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European Journal of Pharmacology 443 (2002) 85–93

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Differential effects of chronic antidepressant treatments on μ- and δ-opioid receptors in rat brain

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Received 20 February 2002; accepted 5 April 2002

Abstract

We performed an autoradiographic study of [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin (DAMGO)-sensitive [^{3}H]naloxone binding to μ-opioid receptors and of [^{3}H][D-Pen^2,D-Pen^5]enkephalin (DPDPE) binding to δ-opioid receptors in the rat brain after 4- or 21-day treatments with paroxetine, reboxetine and moclobemide to investigate the participation of these receptors in the adaptive mechanisms occurring during the delay of action of new generation antidepressants. Paroxetine increased μ-opioid receptor binding site density in cingulate and insular cortices, dorsal endopiriform nucleus (4 days) and olfactory tubercle (21 days) and decreased it in thalamus (21 days). Reboxetine increased it in amygdala (4 days), hippocampus and thalamus (21 days) and decreased it in dorsal raphe (4 days). Moclobemide increased it in hippocampus (4 days) and decreased it in anterior olfactory nucleus, frontal cortex, amygdala and hypothalamus (21 days). Moclobemide increased δ-opioid receptor binding site density in frontal cortex and amygdala (4 days) and decreased it in amygdala and colliculi (21 days). Opioid receptors displayed distinct patterns of adaptations in response to the three antidepressants studied.

Keywords: Paroxetine; Reboxetine; Moclobemide; μ-Opioid receptor; δ-Opioid receptor; Chronic treatment

1. Introduction

Several lines of evidence have suggested the involvement of the opioid system in the mechanisms underlying the physiopathology of depression and the action of antidepressants.

An increased density of μ-opioid receptors has been noted in frontal and temporal cortex, as well as in caudate nucleus in the brain of suicide victims (Gross-Isserof et al., 1990; Gabilondo et al., 1995). On the other hand, μ-opioid receptor agonists such as oxycodone and oxymorphone have been found to improve mood state in patients with highly refractory major depression (Stoll and Rueter, 1999), a finding concurring with the proposal that the activation of central opioid receptors has curative effects in endogenous depression (Emrich et al., 1982).

Behavioral studies revealed antidepressant-like effects of different enkephalinase and/or aminopeptidase inhibitors in classical antidepressant screening tests. These behavioural responses were proved to be mediated by μ- and δ-opioid receptors as they were antagonized by opioid receptor antagonists and mimicked by opioid receptor agonists (Kita et al., 1997; De Felipe et al., 1989; Baamonde et al., 1992; Tejedor-Real et al., 1995, 1998; Besson et al., 1996).

All antidepressants need a delay of at least 2 weeks to elicit their therapeutic effect in human, although they interact within a few hours with their neuronal targets, suggesting that adaptive mechanisms should occur during this delay. It was hypothesized that some adaptations of endogenous opioid system, particularly of μ- and δ-opioid receptors, could participate in these mechanisms.

In rats, chronic treatments with various antidepressants have been found to increase or decrease [Met^5]- and [Leu^5]enkephalin-like immunoreactivity in numerous brain regions (Kurumaji et al., 1988; De Felipe et al., 1985; Hamon et al., 1987; Dziedzicka-Wasylewska and Rogoz, 1995). In the same way, contradictory results were reported concerning the effects of chronic antidepressant treatments on opioid receptors density. Reisine and Soubrié (1982) described a loss of opioid receptors in the cortex of rats

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0014-2999/02/$ - see front matter
PII: S0014-2999(02)01585-6

treated chronically with desipramine and no change in striatum and hippocampus, while Stengaard-Pedersen and Schou (1986) did not observe any change in either region following chronic desipramine treatment (21 days). Chronic amoxapine or amitriptyline treatments (14 days) induced a decrease in μ- and δ-opioid receptors densities in the hypothalamus but not in the cortex (Hamon et al., 1987) and chronic clomipramine treatment (28 days) induced an opioid receptor down-regulation in the whole rat brain (Benkelfat et al., 1989). On the contrary, chronic imipramine was shown to induce an increase in opioid binding sites in the cortex (Antkiewicz-Michaluk et al., 1984), as well as an increase in μ-opioid receptors expression in the caudate putamen, dentate gyrus and cortex (De Gandarias et al., 1998).

