JPET Fast Forward. Published on September 3, 2003 as DOI: 10.1124/jpet.103.053215

JPET #53215

Antidepressant-induced increase in high-affinity rolipram binding sites in rat brain: dependence on noradrenergic and serotonergic function

Yu Zhao, Han-Ting Zhang, and James M. O'Donnell

Department of Pharmacology

The University of Tennessee Health Science Center

Memphis, TN 38163

Running title: Antidepressant effects on inhibitor binding to PDE4

Correspondence should be addressed to:

James M. O'Donnell, Ph.D.

Department of Pharmacology

University of Tennessee Health Science Center

874 Union Avenue

Memphis, TN 38163

Phone: 901-448-3621

Fax: 901-448-3849

Email: jodonnell@utmem.edu

Number of text page: 34

Number of tables: 0

Number of figures: 8

Number of references: 39

Number of words: Abstract (247); Introduction (713); Discussion (1436)

Abbreviations: PDE4, type 4 cyclic AMP phosphodiesterase; HARBS, high-affinity rolipram binding site; LARBS, low-affinity rolipram binding site; DMI, desipramine; FLU, fluoxetine; 6-OHDA, 6-hydroxydopamine; 5,7-DHT, 5,7-dihydroxytryptamine; CREB, cyclic AMP response element binding protein; PKA, protein kinase A

Section: Neuropharmacology

Abstract

The effects of antidepressant treatment on the high- and low-affinity rolipram binding sites on type 4 phosphodiesterase (PDE4) were determined; previous work had shown that repeated antidepressant treatment increases the overall expression of PDE4. Rats were administered different doses of the antidepressant drugs desipramine or fluoxetine, or saline for 1, 7, or 14 days. [^{3}H]-Rolipram and [^{3}H]-piclamilast were used to assess high- and low-affinity rolipram binding sites on type 4 phosphodiesterase (PDE4; the HARBS and LARBS, respectively) in hippocampus and cerebral cortex. Repeated, but not acute, treatment with the antidepressants increased [^{3}H]-rolipram binding to membrane fractions in a dose-dependent manner; the HARBS component of [^{3}H]-piclamilast binding also was increased by these treatments. By contrast, the LARBS component of [^{3}H]-piclamilast binding was not altered. [^{3}H]-Rolipram and [^{3}H]-piclamilast binding to the cytosolic fractions of rat cerebral cortex and hippocampus was not altered by the antidepressant treatments. 6-Hydroxydopamine (6-OHDA; 300 □g, icv) and 5,7-dihydroxytryptamine (5,7-DHT; 200 □g, icv) were used to lesion noradrenergic and serotonergic neurons, respectively. The effects of desipramine, but not fluoxetine, on [^{3}H]-rolipram and [^{3}H]-piclamilast binding to rat hippocampal membranes were blocked by the 6-OHDA-induced lesion. By contrast, the effects of fluoxetine, but not desipramine, were reduced by the 5,7-DHT-induced lesion. This indicates that the up-regulation of the HARBS by desipramine and fluoxetine requires the integrity of noradrenergic and serotonergic neurons, respectively. Collectively, these results suggest that antidepressants, although acting through different pathways, may eventually lead to the regulation of components of the cAMP signal transduction system.

The precise mechanisms of action of antidepressant drugs remain unclear. Early studies focused on their acute effects on serotonergic or noradrenergic systems. The delay of the therapeutic effects of antidepressants led to studies of their longer-term pharmacologic effects (Mongeau et al., 1997). Several hypotheses have been proposed, often involving the down-regulation of postsynaptic receptors, including beta adrenergic and serotonergic receptors. However, it is clear that the acute synaptic effects of antidepressants, although not sufficient by themselves, initiate the cascade of events that produce clinical improvement (Miller et al., 1996).

Duman and co-workers have suggested that there is increased activity of cAMP signal transduction cascades in response to antidepressant treatment (Duman et al., 1997). Chronic electroconvulsive shock and antidepressant drug administration increase the coupling of stimulatory G protein to adenylyl cyclase (Ozawa et al., 1991), the activity of cAMP-dependent protein kinase in crude nuclear fractions of rat cerebral cortex (Nestler et al., 1989), and the expression of cAMP response element binding protein (CREB) mRNA in the rat hippocampus (Nibuya et al., 1996). Furthermore, type 4 phosphodiesterase (PDE4), the primary form of phosphodiesterase hydrolyzing cAMP associated with the central beta adrenergic receptors (Ye and O'Donnell, 1996), has been implicated in the actions of proven antidepressants. Repeated treatment with various antidepressants or induction of electroconvulsive seizure, which has an antidepressant effect, increases the expression of PDE4A and PDE4B, but not PDE4D, in the rat frontal cortex and hippocampus (Suda et al., 1998; Takahashi et al., 1999; Ye et al., 1997, 2000). Furthermore, PDE4 inhibitors, such as rolipram and papaverine, have antidepressant-like effects in several behavioral models and therapeutic effects in depressed patients (Bobon et al., 1988; O'Donnell, 1993; Zhang et al., 2002).

One of the unique characteristics of PDE4 is that the binding of rolipram, the prototypic PDE4 inhibitor, is to two sites, termed the low-affinity rolipram binding site (LARBS) and the high-affinity rolipram binding site (HARBS) (Jacobitz et al., 1996; Schneider et al., 1986); the HARBS and the LARBS are more accurately described as two distinct binding affinity states, rather than separate sites. It should be noted that the terminology of HARBS and LARBS refers specifically to rolipram binding. Some inhibitors bind with high affinity to both HARBS and LARBS (e.g., piclamilast). A study using a series of truncated PDE4A mutants showed that inhibitor binding to both the HARBS and the LARBS is to the catalytic site (Jacobitz et al., 1996). Binding to the HARBS, but not the LARBS, depends on the presence of the N-terminal region of the protein. It has been suggested that the HARBS and the LARBS mediate different effects of PDE4 inhibitors. Some effects, such as induction of head twitches and tremor in mice and emesis in ferrets, are associated with the HARBS. By contrast, inhibition of guinea pig mast cell degranulation and suppression of antigen-induced T-cell proliferation are associated with the LARBS (Barnette et al., 1995; Duplantier et al., 1996; Schmiechen et al., 1990). The finding that repeated treatment with antidepressants from different pharmacological classes increases the expression of PDE4 suggests that these drugs may ultimately affect common signaling pathways (Takahashi et al., 1999; Ye et al., 1997, 2000). It is not known whether antidepressant treatment affects the HARBS and the LARBS differentially. Given that factors such as phosphorylation and interaction with other proteins can affect inhibitor affinity (Hoffmann et al., 1998; McPhee et al., 1999), it appears likely that the HARBS and the LARBS are regulated to different degrees by antidepressant treatment. Such a finding might suggest that antidepressant treatment alters cAMP-mediated signaling in the central nervous system via this mechanism, since the HARBS is present in brain, but not peripheral tissues (Schneider et al., 1986; Zhao et al., 2003).

In the present study, the effects of antidepressant treatment on the HARBS and the LARBS in the rat brain were examined to determine whether these PDE4 inhibitor binding sites were affected differently. Rats were treated repeatedly with the antidepressant desipramine or fluoxetine, relatively selective inhibitors of norepinephrine and serotonin uptake, respectively. The HARBS and the LARBS in preparations of rat cerebral cortex and hippocampus were assessed using [^{3}H]-piclamilast and [^{3}H]-rolipram (Zhao et al., 2003). In order to determine whether their actions depend on enhanced monoaminergic function, the effects of the antidepressants also were determined following noradrenergic or serotonergic lesions.

Materials and Methods

Animals. Male Sprague-Dawley rats (Harlan, Indianapolis, IN, U.S.A.) were housed in a temperature- (22-24°C) and light- (on 6:00 a.m. - 6:00 p.m.) controlled room and were allowed free assess to food pellets and water. Their use in the present studies was in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health and has been approved by the Animal Care and Use Committee of the University of Tennessee Health Science Center.

