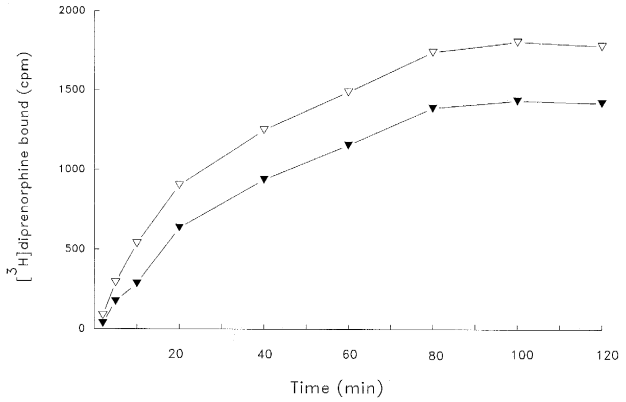

Fig. 2. Association kinetics for the specific binding of [^{3}H]diprenorphine (2.6×10^{-10} M) to mouse brain membranes at 25°C in the absence (△) and presence (▼) of 10^{-6} M of cyproterone acetate. Specific binding was calculated as the difference between the mean of the total binding and the non-specific binding (in the presence of 10^{-5} M of naloxone). Results are from one experiment run in triplicate.

placement induced by cyproterone acetate was $(2.19 \pm 1.01)\times10^{-6}$ M, not significantly different from that obtained at the 60 min incubation time.

4. Discussion

These results show that cyproterone acetate decreases the binding of [^{3}H]diprenorphine to mouse brain, and the inhibition is complete with 5×10^{-5} M of cyproterone acetate. The IC_{50} obtained is in the range of those obtained previously (about 10^{-5} M) with other sex steroids that compete with the binding of opioids in the CNS (LaBella et al., 1978; Schwarz and Pohl, 1994). The displacement of cyproterone acetate is not restricted to a particular type of opioid receptor, and the binding of [^{3}H]diprenorphine to either μ, δ or κ receptors can be displaced by cyproterone acetate. This inhibitory effect is not due to a delay of the time needed to reach the binding equilibrium conditions by [^{3}H]diprenorphine because its association rate constant was unaltered in the presence of the steroid. In fact, the IC_{50} values obtained either after 100 min of incubation (a time at which equilibrium is already attained) or at the more standard conditions of binding studies performed with [^{3}H]diprenorphine (i.e., 60 min incubation) (Sadée et al., 1982), are indistinguishable. Thus, it is likely that cyproterone acetate could directly bind to opioid membrane receptors in the CNS.

This displacement obtained with cyproterone acetate questions two previous statements. Firstly, estrogen derivatives are not the only steroids that can presumably bind to opiate receptors, as cyproterone acetate is an antiandrogen. The IC_{50} values obtained with cyproterone acetate (10^{-6} M) are similar or even lower than those previously reported with estrogens which range from about 10^{-5} M or higher, except for 17α-estradiol, 17α-dihydroquilin or 17α-dihydroequilenin ($(1–3)\times10^{-6}$ M) (LaBella et al., 1978; Schwarz and Pohl, 1994). Secondly, since flutamide and 5α-dihydrotestosterone do not inhibit opiate binding, the competition produced by cyproterone acetate seems independent of its affinity on intracellular androgen receptors and its antiandrogen activity. This effect of cyproterone acetate also seems to be unrelated to its progestational nature since progesterone does not inhibit [^{3}H]diprenorphine binding. Consequently, it does not seem to be a 'group' condition (related to the classification based on their actions on intracellular estrogen, progesterone or androgen receptors), nor does it seem to rely on a phenolic group of the A steroidal ring as has been proposed for the estrogen derivatives (LaBella et al., 1978; Schwarz and Pohl, 1994).

Finally, bearing in mind that the assays take place only in membrane fractions, these results add new insights into the possible direct non-genomic effects of steroids in the central nervous system (Schumacher, 1990; Brann et al., 1995; Mahesh et al., 1996). The majority of steroid-neurotransmitter receptor interactions have mainly been described in channel-coupled receptors for GABA (γ-aminobutyric acid) (Lambert et al., 1995), NMDA (*N*-methyl-D-aspartate) (Wu et al., 1991) or glycine (Wu et al., 1990) all of which act through ligand-gated receptors. Thus, this effect of cyproterone acetate on opiate receptors is one of the few examples of direct binding of steroids to seven-transmembrane, G protein-coupled receptors. A direct interaction of this kind could broaden our understanding of the modulatory roles that steroids exert in the central nervous system at the membrane level, apart from their classical intracellular receptors.

This competition of cyproterone acetate on opiate binding requires further studies on steroid-opiate interactions in order to clarify structure-activity relationships, the subtype of opioid receptor involved or its putative functional relevance. Meanwhile, this particular interaction could perhaps explain the sedation induced by this drug in humans or even its analgesic effect in cluster headache (Sicuteri, 1988).