The comparison between those studies is very difficult because they were performed with different antidepressants for different periods of chronic treatments and because binding to receptors were measured in different conditions. Besides, most of the above-mentioned binding studies were performed on rat brain membrane homogenates, which did not allow a precise mapping of the brain regions involved in changes of μ- and δ-opioid receptors density. Moreover, most studies were performed with tricyclic antidepressants, which display numerous side effects.

These discrepancies led us to perform a systematic comparison of the consequences of chronic treatments with three new generation nontricyclic antidepressants in order to examine whether μ- and δ-opioid receptor changes in particular brain regions would constitute a common mechanism of action.

We chose three antidepressants with distinct specific primary targets: paroxetine, a selective serotonin reuptake inhibitor; reboxetine, a noradrenaline reuptake inhibitor; and moclobemide, a reversible inhibitor of monoamine oxidase type A. Paroxetine is a reference selective serotonin reuptake inhibitor, the administration of which enhances serotonergic transmissions. Moreover, paroxetine shows a greater potency to inhibit serotonin uptake than all other selective serotonin reuptake inhibitors (Nemeroff, 1998) and a great selectivity towards the serotonin transporter (for review, see Hollister, 1994). Reboxetine is the first selective norepinephrine reuptake inhibitor to be introduced (Riva et al., 1989; Berzewski et al., 1997). It displays a 124-fold sensitivity for the norepinephrine uptake site (Wong et al., 2000) compared to the serotonin uptake site. Moclobemide is an inhibitor of monoamine oxidase type A acting as an antidepressant through enhancing brain serotonin, norepinephrine and dopamine levels (Haefely et al., 1993).

Table 1
DAMGO-displaceable [^{3}H]naloxone binding to μ-opioid receptors on brain sections from rats treated during 4 days with antidepressants: paroxetine (10 mg/kg daily), reboxetine (10 mg/kg twice daily) or moclobemide (10 mg/kg daily) compared to their respective controls

Brain regions	Distance from bregma (mm)	Paroxetine		Reboxetine		Moclobemide	
		Controls	Treated	Controls	Treated	Controls	Treated
Cortex							
Frontal	4.2	35 ± 4	40 ± 2	35 ± 3	39 ± 2	43 ± 3	47 ± 3
Cingulate	2.2	34 ± 1	46 ± 2 **	45 ± 2	49 ± 1	47 ± 3	50 ± 3
Insular agranular	1.7	43 ± 3	51 ± 1 *	40 ± 2	45 ± 2	46 ± 3	49 ± 2
Amygdala							
Bed nu. accessory olfactory tract	− 2.12	74 ± 3	72 ± 6	70 ± 3	80 ± 4 *	83 ± 1	72 ± 5
Medial posteroventral nu.	− 2.8	64 ± 4	65 ± 2	76 ± 6	73 ± 3	58 ± 4	54 ± 4
Posteromedial cortical nu.	− 3.8	113 ± 10	114 ± 9	136 ± 5	134 ± 8	136 ± 7	149 ± 10
Thalamus							
Ventrolateral nu.	− 2.12	55 ± 2	57 ± 3	42 ± 2	43 ± 2	58 ± 4	60 ± 4
Mediodorsal nu.	− 2.8	85 ± 5	88 ± 7	65 ± 2	59 ± 4	87 ± 4	76 ± 4
Central medial nu.	− 2.8	86 ± 5	87 ± 5	89 ± 5	80 ± 6	102 ± 7	94 ± 6
Laterodorsal nu.	− 2.8	60 ± 6	56 ± 4	42 ± 3	38 ± 3	61 ± 4	58 ± 4
Miscellaneous							
Anterior olfactory nu.	4.2	19 ± 2	21 ± 2	24 ± 2	27 ± 2	23 ± 2	26 ± 3
Olfactory tubercle	0.2	34 ± 3	41 ± 3	44 ± 2	46 ± 2	42 ± 4	43 ± 4
Dorsal endopiriform nu.	0.2	41 ± 2	51 ± 3 *	48 ± 3	50 ± 2	56 ± 3	57 ± 5
Anterior hippocampus	− 2.12	45 ± 3	46 ± 3	35 ± 2	35 ± 2	43 ± 2	40 ± 3
Posterior hippocampus	− 5.3	33 ± 3	40 ± 3	46 ± 2	42 ± 2	34 ± 3	46 ± 5 *
Ventromedial hypothalamus	− 2.8	19 ± 3	22 ± 3	22 ± 4	21 ± 2	30 ± 4	38 ± 5
Dorsal raphe nu.	− 7.3	21 ± 3	26 ± 2	30 ± 2	25 ± 2 *	24 ± 2	31 ± 4

Mean ± S.E.M. (expressed as fmol/mg tissue eq.) from five to seven controls and five to seven treated rats.
* $P < 0.05$ compared to the corresponding controls.
** $P < 0.001$ compared to the corresponding controls.