Surgical procedures. Rats subjected to monoaminergic lesions (see below) were anesthetized with ketamine/xylazine, and placed in a stereotaxic frame. Cannulae were implanted bilaterally into the lateral ventricles (0.5 mm posterior to Bregma, ±1.6 mm lateral to the midline, and 3.9 mm ventral to dura; O'Donnell et al., 1994). Rats were allowed at least five days to recover before use in experiments.

Central noradrenergic lesions. Noradrenergic lesions were produced by bilateral icv administration of 6-hydroxydopamine (6-OHDA; 150 μg dissolved in 10 μl of 0.2% ascorbic acid/0.9% NaCl per side). The noradrenergic lesions were verified by measurement of norepinephrine uptake sites using [^{3}H]-nisoxetine binding (Tejani-Butt, 1992).

Central serotonergic lesions. Serotonergic lesions were produced by bilateral icv administration of 5,7-dihydroxytryptamine (5,7-DHT; 100 μg dissolved in 10 μl of 0.2% ascorbic acid/0.9% NaCl per side). To protect the noradrenergic neurons, rats were pretreated

with 25 mg/kg desipramine (i.p.) 30 min before icv infusion of 5,7-DHT (Breese and Traylor, 1971). The serotonergic lesions were verified by measurement of serotonin uptake sites using [^{3}H]-citalopram binding (D'Amato et al., 1987).

Treatment of animals. Rats were administered 1, 3, or 10 mg/kg desipramine or fluoxetine (twice daily, i.p.) for 1, 7, or 14 days. Rats used in the experiment to test the effects of monoamine depletion were administered 6 mg/kg desipramine or fluoxetine (twice daily, i.p.) for 14 days. These treatments started 20 days after the icv infusion of 6-OHDA or 5,7-DHT.

Radioligand binding assays. Rats were killed by decapitation 24 hours after the last injection. The cerebral cortex and hippocampus were dissected on ice and homogenized in incubation buffer (50 mM Tris HCl, 5 mM $MgCl_2$, pH 7.5) using a Polytron homogenizer. Samples were centrifuged at 15,000 g for 15 minutes; the pellets were resuspended in incubation buffer.

[^{3}H]-Rolipram and [^{3}H]-piclamilast binding was measured as described previously (Zhao et al., 2003). Membrane or cytosolic preparations containing 200 – 300 □g protein were incubated at 30°C in 250 □l of incubation buffer containing 2 nM [^{3}H]-rolipram or [^{3}H]-piclamilast. Nonspecific binding was determined in the presence of 10 □M unlabeled Ro 20-1724 for [^{3}H]-rolipram binding or 1 mM unlabeled rolipram for [^{3}H]-piclamilast binding.

For the saturation binding studies, different concentrations of [^{3}H]-rolipram (0.5 - 50 nM) and [^{3}H]-piclamilast (0.01 - 20 nM) were used. The saturation curves were determined only using preparations of cerebral cortex.

For calculating the percentage of the HARBS and the LARBS fractions, rolipram competition of [^{3}H]-piclamilast binding was determined. For the competition assay, a 2 nM

concentration of [^{3}H]-piclamilast was used in the presence of different concentrations of unlabeled rolipram (Zhao et al., 2003).

For [^{3}H]-nisoxetine binding, which provides an index of the density of noradrenergic terminals, brain tissue was homogenized in buffer (50 mM Tris, 120 mM NaCl, 5 mM KCl, pH 7.4). Membrane preparations (200 – 300 μg protein) were incubated in 250 μl incubation buffer (50 mM Tris, 300 mM NaCl, 5 mM KCl, pH 7.4) containing 5 nM [^{3}H]-nisoxetine for 4 hr at 0°C. Nonspecific binding was determined using 25 μM desipramine.

For [^{3}H]-citalopram binding, which provides and index of the density of serotonergic terminals, brain tissue was homogenized in buffer (50 mM Tris, 120 mM NaCl, 5 mM KCl, pH 7.4). Membrane preparations (200 – 300 μg protein) were incubated in 250 μl of the same buffer containing 1 nM [^{3}H]-citalopram for 60 minutes at 25°C. Nonspecific binding was determined using 25 μM fluoxetine.

All the radioligand binding assays were carried out in duplicate. At the end of the incubation period, the reactions were stopped by addition of 5 ml of ice-cold buffer and then rapidly filtered through glass-fiber filters that had been soaked in 0.3% polyethyleneimine. The filters were washed twice with 5 ml of ice-cold buffer, and radioactivity measured using a liquid scintillation counter. Binding was normalized to protein content, which was determined using the bicinchoninic acid assay (Smith et al., 1985).

Statistical analysis. Data were analyzed by nonlinear regression (O'Donnell et al., 1984; Zhao et al., 2003). B_{max} and K_d values were determined for saturation experiments. The equation used for the two-site model was $B = [B_H/(1 + C/IC_H)] + [(B_L/(1 + C/IC_L)]$ where B is equal to the amount of radioligand bound, B_H and B_L are the percentage of competitor binding to high- and

low-affinity sites, respectively, C is the competitor concentration, and IC_H and IC_L are the IC_{50} values for the high- and low-affinity sites, respectively. For the one-site model, B_H equaled zero and the equation reduced to $B = B_L/(1 + C/IC_L)$. The percentages of the HARBS and the LARBS were calculated from rolipram competition curves for inhibition of [^{3}H]-piclamilast binding by nonlinear regression using a two-site model. All values are expressed as means ± SEM from at least four independent experiments carried out in duplicate. Differences between the treatment and control groups were analyzed using one-way ANOVA followed by Dunnett's test.

Materials. [^{3}H]-Rolipram was purchased from American Radiolabeled Chemicals Inc. (St. Louis, MO, U.S.A.). [^{3}H]-Piclamilast was a gift from GlaxoSmithKline (Valley Forge, PA, U.S.A.). [^{3}H]-Nisoxetine and [^{3}H]-citalopram were purchased from PerkinElmer (Boston, MA, U.S.A.). Rolipram was provided by Schering AG (Berlin, Germany). Other chemicals were obtained from Fisher Scientific (Pittsburgh, PA, U.S.A.) or Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Results

Dose- and time-dependent effects of antidepressant treatments on [^{3}H]-rolipram binding to rat hippocampus and cerebral cortex. [^{3}H]-Rolipram binding, an index of the HARBS, was measured using membrane and cytosolic fractions of both hippocampus and cerebral cortex from rats treated with either desipramine or fluoxetine. [^{3}H]-Rolipram binding was increased for both hippocampal and cerebral cortical membranes after 14 days of treatment with either antidepressant (Figs. 1, 2); treatment for 1 or 7 days had no effect (Fig. 3). Repeated treatment with 3 or 10 mg/kg desipramine for 14 days increased [^{3}H]-rolipram binding to hippocampal membranes; the 1 mg/kg dose had no effect (Fig. 2). Fluoxetine treatment for 14 days at doses of 3 and 10 mg/kg also increased [^{3}H]-rolipram binding; the 1 mg/kg dose had no effect (Fig. 2). Saturation analysis was carried out using cerebral cortical preparations. Repeated, but not acute, treatment with desipramine and fluoxetine at doses of 3 or 10 mg/kg increased the B_{max} values for [^{3}H]-rolipram binding (Fig. 2); the K_d values were not altered by the treatments. Antidepressant treatments did not alter [^{3}H]-rolipram binding to cytosolic fractions of hippocampus or cerebral cortex (data not shown).