Acknowledgements

This work was supported by grants from the Fondo de Investigaciones de la Seguridad Social FIS 95/1718. M.G. is under contract with the Ministerio de Educación y Ciencia (Contrato de Incorporación de Doctores a Equipos de Investigación en España).

References

Baamonde, A., Hidalgo, A., Andrés-Trelles, F., 1989. Sex related differences in the effects of morphine and stress on visceral pain. Neuropharmacology 28, 967–970.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye-binding. Anal. Biochem. 72, 248–254.

Brann, D.W., Hendry, L.B., Mahesh, V.B., 1995. Emerging diversities in the mechanism of action of steroid hormones. J. Steroid Biochem. Mol. Biol. 52, 113–133.

Candido, J., Lufti, K., Billinges, B., Sierra, V., Dutaroy, A., Inturrisi, C.E., Yoburn, B.C., 1992. Effect of adrenal and sex hormones on opioid analgesia and opioid receptor regulation. Pharmacol. Biochem. Behav. 42, 685–692.

Goldstein, A., 1987. Binding selectivity profiles for ligands of multiple receptor types: focus on opioid receptors. Trends Pharmacol. Sci. 8, 456–459.

Hammer, R.P., 1990. μ-Opiate receptor binding in medial preoptic area is cyclical and sexually dimorphic. Brain Res. 515, 187–192.

Kavaliers, M., Galea, L.A., 1995. Sex differences in the expression and antagonism of swim stress-induced analgesia in deer mice vary with the breeding season. Pain 63, 327–334.

Kepler, K.L., Standifer, K.M., Paul, D., Kest, B., Pasternak, G.W., Bodnar, R.J., 1991. Gender effects and central opioid analgesia. Pain 45, 87–94.

LaBella, F.S., Kim, R.S., Templeton, J., 1978. Opiate receptor binding activity of 17-alpha estrogenic steroids. Life Sci. 23, 1797–1804.

Lambert, J.J., Belelli, D., Hill-Venning, C., Peters, J.A., 1995. Neurosteroids and $GABA_A$ receptor function. Trends Pharmacol. Sci. 16, 295–303.

Lukas, S.E., 1993. Current perspectives on anabolic-androgenic steroid abuse. Trends Pharmacol. Sci. 14, 61–68.

Mahesh, V.B., Brann, D.W., Hendry, L.W., 1996. Diverse modes of action of progesterone and its metabolites. J. Steroid Biochem. Mol. Biol. 56, 209–219.

McPherson, G.A. (Ed.), 1994. RADLIG, a collection of programs for the analysis of radioligand binding experiments. Biosoft, Cambridge.

Pang, C.N., Zimmermann, E., Sawyer, C.H., 1977. Morphine inhibition of the preovulatory surges of plasma luteinizing hormone and follicle stimulating hormone in the rat. Endocrinology 101, 1726–1732.

Piva, F., Limonta, P., Dondi, D., Pimpinelli, F., Martini, L., Maggi, R., 1995. Effects of steroids on the brain opioid system. J. Steroid Biochem. Mol. Biol. 53, 343–348.

Ruda, M.A., 1993. Gender and pain. Pain 53, 1–2.

Sadée, W., Perry, D.C., Rosenbaum, J.S., Herz, A., 1982. [^{3}H]Dipre-norphine receptor binding in vivo and in vitro. Eur. J. Pharmacol. 81, 431–440.

Schumacher, M., 1990. Rapid membrane effects of steroid hormones: an emerging concept in neuroendocrinology. Trends Pharmacol. Sci. 13, 359–362.

Schwarz, S., Pohl, P., 1994. Steroids and opioid receptors. J. Steroid Biochem. Mol. Biol. 48, 391–402.

Sicuteri, F., 1988. Antiandrogenic medication of cluster headache. Int. J. Clin. Pharmacol. Res. 8, 21–24.

Unruh, A.M., 1996. Gender variations in clinical pain experience. Pain 65, 123–167.

Veldhuis, J.D., Rogol, A.D., Samojlick, E., Estel, N.H., 1984. Role of endogenous opiates in the expression of negative feedback actions of androgen and estrogen on pulsatile properties of luteinizing hormone secretion in man. J. Clin. Invest. 74, 47–55.

Wu, F.S., Gibbs, T.T., Farb, D.H., 1990. Inverse modulation of γ-amino-butyric acid- and glycine-induced currents by progesterone. Mol. Pharmacol. 37, 597–602.

Wu, F.S., Gibbs, T.T., Farb, D.H., 1991. Pregnenolone sulfate: a positive allosteric modulator at the *N*-methyl-D-aspartate receptor. Mol. Pharmacol. 40, 333–336.

Zhou, L., Hammer, R.P. Jr., 1995. Gonadal steroid hormones upregulate medial preoptic μ-opioid receptors in the rat. Eur. J. Pharmacol. 278, 271–274.