The effects of paroxetine, reboxetine and moclobemide on μ- and δ-opioid receptor binding sites densities have been studied after short-term (4 days) and long-term (21 days) daily treatments. We have undertaken quantitative autoradiography to perform detailed neuroanatomical analysis of the effects of antidepressants. We analysed many brain regions including limbic system and functionally related regions, which are especially relevant in mood regulation and antidepressants action (Drevets and Raichle, 1992; Soares and Mann, 1997) and have not been so far investigated in this context.

2. Materials and methods

2.1. Animals and treatments

The study was performed on male Sprague–Dawley rats, purchased from Charles River (Saint-Aubin lès Elbeuf, France). The animals were housed five per cage, under controlled temperature and lighting conditions (23 ± 1 °C; lights on 7:30 a.m.–7:30 p.m.) and had access to food and water ad libitum. This study was performed in accordance with guidelines for the use of animals in research (French Decree no. 87.848).

Three sets of experiments were performed. In each of them, a first group of rats was treated for 4 days and a second group for 21 days.

In the first set of experiments, rats were injected intraperitoneally, once daily, with paroxetine 10 mg/kg or saline (5 ml/kg) (Vilpoux et al., 2000). Each group was constituted with seven treated rats and seven control rats. They were sacrificed 24 h after the last injection. Paroxetine was kindly provided by Smithkline-Beecham Pharmaceuticals (UK).

In the second set of experiments, rats were injected intraperitoneally, twice daily, with reboxetine 10 mg/kg (the first injection at 10:00 a.m. and the second at 6:00 p.m.) or saline (5 ml/kg) (Harkin et al., 1999; Riva et al., 1989). The twice-daily dosing of reboxetine was performed on account of its short half-life (Dostert et al., 1997). Each group was constituted with eight treated rats and eight control rats. They were sacrificed 16 h after the last injection. Reboxetine was kindly provided by Pharmacia & Upjohn (USA).

In the third set of experiments, rats were injected intraperitoneally, once daily, with moclobemide 10 mg/kg or the corresponding vehicle (distilled water 5 ml/kg) (Miura et al., 1996). Each group was constituted with eight treated rats and eight control rats. They were sacrificed 24 h after the last injection. Moclobemide was kindly provided by Roche (Switzerland).

Table 2
DAMGO-displaceable [^{3}H]naloxone binding to μ-opioid receptors on brain sections from rats treated during 21 days with antidepressants: paroxetine (10 mg/kg daily), reboxetine (10 mg/kg twice daily) or moclobemide (10 mg/kg daily) compared to their respective controls

Brain regions	Distance from bregma (mm)	Paroxetine		Reboxetine		Moclobemide	
		Controls	Treated	Controls	Treated	Controls	Treated
Cortex							
Frontal	4.2	47 ± 3	44 ± 4	38 ± 2	40 ± 3	48 ± 3	37 ± 2**
Cingulate	2.2	59 ± 2	58 ± 2	49 ± 2	48 ± 2	56 ± 1	49 ± 4
Insular agranular	1.7	54 ± 2	57 ± 6	46 ± 2	45 ± 3	50 ± 2	50 ± 3
Amygdala							
Bed nu. accessory olfactory tract	−2.12	88 ± 3	92 ± 6	72 ± 4	81 ± 3	83 ± 6	81 ± 5
Medial posteroventral nu.	−2.8	98 ± 10	92 ± 6	55 ± 3	56 ± 2	58 ± 3	47 ± 2*
Posteromedial cortical nu.	−3.8	130 ± 12	143 ± 16	119 ± 8	127 ± 5	133 ± 14	149 ± 14
Thalamus							
Ventrolateral nu.	−2.12	55 ± 2	45 ± 2**	43 ± 2	46 ± 2	44 ± 5	47 ± 3
Mediodorsal nu.	−2.8	113 ± 10	80 ± 7*	65 ± 4	79 ± 4*	91 ± 4	82 ± 8
Central medial nu.	−2.8	118 ± 6	90 ± 6**	74 ± 4	90 ± 3**	103 ± 4	83 ± 10
Laterodorsal nu.	−2.8	69 ± 6	55 ± 7	50 ± 3	58 ± 1*	57 ± 4	52 ± 5
Miscellaneous							
Anterior olfactory nu.	4.2	26 ± 5	24 ± 3	21 ± 1	23 ± 2	27 ± 3	17 ± 2*
Olfactory tubercle	0.2	41 ± 2	49 ± 3*	41 ± 1	41 ± 2	38 ± 2	39 ± 3
Dorsal endopiriform nu.	0.2	47 ± 4	57 ± 3	50 ± 2	51 ± 3	56 ± 2	51 ± 4
Anterior hippocampus	−2.12	47 ± 2	47 ± 3	31 ± 2	39 ± 2*	37 ± 3	35 ± 4
Posterior hippocampus	−5.3	41 ± 3	40 ± 3	39 ± 3	44 ± 2	34 ± 2	35 ± 4
Ventromedial hypothalamus	−2.8	42 ± 4	40 ± 3	25 ± 2	31 ± 4	35 ± 3	24 ± 2**
Dorsal raphe nu.	−7.3	28 ± 3	36 ± 4	24 ± 2	24 ± 3	25 ± 3	24 ± 3