Dose- and time-dependent effects of antidepressant treatments on [^{3}H]-piclamilast binding to rat hippocampus and cerebral cortex. [^{3}H]-Piclamilast binding was measured using the membrane and cytosolic fractions of both hippocampus and cerebral cortex from rats treated with either desipramine or fluoxetine. Rolipram inhibition of [^{3}H]-piclamilast binding was performed to calculate the percentage of the HARBS and the LARBS comprising the total binding. Of the total [^{3}H]-piclamilast binding to both hippocampal and cerebral cortical

preparations, about 60 to 70% was to the HARBS, while about 30 to 40% was to the LARBS (data not shown). Similar to what was observed for [^{3}H]-rolipram binding, the effects of desipramine and fluoxetine treatment on [^{3}H]-piclamilast binding were time-dependent. Repeated, but not acute, treatment with either antidepressant increased [^{3}H]-piclamilast binding (Figs. 4, 5). Repeated treatment with either desipramine or fluoxetine, at doses of 3 or 10 mg/kg, increased the HARBS in hippocampal membranes; treatment with 1 mg/kg of either antidepressant had no effect (Fig. 4). By contrast, neither acute nor repeated antidepressant treatments increased the LARBS in hippocampal membranes (Fig. 4). Saturation analysis was carried out using cerebral cortical preparations. Repeated treatment with 10 mg/kg desipramine for 14 days, but not 1 or 7 days, increased the B_{max} values for the HARBS (Figs. 4, 5); fluoxetine treatment increased the B_{max} value for the HARBS, when administered at doses of 3 or 10 mg/kg for 14 days (Fig. 4). The B_{max} values for the LARBS in the cerebral cortical membranes were not affected by the antidepressant treatments (Fig. 4). The K_d values were not altered by the treatments. Similar to what was observed when assessing [^{3}H]-rolipram binding, the antidepressant treatments did not alter [^{3}H]-piclamilast binding to cytosolic fractions of rat hippocampus or cerebral cortex (data not shown).

Noradrenergic lesions blocked the effect of desipramine, but not fluoxetine, on [^{3}H]-rolipram and [^{3}H]-piclamilast binding to rat hippocampal membranes. [^{3}H]-Rolipram binding to hippocampal membranes from control rats was increased by desipramine or fluoxetine treatment (Fig. 6). For [^{3}H]-piclamilast binding, the HARBS component was increased by desipramine or fluoxetine treatment; the LARBS was not affected (Fig. 6). The ability of fluoxetine to increase [^{3}H]-rolipram and the HARBS component of [^{3}H]-piclamilast binding was

not affected by the noradrenergic lesions. By contrast, desipramine treatment did not increase [^{3}H]-rolipram binding or the HARBS component of [^{3}H]-piclamilast binding to hippocampal membranes prepared from 6-OHDA-treated rats (Fig. 6). The magnitude of the 6-OHDA-induced lesions on noradrenergic neurons was shown by the reduction of [^{3}H]-nisoxetine binding to hippocampal membranes, which indicates a significant loss of noradrenergic terminals (Fig. 7).

Serotonergic lesions reduced the effect of fluoxetine, but not desipramine, on [^{3}H]-rolipram and [^{3}H]-piclamilast binding to rat hippocampal membranes. [^{3}H]-Rolipram binding to hippocampal membranes from control rats was increased by desipramine or fluoxetine treatment (Fig. 8). For [^{3}H]-piclamilast binding, the HARBS component was increased by desipramine or fluoxetine treatment; the LARBS was not affected (Fig. 8). The ability of desipramine to increase [^{3}H]-rolipram and the HARBS component of [^{3}H]-piclamilast binding was not affected by serotonergic lesions. By contrast, the ability of fluoxetine treatment to increase [^{3}H]-rolipram binding or the HARBS component of [^{3}H]-piclamilast binding was reduced in hippocampal membranes prepared from 5,7-DHT-treated rats (Fig. 8). The magnitude of the 5,7-DHT-induced lesions on serotonergic neurons was shown by the reduction of [^{3}H]-citalopram binding to hippocampal membranes, which indicates a significant loss of serotonergic terminals (Fig. 7).

Discussion

The results of this study demonstrate that repeated treatment with antidepressants, specifically the norepinephrine reuptake inhibitor desipramine and the serotonin reuptake inhibitor fluoxetine, increased the HARBS, but not the LARBS, in rat hippocampal and cerebral cortical membranes. The up-regulation of the HARBS, however, was not observed in the cytosolic fractions of these brain regions. The effects of desipramine, but not fluoxetine, on the HARBS were blocked by 6-OHDA-induced noradrenergic lesions. By contrast, the effects of fluoxetine, but not desipramine, were reduced by 5,7-DHT-induced serotonergic lesions.

The HARBS and the LARBS in preparations of rat brain were assessed using [^{3}H]-rolipram and [^{3}H]-piclamilast binding. [^{3}H]-Piclamilast binding has been demonstrated to label both the HARBS and the LARBS at nanomolar concentrations; [^{3}H]-rolipram binds only to the HARBS at this concentration range (Jacobitz et al., 1996; Zhao et al., 2003). The fractions of the HARBS and the LARBS are obtained by determining rolipram competition of [^{3}H]-piclamilast binding. [^{3}H]-Rolipram binding, an index of the HARBS, was increased by the repeated antidepressant treatments. Similarly, the HARBS fraction of the [^{3}H]-piclamilast binding also was increased by these treatments; the LARBS fraction of [^{3}H]-piclamilast binding was not affected. However, the magnitude of the increase of [^{3}H]-rolipram binding differed from that of the HARBS fraction of [^{3}H]-piclamilast binding; the up-regulation of [^{3}H]-rolipram binding was somewhat greater than that of the HARBS fraction of the [^{3}H]-piclamilast binding.

The up-regulation of the HARBS was observed using both rat hippocampal and cerebral cortical membranes, with the effect in the hippocampus being somewhat greater. These results suggest that the antidepressant treatments regulate cAMP-mediated signal transduction systems

in both brain regions. Studies have shown antidepressant-induced regulation of the cAMP signaling systems at several levels, including G proteins, adenylyl cyclase, cAMP-dependent protein kinase (PKA), PDE4, and CREB in hippocampus and cerebral cortex (Nestler et al., 1989; Nibuya et al., 1996; Ozawa et al., 1991; Suda et al., 1998; Takahashi et al., 1999; Ye et al., 1997, 2000). The hippocampus has been implicated in both the pathophysiology and pharmacotherapy of depression and related illnesses (Bremner et al., 1995; Mongeau et al., 1997). Posttraumatic stress disorder patients exhibit reduced right hippocampus volume relative to that of control subjects, but no difference in the volume of other brain regions (Bremner et al., 1995). Shah and co-workers (1998) reported a correlation between hippocampal atrophy and impaired verbal learning in depression patients. Cerebral cortex, especially frontal cortex, also appears to be involved in depressive disorders; brain imaging studies have demonstrated abnormalities in the volume and function of cortical areas in depressed patients (Drevets et al., 1997). Thus, antidepressant-induced regulation of cAMP system in hippocampus and cerebral cortex might contribute to their clinical effects.

Rolipram, the prototypic PDE4 inhibitor, binds to two affinity states of the PDE4 enzyme, a low-affinity site (K_i of approximately 500 nM; the LARBS) and a high-affinity site (K_i of approximately 1 nM; the HARBS) (Jacobitz et al., 1996). It has been reported that the HARBS and the LARBS mediate different constellations of effects of PDE4 inhibitors (Barnette et al., 1995; Duplantier et al., 1996). The present study showed that the HARBS and the LARBS were differentially regulated by the antidepressant treatments. The HARBS, but not the LARBS, was up-regulated by repeated antidepressant administration. This suggests the HARBS might be implicated in signaling pathways regulated by antidepressants. In support of this result, Wachtel and co-workers (1990) reported that there are significant correlations between the potency of a

number of PDE4 inhibitors for antagonizing reserpine-induced hypothermia in mice, a classic model used to predict antidepressant activity, and their potency for inhibiting of [^{3}H]-rolipram binding in vivo. However, some evidence suggests that the antidepressant-like effects of PDE4 inhibitors are mediated by interactions with the LARBS. It has been reported that the PDE4 inhibitor CP 76,593, even though more potent than rolipram for the inhibition of high-affinity [^{3}H]-rolipram binding, is considerably less potent than rolipram for producing antidepressant-like effects on differential reinforcement of low response rate (DRL) behavior (O'Donnell, 1993). In addition, the potency order of a series of drugs for inhibition of high-affinity [^{3}H]-rolipram binding is only moderately correlated with their potency order for reducing immobility of mice in the forced-swim test (Saccomano et al., 1991).

The preferential up-regulation of the HARBS may involve several processes. Antidepressants have been reported to differentially regulate PDE4 subtypes. Expression of PDE4A and PDE4B, but not PDE4D, in brain is increased by repeated treatment with antidepressants from different pharmacological classes, including selective reuptake inhibitors of serotonin (sertraline and fluoxetine) or norepinephrine (desipramine), monoamine oxidase inhibitors (phenelzine and tranylcypromine), or atypical antidepressant (trazodone), or by electroconvulsive shock treatment. This is evidenced by increases in both mRNA and protein expression (Suda et al., 1998; Takahashi et al., 1999; Ye et al., 1997, 2000). It is also possible that antidepressant treatment alters the intracellular targeting or phosphorylation state of PDE4 subtypes, which changes their conformational states. PDE4 isoforms can exhibit varied sensitivity to inhibition by rolipram under different conditions. The particulate form of PDE4A4 is more sensitive to inhibition by rolipram than is its cytosolic form (Huston et al., 1996). Complexing PDE4A4 with the SRC homology (SH3) domains of tyrosyl protein kinases, such as

LYN, FYN, and SRC, increases its sensitivity to inhibition by rolipram (McPhee et al., 1999). Treatment of guinea pig eosinophil membranes with deoxycholate and high salt or with a vanadate/glutathione complex, increases the potency of rolipram by greater than 10-fold (Souness et al., 1992). PKA-mediated phosphorylation of PDE4D3 increases its sensitivity to rolipram inhibition (Hoffmann et al., 1998; Sette and Conti, 1996). In addition, reversible divalent metal binding to PDE4 is involved in the mediation of differential rolipram interactions (Laliberte et al., 2000). Mg^{2+}, Mn^{2+}, and Co^{2+} all stabilize similar, high-affinity (K_d values of 3-8 nM) rolipram binding to the PDE4 holoenzyme. In the absence of the divalent cations, only low-affinity rolipram binding to the apoenzyme is detected (Liu et al., 2001).

Antidepressant treatment up-regulated the HARBS in the membrane fractions of the hippocampus and cerebral cortex, but not in the cytosolic fractions. These results suggest the compartmentalization of cAMP signaling components. PDE4 subtypes exhibit different intracellular distribution and protein interactions because of their unique N-terminals. While PDE4A1 is totally membrane-associated, PDE4A5 exist in both soluble and particulate fractions (Huston et al., 1996, 2000). PDE4A5 contains motifs that interact with SH3 domains (Dalgarno et al., 1997). Apoptosis-induced cleavage within the unique N-terminal region containing the SH3 domain-binding site of PDE4A5 leads to its intracellular redistribution (Huston et al., 2000). The PDE4D5 isoform interacts with the scaffold protein RACK1 (Yarwood et al., 1999). It is possible that the increased cAMP signaling associated with the antidepressant treatments stimulates the expression of the membrane-associated PDE4 subtypes or affects their interaction with other cellular proteins, resulting in an increase in the HARBS.

Noradrenergic and serotonergic lesions alone had no effect on the [^{3}H]-rolipram and [^{3}H]-piclamilast binding to rat hippocampal membranes. It has been reported that diminished

noradrenergic activity reduces the expression of PDE4A and PDE4B, but not PDE4D subtypes (Ye et al., 1997). One explanation for this discrepancy is the use of the different detection systems. PDE4A and PDE4B comprise only a portion of the total PDE4 pool; lesion-induced down-regulation may be difficult to detect using binding assays. Destruction of noradrenergic neurons blocked the ability of repeated desipramine, but not fluoxetine, treatment to increase the HARBS; by contrast, the lesion of the serotonergic neurons reduced the effect of fluoxetine, but not desipramine, treatment. The results indicate that the effects of desipramine and fluoxetine on the HARBS require the integrity of noradrenergic and serotonergic nerve terminals, respectively. This suggests that although desipramine and fluoxetine have similar effects on the HARBS in rat brain, they act through different neurochemical pathways. Their acute effects on noradrenergic or serotonergic neurotransmitters, respectively, seem necessary to initiate the cascade of events that lead to an alteration of PDE4 expression and an increase in the HARBS. This dependence on monoamine function also is reported for the therapeutic effects in depression (Miller et al., 1996).

In the present study, it is shown that repeated antidepressant treatment selectively increases the HARBS in membrane fractions of hippocampus and cerebral cortex. The desipramine- and fluoxetine-induced increases in the HARBS are dependent on the integrity of noradrenergic and serotonergic neurons, respectively. The mechanism by which antidepressant treatment preferentially up-regulates the HARBS is not clear. Increased expression of certain PDE4 subtypes or changes in their interactions with other cellular proteins may be involved. The increased HARBS may be a compensatory response to enhanced cAMP concentrations and may represent another component of cAMP-mediated signal transduction systems affected by repeated administration of antidepressants from different pharmacological classes.

Acknowledgements

The authors thank Dr. David Edwards (GlaxoSmithKline Pharmaceuticals) for providing [^{3}H]-piclamilast and Kathy Mishler for expert technical assistance.

References

Barnette MS, Grous M, Cieslinski LB, Burman M, Christensen SB, Torphy TJ (1995) Inhibitors of phosphodiesterase IV (PDE IV) increase acid secretion in rabbit isolated gastric glands: correlation between function and interaction with a high-affinity rolipram binding site. J Pharmacol Exp Ther 273:1396-1402.

Bobon D, Breulet M, Gerard-Vandenhove MA, Guiot-Goffioul F, Plomteux G, Sastre-y-Hernandez M, Schratzer M, Troisfontaines B, von Frenckell R, Wachtel H (1988) Is phosphodiesterase inhibition a new mechanism of antidepressant action? A double blind double-dummy study between rolipram and desipramine in hospitalized major and/or endogenous depressives. Eur Arch Psychiatry Neurol Sci 238:2-6.

Breese GR, Traylor TD (1971) Depletion of brain noradrenaline and dopamine by 6-hydroxydopamine. Br J Pharmacol 42:88-99.

Bremner JD, Randall P, Scott TM, Bronen RA, Seibyl JP, Southwick SM, Delaney RC, McCarthy G, Charney DS, Innis RB (1995) MRI-based measurement of hippocampal volume in patients with combat-related posttraumatic stress disorder. Am J Psychiatry. 152:973-981.

Dalgarno DC, Botfield MC, Rickles RJ (1997) SH3 domains and drug design: ligands, structure, and biological function. Biopolymers 43:383-400.

D'Amato RJ, Largent BL, Snowman AM, Snyder SH (1987) Selective labeling of serotonin uptake sites in rat brain by [^{3}H]-citalopram contrasted to labeling of multiple sites by [^{3}H]-imipramine. J Pharmacol Exp Ther 242:364-371.

Drevets WC, Price JL, Simpson JR Jr, Todd RD, Reich T, Vannier M, Raichle ME (1997) Subgenual prefrontal cortex abnormalities in mood disorders. Nature 386:824-827.

Duman RS, Heninger GR, Nestler EJ (1997) A molecular and cellular theory of depression. Arch Gen Psychiatry 54:597-606.

Duplantier AJ, Biggers MS, Chambers RJ, Cheng JB, Cooper K, Damon DB, Eggler JF, Kraus KG, Marfat A, Masamune H, Pillar JS, Shirley JT, Umland JP, Watson JW (1996) Biarylcarboxylic acids and -amides: inhibition of phosphodiesterase type IV versus [3H]rolipram binding activity and their relationship to emetic behavior in the ferret. J Med Chem 39:120-125.