Mean ± S.E.M. (expressed as fmol/mg tissue eq.) from five to eight controls and five to eight treated rats.
* $P<0.05$ compared to the corresponding controls.
** $P<0.01$ compared to the corresponding controls.

In each group, rats weighed approximately 300 g on the day of sacrifice. Brains were dissected out between 10:00 a.m. and 12:00.

2.2. Preparation of brain sections

After sacrifice, the brains were quickly removed, frozen in isopentane at −30 °C and stored at −80 °C until used. Coronal 14-μm thick tissue sections were cut in a cryostat at −22 °C (Leica) throughout the brain from anterior cortex to brainstem and thaw-mounted onto chrome-alum 5%–gelatine 0.5%-coated glass slides, dehydrated at room temperature for 15 min and stored at −80 °C.

2.3. μ-Opioid receptors autoradiographic binding

For the autoradiographic determination of the [D-Ala^2,-MePhe^4,Gly-ol^5]enkephalin (DAMGO)-sensitive [^{3}H]naloxone binding site density, brain sections were brought to room temperature and preincubated for 2 min at 4 °C in Tris buffer (Tris HCl 50 mM; NaCl 100 mM; pH 7.4) (Delfs et al., 1994). Incubation was performed for 1 h at 4 °C in the same buffer containing 2.5 nM [^{3}H]naloxone (NEN Life-Science Products, Paris, France, Spec. Act. 54.6 Ci/mmol) to assess total binding to the sections. Nonspecific binding was evaluated on adjacent sections incubated in the same conditions plus 1 μM unlabelled DAMGO (Bachem Feinchemikalien, Bubendorf, Switzerland), which is a specific μ-opioid receptor agonist. The slides were then rinsed six times for 10 s at 4 °C in phosphate-buffered saline (NaH_2PO_4 0.1 M; Na_2HPO_4 0.1 M; pH 7.4). The slides were rinsed once rapidly in ice-cold distilled water, dried under a cold air stream for 20 min and allowed to dehydrate overnight. The sections were apposed onto Hyperfilm-[^{3}H] (Amersham, Les Ulis, France) for 3.5 weeks.

2.4. δ-Opioid receptors autoradiographic binding

For the autoradiographic determination of the [^{3}H][D-Pen^2,D-Pen^5]enkephalin (DPDPE) binding site density (Tao et al., 1993), brain sections were brought to room temperature and preincubated for 15 min at room temperature in Tris buffer (Tris Base 50 mM; NaCl 100 mM; pH 7.6) containing 50 μM GTP (Sigma-Aldrich, Saint Quentin-Fallavier, France) to facilitate removal of endogenous opioid ligands from δ-opioid receptors. Brain sections were rinsed twice for 5 min in the same Tris buffer devoid of GTP. Incubation was performed for 90 min at room temperature in a Tris buffer (Tris base 50 mM; Bovine Serum Albumin 0.2%; Bacitracin 0.004%, pH 7.6) containing 4.7 nM [^{3}H]DPDPE ((D-Pen^2,D-Pen^5), [tyrosyl-2,6-^{3}H (*N*)]-enkephalin; NEN Life-Science Products, Spec. Act. 45 Ci/mmol) to assess total binding to the sections. Nonspecific binding was evaluated on adjacent sections incubated in the same conditions plus 4.7 μM unlabelled DPDPE (Sigma-Aldrich). The slides were rinsed three times for 10 min at 4 °C in Tris buffer (Tris base 50 mM; pH 7.2), then rinsed once rapidly in ice-cold distilled water, dried under a cold air stream for 20 min and allowed to dehydrate overnight. The sections were apposed onto Hyperfilm-[^{3}H] (Amersham) for 9 weeks.