Hoffmann R, Wilkinson IR, McCallum JF, Engels P, Houslay MD (1998) cAMP-specific phosphodiesterase HSPDE4D3 mutants which mimic activation and changes in rolipram inhibition triggered by protein kinase A phosphorylation of Ser-54: generation of a molecular model. Biochem J 333:139-149.

Huston E, Pooley L, Julien P, Scotland G, McPhee I, Sullivan M, Bolger G, Houslay MD (1996) The human cyclic AMP-specific phosphodiesterase PDE-46 (HSPDE4A4B) expressed in

transfected COS7 cells occurs as both particulate and cytosolic species that exhibit distinct kinetics of inhibition by the antidepressant rolipram. J Biol Chem 271:31334-31344.

Huston E, Beard M, McCallum F, Pyne NJ, Vandenabeele P, Scotland G, Houslay MD (2000) The cAMP-specific phosphodiesterase PDE4A5 is cleaved downstream of its SH3 interaction domain by caspase-3. Consequences for altered intracellular distribution. J Biol Chem 275:28063-28074.

Jacobitz S, McLaughlin MM, Livi GP, Burman M, Torphy TJ (1996) Mapping the functional domains of human recombinant phosphodiesterase 4A: structural requirements for catalytic activity and rolipram binding. Mol Pharmacol 50:891-899.

Laliberte F, Han Y, Govindarajan A, Giroux A, Liu S, Bobechko B, Lario P, Bartlett A, Gorseth E, Gresser M, Huang Z (2000) Conformational difference between PDE4 apoenzyme and holoenzyme. Biochemistry 39:6449-6458.

Liu S, Laliberte F, Bobechko B, Bartlett A, Lario P, Gorseth E, Van Hamme J, Gresser MJ, Huang Z (2001) Dissecting the cofactor-dependent and independent bindings of PDE4 inhibitors. Biochemistry 40:10179-10186.

McPhee I, Yarwood SJ, Scotland G, Huston E, Beard MB, Ross AH, Houslay ES, Houslay MD (1999) Association with the SRC family tyrosyl kinase LYN triggers a conformational change in

the catalytic region of human cAMP-specific phosphodiesterase HSPDE4A4B. Consequences for rolipram inhibition. J Biol Chem 274:11796-11810.

Miller HL, Delgado PL, Salomon RM, Berman R, Krystal JH, Heninger GR, Charney DS (1996) Clinical and biochemical effects of catecholamine depletion on antidepressant-induced remission of depression. Arch Gen Psychiatry 53:117-128.

Mongeau R, Blier P, de Montigny C (1997) The serotonergic and noradrenergic systems of the hippocampus: their interactions and the effects of antidepressant treatments. Brain Res Brain Res Rev 23:145-195.

Nestler EJ, Terwilliger RZ, Duman RS (1989) Chronic antidepressant administration alters the subcellular distribution of cyclic AMP-dependent protein kinase in rat frontal cortex. J Neurochem 53:1644-1647.

Nibuya M, Nestler EJ, Duman RS (1996) Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. J Neurosci 16:2365-2372.

O'Donnell JM, Wolfe BB, Frazer A (1984) Agonist interactions with beta adrenergic receptors in rat brain. J Pharmacol Exp Ther 228:640-647.

O'Donnell JM (1993) Antidepressant-like effects of rolipram and other inhibitors of cyclic adenosine monophosphate phosphodiesterase on behavior maintained by differential reinforcement of low response rate. J Pharmacol Exp Ther 264:1168-1178.

O'Donnell JM, Frith S, Wilkins J (1994) Involvement of beta-1 and beta-2 adrenergic receptors in the antidepressant-like effects of centrally administered isoproterenol. J Pharmacol Exp Ther 271:246-254.

Ozawa H, Rasenick MM, Takahata N, Saito T (1991) The effects of electroconvulsive shock on receptor-G protein-adenylate cyclase coupling. Jpn J Psychiatry Neurol 45:137-138.

Schmiechen R, Schneider HH, Wachtel H (1990) Close correlation between behavioural response and binding in vivo for inhibitors of the rolipram-sensitive phosphodiesterase. Psychopharmacology (Berl) 102:17-20.

Schneider HH, Schmiechen R, Brezinski M, Seidler J (1986) Stereospecific binding of the antidepressant rolipram to brain protein structures. Eur J Pharmacol 127:105-115.

Sette C, Conti M (1996) Phosphorylation and activation of a cAMP-specific phosphodiesterase by the cAMP-dependent protein kinase. Involvement of serine 54 in the enzyme activation. J Biol Chem 271:16526-16534.

Shah PJ, Ebmeier KP, Glabus MF, Goodwin GM (1998) Cortical grey matter reductions associated with treatment-resistant chronic unipolar depression. Controlled magnetic resonance imaging study. Br J Psychiatry 172:527-532.

Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC (1985) Measurement of protein using bicinchoninic acid. Anal Biochem 150:461-480.

Souness JE, Maslen C, Scott LC (1992) Effects of solubilization and vanadate/glutathione complex on inhibitor potencies against eosinophil cyclic AMP-specific phosphodiesterase. FEBS Lett 302:181-184.

Suda S, Nibuya M, Ishiguro T, Suda H (1998) Transcriptional and translational regulation of phosphodiesterase type IV isozymes in rat brain by electroconvulsive seizure and antidepressant drug treatment. J Neurochem 71:1554-1563.

Takahashi M, Terwilliger R, Lane C, Mezes PS, Conti M, Duman RS (1999) Chronic antidepressant administration increases the expression of cAMP-specific phosphodiesterase 4A and 4B isoforms. J Neurosci 19:610-618.

Tejani-Butt SM (1992) [^{3}H] Nisoxetine: a radioligand for quantitation of norepinephrine uptake sites by autoradiography or by homogenate binding. J Pharmacol Exp Ther 260:427-436.

Yarwood SJ, Steele MR, Scotland G, Houslay MD, Bolger GB (1999) The RACK1 signaling scaffold protein selectively interacts with the cAMP-specific phosphodiesterase PDE4D5 isoform. J Biol Chem 274:14909-14917.

Ye Y, O'Donnell JM (1996) Diminished noradrenergic stimulation reduces the activity of rolipram-sensitive, high-affinity cyclic AMP phosphodiesterase in rat cerebral cortex. J Neurochem 66:1894-1902.

Ye Y, Conti M, Houslay MD, Farooqui SM, Chen M, O'Donnell JM (1997) Noradrenergic activity differentially regulates the expression of rolipram-sensitive, high-affinity cyclic AMP phosphodiesterase (PDE4) in rat brain. J Neurochem 69:2397-2404.

Ye Y, Jackson K, O'Donnell JM (2000) Effects of repeated antidepressant treatment on type 4A phosphodiesterase (PDE4A) in rat brain. J Neurochem 74:1257-1262.

Zhang HT, Huang Y, Jin SL, Frith SA, Suvarna N, Conti M, O'Donnell JM (2002) Antidepressant-like profile and reduced sensitivity to rolipram in mice deficient in the PDE4D phosphodiesterase enzyme. Neuropsychopharmacology 27:587-595.

Zhao Y, Zhang HT, O'Donnell JM (2003) Inhibitor binding to type 4 phosphodiesterase (PDE4) assessed using [^{3}H]-piclamilast and [^{3}H]-rolipram. J Pharmacol Exp Ther 305:565-572.

Footnotes

a. This work was supported by research grants and an Independent Scientist Award from the National Institute of Mental Health.

b. Send reprint requests to:

Dr. James M. O'Donnell

Department of Pharmacology

University of Tennessee Health Science Center

874 Union Ave.

Memphis, TN 38163

Figure legends

Figure 1. Saturation curves for [^{3}H]-rolipram binding to rat cerebral cortical membranes. Rats were treated with desipramine or fluoxetine (10 mg/kg, i.p., twice daily) for 14 days, and killed 24 hours after the last injection. The saturation curves are representative of 4 experiments conducted in duplicate.

Figure 2. Dose-response of the effects of the antidepressants desipramine (DMI) and fluoxetine (FLU) on [^{3}H]-rolipram binding to rat hippocampal (A) and cerebral cortical (B) membranes. Rats were treated with desipramine or fluoxetine (1, 3 or 10 mg/kg, i.p., twice daily) for 14 days, and killed 24 hours after the last injection. Saturation curves were generated using rat cerebral cortical membranes and B_{max} values were calculated. Data are means ± SEM of [^{3}H]-rolipram binding expressed as a percentage of control [control values (fmol/mg protein): hippocampus, 31.2±9.8; cerebral cortex, B_{max} = 166.8±4.3] (n = 4 per group). Significantly different from saline-treated group, *$p<0.05$; **$p<0.01$.

Figure 3. Time-course of the effects of the antidepressants desipramine (DMI) and fluoxetine (FLU) on [^{3}H]-rolipram binding to rat hippocampal (A) and cerebral cortical (B) membranes. Rats were treated with desipramine or fluoxetine (10 mg/kg, i.p., twice daily) for 1, 7, or 14 days, and killed 24 hours after the last injection. Saturation curves were generated using rat cerebral cortical membranes and B_{max} values were calculated. Data are means ± SEM of [^{3}H]-rolipram binding expressed as a percentage of control [control values (fmol/mg protein): 1 day, hippocampus, 36.0±4.0, cerebral cortex, B_{max} = 327.1±63.3; 7 days, hippocampus, 19.2±1.9,

cerebral cortex, B_{max} = 310.0±36.1; 14 days, see legend to Fig. 2] (n = 4 per group). Significantly different from saline-treated group, *p<0.05; **p<0.01.

Figure 4. Dose-response of the effects of the antidepressants desipramine (DMI) and fluoxetine (FLU) on [^{3}H]-piclamilast binding to rat hippocampal (A, B) and cerebral cortical (C, D) membranes. Rats were treated with desipramine or fluoxetine (1, 3 or 10 mg/kg, i.p., twice daily) for 14 days, and killed 24 hours after the last injection. Rolipram inhibition of [^{3}H]-piclamilast binding was measured using both rat hippocampal and cerebral cortical membranes to determine the HARBS and the LARBS. Saturation curves were generated using rat cerebral cortical membranes and B_{max} values were calculated. Data are means ± SEM of [^{3}H]-piclamilast binding expressed as a percentage of control [control values (fmol/mg protein): HARBS, hippocampus, 85.8±6.8, cerebral cortex, B_{max} = 181.6±23.4; LARBS, hippocampus, 80.9±10.4, cerebral cortex, B_{max} = 115.6±8.4] (n = 4 per group). Significantly different from saline-treated group, *p<0.05; **p<0.01.

Figure 5. Time-course of the effects of the antidepressants desipramine (DMI) and fluoxetine (FLU) on [^{3}H]-piclamilast binding to rat hippocampal (A) and cerebral cortical (B) membranes. Rats were treated with desipramine or fluoxetine (10 mg/kg, i.p., twice daily) for 1, 7, or 14 days, and killed 24 hours after the last injection. Rolipram inhibition of [^{3}H]-piclamilast binding was measured using both rat hippocampal and cerebral cortical membranes to calculate the HARBS and the LARBS. Saturation curves were generated using rat cerebral cortical membranes and B_{max} values were calculated. Data are means ± SEM of [^{3}H]-piclamilast binding expressed as a percentage of control [control values (fmol/mg protein): 1 day, hippocampus, 85.0±8.5, cerebral

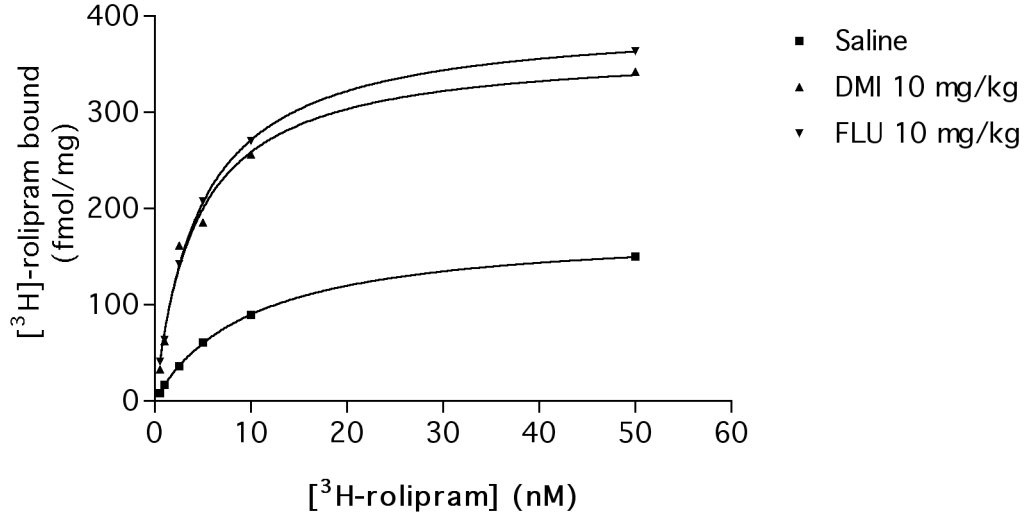
cortex, B_{max} = 195.3±18.5; 7 days, hippocampus, 109.9±27.6, cerebral cortex, B_{max} = 229.7±12.5; 14 days, see legend to Fig. 4] (n = 4 per group). Significantly different from saline-treated group, *p<0.05; **p<0.01.

Figure 6. 6-OHDA-induced noradrenergic lesions blocked the effects of desipramine (DMI), but not fluoxetine (FLU), on [^{3}H]-rolipram (A) and [^{3}H]-piclamilast (B) binding to rat hippocampal membranes. Rats received icv infusions of either vehicle or 300 μg 6-OHDA 20 days before the initiation of repeated desipramine or fluoxetine treatment (6 mg/kg, i.p., twice daily for 14 days). Rats were killed 24 hours after the last injection. Rolipram inhibition of [^{3}H]-piclamilast binding was measured using rat hippocampal membranes to calculate the HARBS and the LARBS. Data are means ± SEM of [^{3}H]-rolipram and [^{3}H]-piclamilast binding expressed as a percentage of control [control values (fmol/mg protein): [^{3}H]-rolipram, 31.5±6.1; [^{3}H]-piclamilast, HARBS, 72.3±4.9, LARBS: 41.5±3.6] (n = 4 - 6 per group). Significantly different from saline-treated group, *p<0.05.

Figure 7. The effects of 6-OHDA-induced noradrenergic lesions and 5,7-DHT-induced serotonergic lesions on [^{3}H]-nisoxetine (A) and [^{3}H]-citalopram (B) binding to rat hippocampal membranes. Rats received icv infusion of vehicle, 300 μg 6-OHDA, or 200 μg 5,7-DHT. Data are means ± SEM of [^{3}H]-nisoxetine and [^{3}H]-citalopram binding expressed as a percentage of control [control values (fmol/mg protein): [^{3}H]-nisoxetine, 22.8±1.5; [^{3}H]-citalopram, 21.0±4.3] (n = 4 - 6 per group). Significantly different from saline-treated group, **p<0.01.

Figure 8. 5,7-DHT-induced serotonergic lesions reduced the effect of fluoxetine (FLU), but not desipramine (DMI), on [^{3}H]-rolipram (A) and [^{3}H]-piclamilast (B) binding to rat hippocampal membranes. Rats received icv infusions of either vehicle or 200 μg 5,7-DHT 20 days before the initiation of repeated desipramine or fluoxetine treatment (6 mg/kg, i.p., twice daily for 14 days). Rats were killed 24 hours after the last injection. Rolipram inhibition of [^{3}H]-piclamilast binding was measured using rat hippocampal membranes to calculate the HARBS and the LARBS. Data are means ± SEM of [^{3}H]-rolipram and [^{3}H]-piclamilast binding expressed as a percentage of control [control values (fmol/mg protein): [^{3}H]-rolipram, 31.5±6.1; [^{3}H]-piclamilast, HARBS, 72.3±4.9, LARBS, 41.5±3.6] (n = 4 - 6 per group). Significantly different from saline-treated group, *$p<0.05$.