2.5. Quantification of the autoradiograms

DAMGO-sensitive [^{3}H]naloxone and [^{3}H]DPDPE binding areas were analyzed at different rostrocaudal levels, defined from their anteriority from bregma, according to Paxinos and Watson (1986).

Quantification of the autoradiograms was performed on a computerized image analysis system (ALCATEL TITN ANSWARE). For autoradiographic studies, nonspecific binding was subtracted from total binding to evaluate specific binding in each brain region. The DAMGO-displaceable [^{3}H]naloxone and [^{3}H]DPDPE binding sites densities were expressed as fentomoles per milligram tissue equivalent. The standardization was done according to a calibration curve established from measurements made on tritiated standard strips (Amersham) and fitted to a polynomial regression. The statistical analysis were performed by means of a Student's *t*-test performed on treated rats ($n = 5–8$) versus control rats.

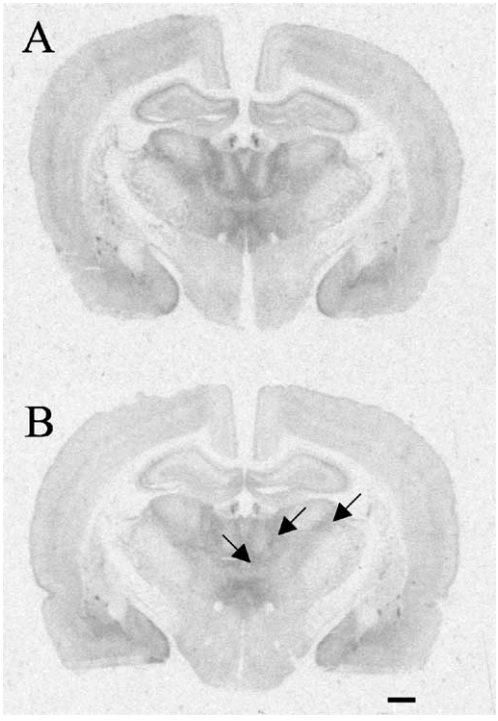

Fig. 1. Autoradiographic images of [^{3}H]naloxone binding on rat brain slices at the level of thalamus (anteriority from bregma −2.8 mm). (A) Control (21 days saline). (B) Paroxetine-treated (21 days, 10 mg/kg). Bar = 1 mm. The arrows indicate the regions where a significant decrease in specific binding site density was observed in paroxetine-treated versus control rats.

3. Results

3.1. Effects of chronic treatments by antidepressants on DAMGO-sensitive [^{3}H]naloxone autoradiographic binding to μ-opioid receptors

Tables 1 and 2 show brain regions displaying changes in μ-opioid receptor binding density at least at one considered time during the course of the study.

3.1.1. Paroxetine treatments

A 4-day paroxetine treatment led to significant increases in μ-opioid receptor binding site density in the cingulate cortex (+35%, $P<0.001$), in the insular agranular cortex (+19%, $P<0.05$) and in the dorsal endopiriform nucleus (+24%, $P<0.05$) (Table 1). After a 21-day treatment, the μ-opioid receptor binding site density was significantly increased in the olfactory tubercle (+19%, $P<0.05$) and was decreased in three thalamic nuclei: the ventrolateral thalamus (−18%, $P<0.01$), the mediodorsal thalamus (−29%, $P<0.05$) and the central medial thalamus (−24%, $P<0.01$) (Table 2). These latter changes are illustrated in Fig. 1.

In all other analyzed regions, no significant change was observed at any time (data presented in Tables 1 and 2).