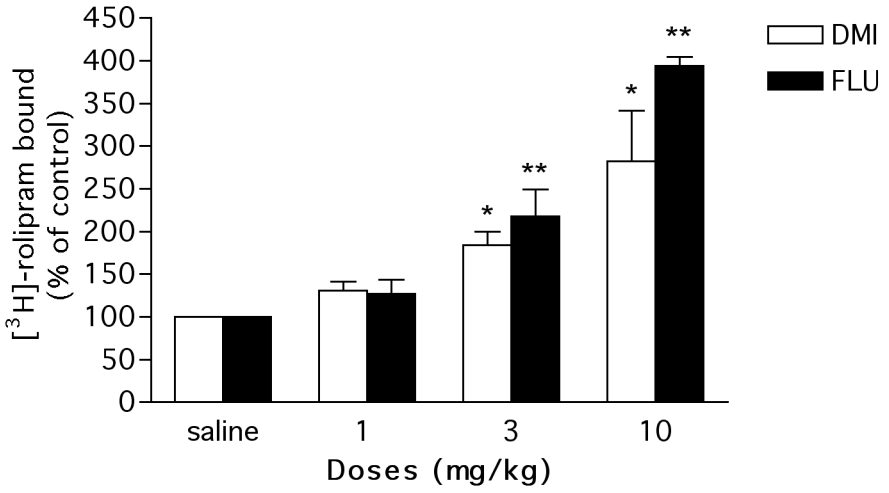
Figure 1



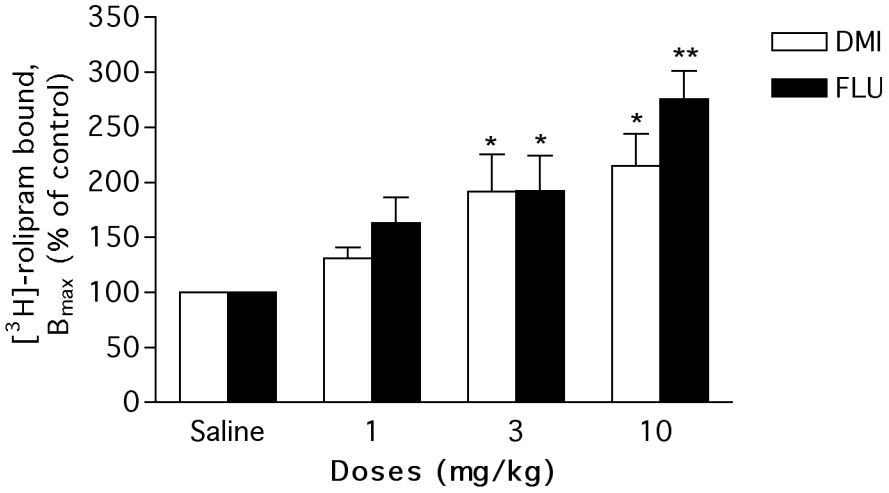
JPET Fast Forward. Published on September 3, 2003 as DOI: 10.1124/jpet.103.053215
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 2

A. Hippocampus, 14 days treatment



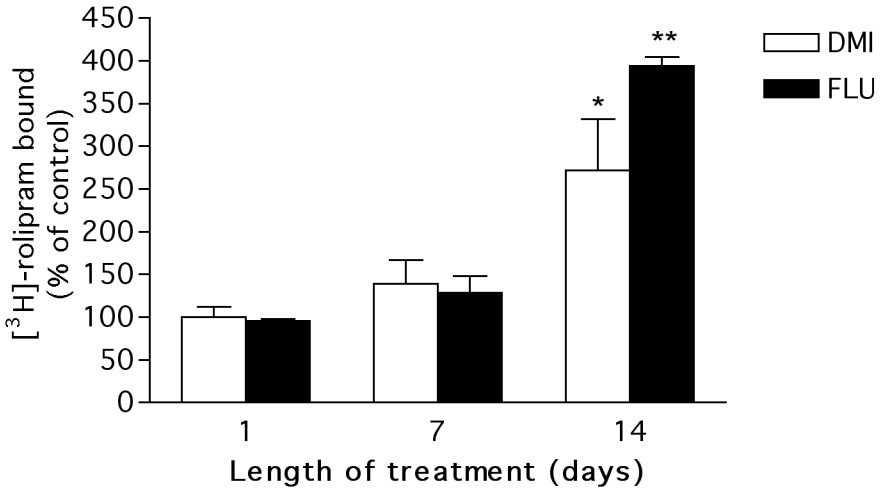
B. Cerebral cortex, 14 days treatment



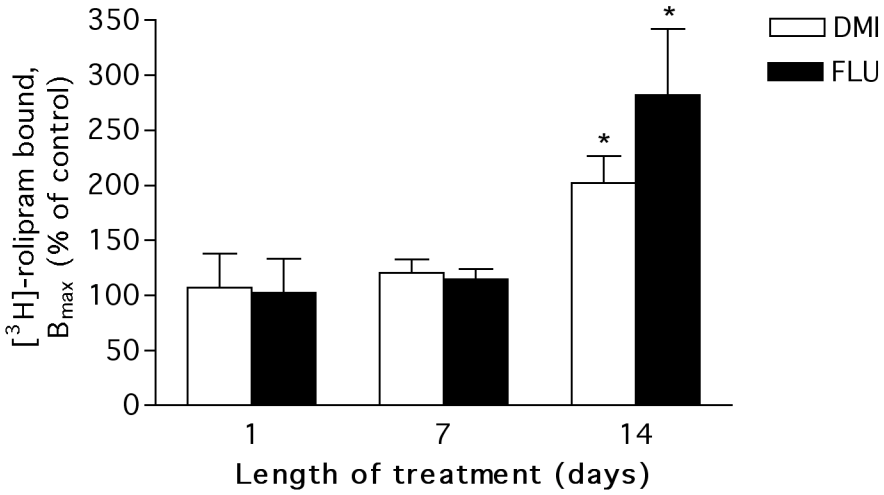
JPET Fast Forward. Published on September 3, 2003 as DOI: 10.1124/jpet.103.053215
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 3

A. Hippocampus, 10 mg/kg dose



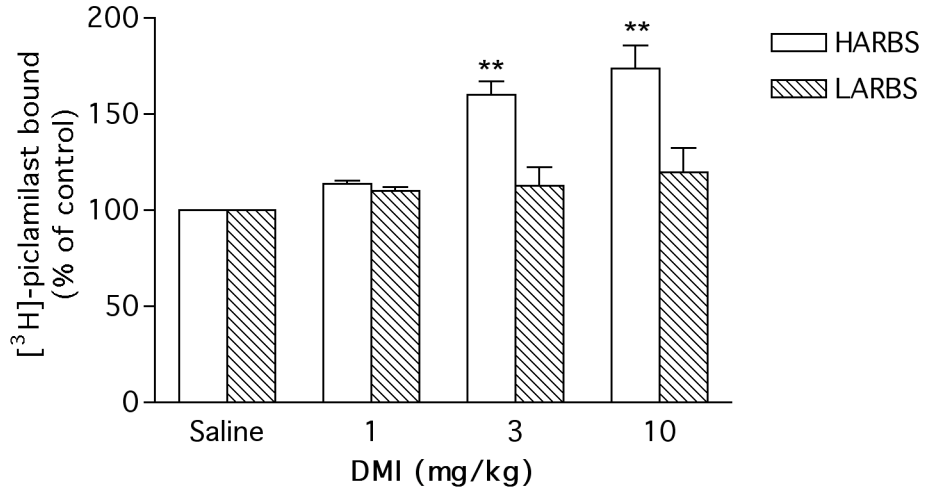
B. Cerebral cortex, 10 mg/kg dose



JPET Fast Forward. Published on September 3, 2003 as DOI: 10.1124/jpet.103.053215
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 4