3.1.2. Reboxetine treatments

A short-term (4 days) reboxetine treatment induced a decrease in DAMGO-sensitive [^{3}H]naloxone binding sites in the dorsal raphe nucleus (−17%, $P<0.05$) and an increase in the bed nucleus accessory olfactory tract (+14%, $P<0.05$) (Table 1). A long-term (21 days) reboxetine treatment led to an increase in the hippocampus (+26%, $P<0.05$) and several thalamic regions: mediodorsal thalamus (+22%, $P<0.05$), central medial thalamus (+22%, $P<0.01$) and laterodorsal thalamus (+16%, $P<0.05$) (Table 2). No change was observed in any other analyzed regions (data presented in Tables 1 and 2).

3.1.3. Moclobemide treatments

A 4-day moclobemide treatment resulted in an increase in DAMGO-sensitive [^{3}H]naloxone binding site density in the hippocampus (+35%, $P<0.05$) (Table 1). A 21-day treatment with this antidepressant induced a decrease in DAMGO-sensitive [^{3}H]naloxone binding site density in frontal cortex (−23%, $P<0.01$), anterior olfactory nucleus (−37%, $P<0.05$), medial posteroventral amygdaloid nucleus (−19%, $P<0.05$) and ventromedial hypothalamus (−31%, $P<0.01$) (Table 2). No change in binding site density was found in the other studied regions (data presented in Tables 1 and 2).

Table 3
[^{3}H]DPDPE binding to δ-opioid receptors on brain sections from rats treated during 4 or 21 days with moclobemide (10 mg/kg daily) compared to their respective controls

Brain regions	Distance from bregma (mm)	Moclobemide 4 days		Moclobemide 21 days	
		Controls	Treated	Controls	Treated
Cortex					
Cortical layers I, II	1.7	49 ± 2	53 ± 2	43 ± 3	39 ± 2
Cortical layers III, IV, V	1.7	39 ± 2	39 ± 2	36 ± 3	34 ± 1
Cortical layer VIa	1.7	48 ± 2	55 ± 2*	41 ± 4	41 ± 2
Cingulate	1.7	43 ± 3	43 ± 2	41 ± 4	36 ± 2
Parietal	−2.8	45 ± 2	48 ± 2	39 ± 3	38 ± 3
Occipital	−5.3	37 ± 1	35 ± 7	36 ± 3	34 ± 3
Amygdala					
Medial posteroventral nu.	−2.8	34 ± 2	33 ± 3	31 ± 2	23 ± 2*
Medial posterodorsal nu.	−2.8	25 ± 1	27 ± 1*	24 ± 1	27 ± 2
Basolateral nu.	−2.8	58 ± 1	61 ± 2	43 ± 3	41 ± 3
Posteromedial cortical nu.	−5.3	22 ± 1	28 ± 3*	19 ± 1	17 ± 1
Basal ganglia					
Striatum	1.7	77 ± 3	80 ± 4	61 ± 4	59 ± 4
Nucleus accumbens	1.7	57 ± 3	62 ± 4	47 ± 3	42 ± 4
Miscellaneous					
Olfactory tubercle	1.7	62 ± 3	67 ± 4	45 ± 4	49 ± 2
Claustrum	1.7	85 ± 4	88 ± 3	71 ± 5	74 ± 6
Hypothalamus	−2.12	22 ± 1	23 ± 2	13 ± 2	14 ± 1
Hippocampus	−3.8	16 ± 1	17 ± 1	14 ± 1	15 ± 1
Interpeduncular nu.	−5.3	39 ± 5	35 ± 5	27 ± 5	28 ± 2
External cortex of inferior colliculi	−8.3	34 ± 3	38 ± 3	25 ± 3	19 ± 2*

Mean ± S.E.M. (expressed as fmol/mg tissue eq.) from five to eight controls and five to eight treated rats.
* $P<0.05$ compared to the corresponding controls.

3.2. Effects of chronic antidepressant treatments on [^{3}H]DPDPE autoradiographic binding to δ-opioid receptors

3.2.1. Paroxetine treatments

None of the regions analysed displayed any modification of the [^{3}H]DPDPE binding site density after a short-term (4 days) or a long-term (21 days) paroxetine treatment (data not shown).

3.2.2. Reboxetine treatments

A short-term (4 days) or long-term (21 days) treatment with reboxetine did not modify the [^{3}H]DPDPE binding site density in either studied brain region (data not shown).

3.2.3. Moclobemide treatments

A 4-day moclobemide treatment induced an increase in [^{3}H]DPDPE binding site density in layer VIa of frontal cortex (+15%, $P<0.05$), medial posterodorsal amygdaloid nucleus (+8%, $P<0.05$) and cortical posteromedial amygdaloid nucleus (+27%, $P<0.05$). On the other hand, a 21-day treatment resulted in a decrease in [^{3}H]DPDPE binding site density in medial posteroventral amygdaloid nucleus (−26%, $P<0.05$) and in the external cortex of inferior colliculi (−24%, $P<0.05$). No modification was noticed in any other studied region (Table 3).

4. Discussion

Our study was undertaken to investigate the modifications of μ- and δ-opioid receptors which occur during the delay of action of new generation antidepressants and which could be associated with the therapeutic efficacy.

Autoradiographic studies display high anatomical resolution, thus, allowing investigations in numerous limbic regions, particularly relevant in the mode of action of antidepressants, which had not been investigated so far as regards opioid receptors.

4.1. Changes in μ-opioid receptor binding density after paroxetine, reboxetine and moclobemide treatments

Our results showed the development of a time-related response of μ-opioid receptors density throughout the 3 weeks of the treatments with paroxetine, reboxetine and moclobemide.

However, there was a certain heterogeneity in μ-opioid receptors adaptations during these treatments, and these varied according to the regions. Particularly, in thalamic nuclei, paroxetine for 21 days induced a decreased DAMGO-displaceable [^{3}H]naloxone binding, while reboxetine for 21 days induced an increase in this binding and the 21-day moclobemide treatment induced no change in thalamic μ-opioid receptors density. Our results, comparing the effects of three antidepressants which act on different primary targets, evidenced that there is no common pattern of adaptations of μ- or δ-opioid receptors in any brain regions, neither at the beginning nor at the end of a 3-week treatment. Autoradiographic studies were performed with a unique concentration of each ligand. In the light of previous studies using rat brain homogenates and saturation analysis (Reisine and Soubrié, 1982; Hamon et al., 1987; Antkiewicz-Michaluk et al., 1984), antidepressant-induced changes seem to be more likely in the density of receptors, rather than in their affinity.

The decrease in thalamic μ-opioid receptor binding site density after the 3-week paroxetine treatment is consistent with the observation made after a chronic treatment with clomipramine, which is also a serotonin reuptake blocker. Benkelfat et al. (1989) indeed showed, on whole brain homogenates, that chronic clomipramine treatment for 28 days induced a 15% decrease in the number of μ-opioid receptor binding sites.

De Gandarias et al. (1998, 1999, 2000) described a common pattern of changes in μ-opioid receptors immunoreactivity after 14 days of chronic treatments with imipramine, fluoxetine and lithium. All these treatments increased the density of neural cells immunostained for μ-opioid receptors in the piriform, frontal and parietal cortices, the lateral septum, the dentate gyrus and the caudate putamen in the rat brain. Accordingly, Antkiewicz-Michaluk et al. (1984) showed that chronic citalopram for 24 days elevated [^{3}H]naloxone binding to opioid receptors in rat cerebral cortical membranes. Chronic antidepressants used in our study failed to produce the same effects as chronic fluoxetine, citalopram, imipramine or lithium in these regions. Moreover, inversely, in the frontal cortex, moclobemide treatment for 21 days decreased the cortical μ-opioid receptor binding site density. The systematic comparison of the three antidepressants described in our study shows that changes in μ-opioid receptors do not constitute a common feature of the mode of action of antidepressants.

However, it is worthy of note that the changes in μ-opioid receptor binding sites elicited by the three antidepressants, although heterogeneous, took place in regions belonging to the limbic system: the frontal, cingulate, insular agranular and prefrontal cortices, the amygdala, the hippocampus and the mediodorsal thalamus, or in areas functionally related to the limbic system such as hypothalamus.

Some of these regions, the prefrontal cortex, amygdala and medial thalamus constitute a limbic–thalamo-cortical circuit that may be engaged in abnormal activity that maintains the cognitive and emotional set of depression (for review, see Drevets and Raichle, 1992; Soares and Mann, 1997). This circuit is back-controlled by a limbic–striatal–pallidal–thalamic circuit that connects to the former one (for review, see Drevets and Raichle, 1992; Soares and Mann, 1997). The mediodorsal thalamus plays a crucial role as it integrates the inputs from the two circuits and sends, in fine, excitatory inputs towards the prefrontal cortex (for review, see Drevets and Raichle, 1992; Soares and Mann,

1997). Moreover, the mediodorsal thalamus, where the 21-day paroxetine and reboxetine treatments modified μ-opioid receptors density, has been described as a particular target of selective serotonin reuptake inhibitors antidepressants and as a brain region especially involved in mood regulation (Smith, 1999). Drevets and Raichle (1992) proposed that antidepressant treatments may correct or compensate for the pathophysiology of unipolar depression through modulation of limbic–thalamo-cortical activity and suggested that changes in monoaminergic systems may modulate activity in these circuits.

There is evidence that opioid systems do modulate serotonergic and noradrenergic transmissions, at least in some regions of these neuroanatomic circuits of depression. Marek and Aghajanian (1998) demonstrated that μ-opioid receptors activation blocks the serotonin-induced excitatory postsynaptic currents in the medial prefrontal cortex of the rat. In vivo microdialysis study (Yoshioka et al., 1993) and superfusion experiments (Passarelli and Costa, 1989) proved that μ-opioid receptors stimulation decreases the K+-induced release of serotonin from serotonergic neurons in the rat hippocampus. Morphine enhances brain serotonin synthesis and metabolism (for references, see Tao and Auerbach, 1995), increases extracellular serotonin in the amygdala, frontal cortex, thalamus and ventral hippocampus (Tao and Auerbach, 1995) and inhibits the spontaneous neuronal firing of serotonin-containing neurons in the amygdala (Haigler, 1978). On the other hand, μ-opioid receptors stimulation decreases the K+-induced release of norepinephrine from noradrenergic neurons in the rat hippocampus (Matsumoto et al., 1994), in the cortex (Trendelenburg et al., 2000) and in the hypothalamus (Yilmaz and Gilmore, 1999).

On account of these neuromodulatory effects of opioid systems, it is conceivable that changes in μ-opioid receptors density during an antidepressant therapy would have direct effectiveness in modulating monoaminergic transmissions in the limbic–thalamo-cortical circuit. However as no common pattern of response appeared, changes in μ-opioid receptors would not constitute a common pathway for the action of antidepressants belonging to distinct families.

4.2. δ-Opioid receptors density changes after paroxetine, reboxetine and moclobemide treatments

The study of δ-opioid receptors in the course of antidepressants treatments showed that neither paroxetine nor reboxetine did induce any change in the [^{3}H]DPDPE binding site density either in the short-term (4 days) or the long-term (21 days) of the treatment. Moclobemide induced an increase in δ-opioid receptor binding density in the frontal cortex and in the amygdala after 4 days of treatment and a decrease in the amygdala and external cortex of inferior colliculi after 21 days of treatment.

Few data have been available so far concerning the involvement of δ-opioid receptors in the response to chronic antidepressant treatments. Chronic amoxapine and amitryptiline treatments for 14 days have been found to produce a decrease in δ-opioid receptor binding sites densities in the hypothalamus (Hamon et al., 1987), a result that the three antidepressants tested here failed to reproduce.

Moclobemide was the sole antidepressant tested here that induced some changes in δ-opioid receptor binding sites densities. Moclobemide is, as tricyclics, a mixed serotonin, norepinephrine and dopamine-targeted antidepressant. This particular trait could be responsible for its action on δ-opioid receptor binding site density.

According to this, δ-opioid receptors density changes do not seem to be a common trait of action of antidepressant treatments in any brain region. Particularly, they do not seem to be involved in the mode of action of antidepressants with specific primary targets, but rather in that of antidepressants acting on serotonin, norepinephrine and/or dopamine systems.

5. Conclusion

In summary, the three antidepressants studied here, when administered chronically, induced adaptations in the density of μ-opioid receptor binding sites in various brain regions. Among them, only moclobemide modified the density of binding to δ-opioid receptors.

In our study, there was no particular brain region where all tested antidepressants induced the same pattern of changes in opioid receptor binding density. Nevertheless, nearly all changes in μ-opioid receptor binding site density occurred in limbic and thalamic regions, which take part in a neuroanatomical circuit of depression. Those changes could therefore participate in the modulation of monoaminergic transmissions in these regions along antidepressant treatments.

Acknowledgements

We wish to thank Smithkline-Beecham Pharmaceuticals, Pharmacia & Upjohn and Roche for the gifts of paroxetine, reboxetine and moclobemide, respectively. We thank Nicolas Feraille for his help in experiments concerning paroxetine treatments. This study was supported by grants from the Région Haute-Normandie, France.

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