A. Hippocampus, 14 days desipramine treatment



B. Hippocampus, 14 days fluoxetine treatment

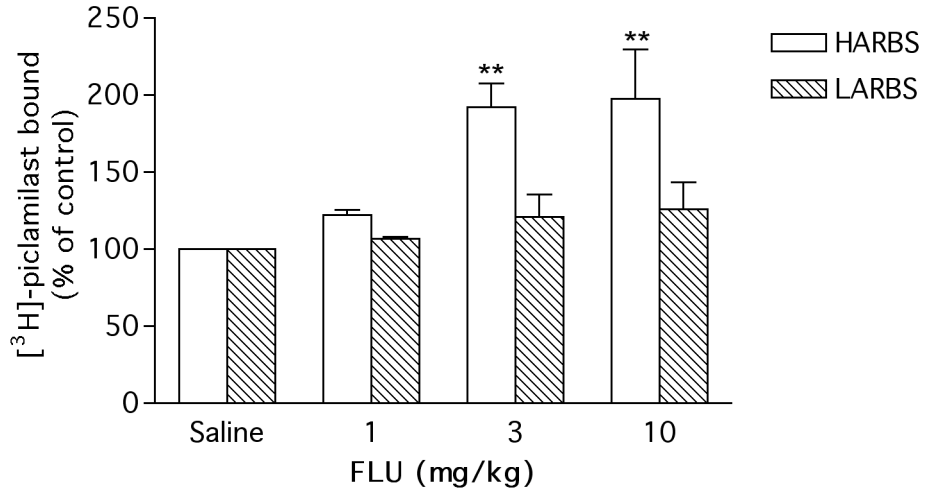
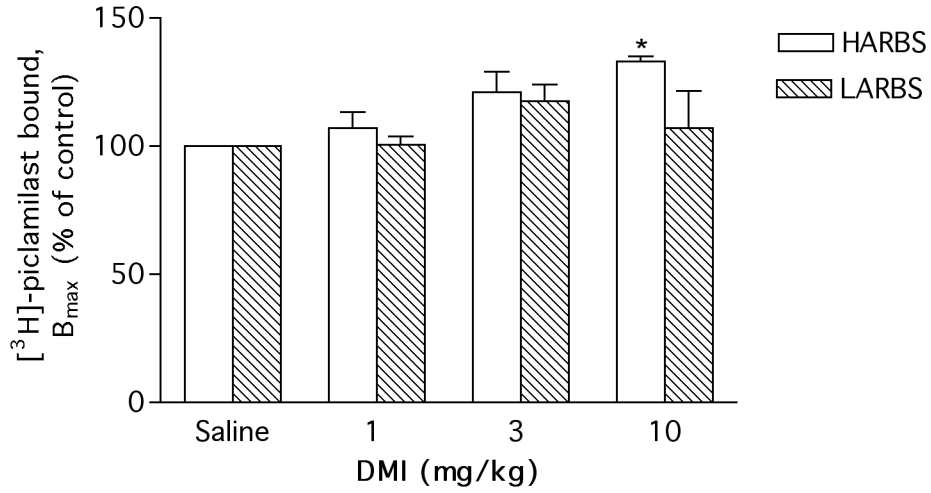
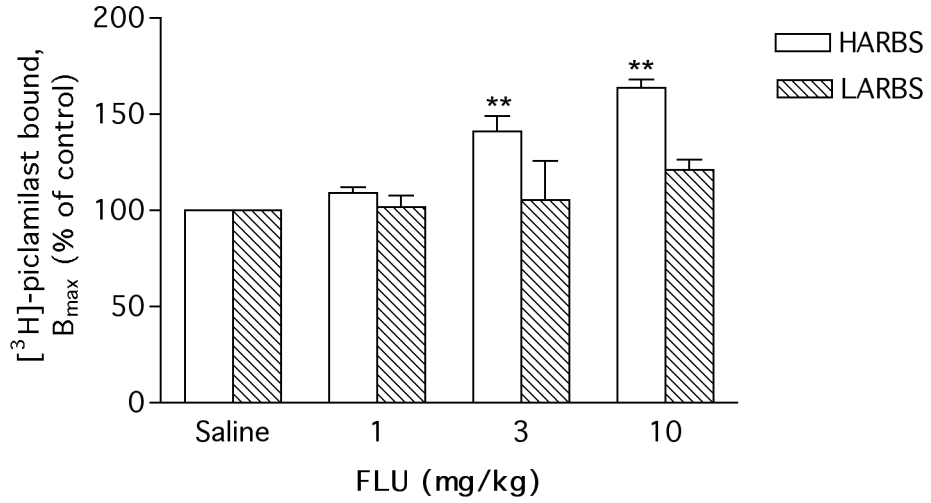


Figure 4 (continued)

C. Cerebral cortex, 14 days desipramine treatment



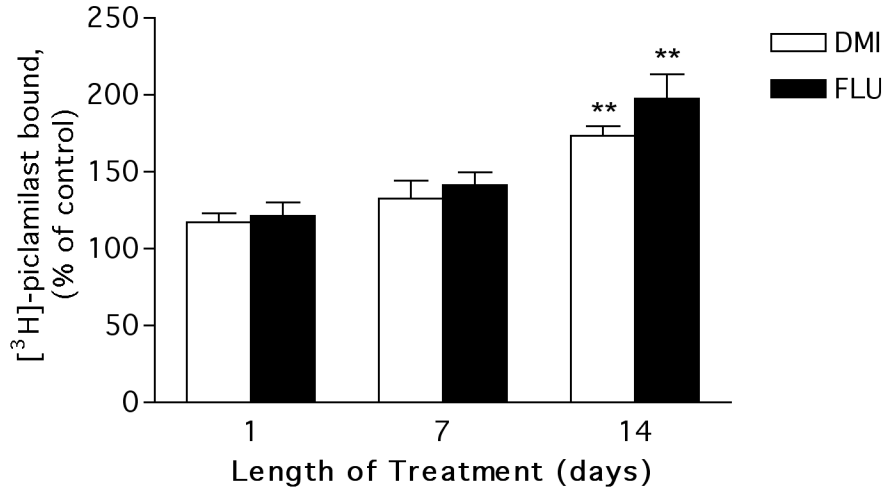
D. Cerebral cortex, 14 days fluoxetine treatment



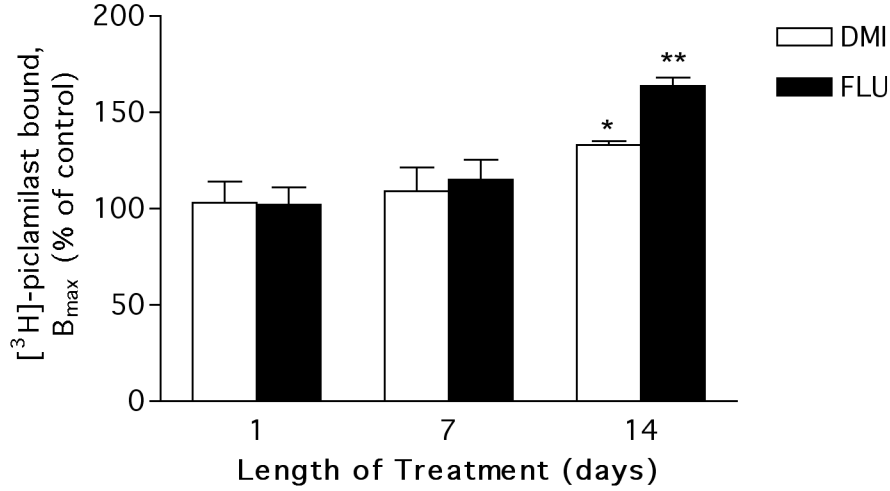
JPET Fast Forward. Published on September 3, 2003 as DOI: 10.1124/jpet.103.053215
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 5

A. Hippocampus, 10 mg/kg dose, HARBS



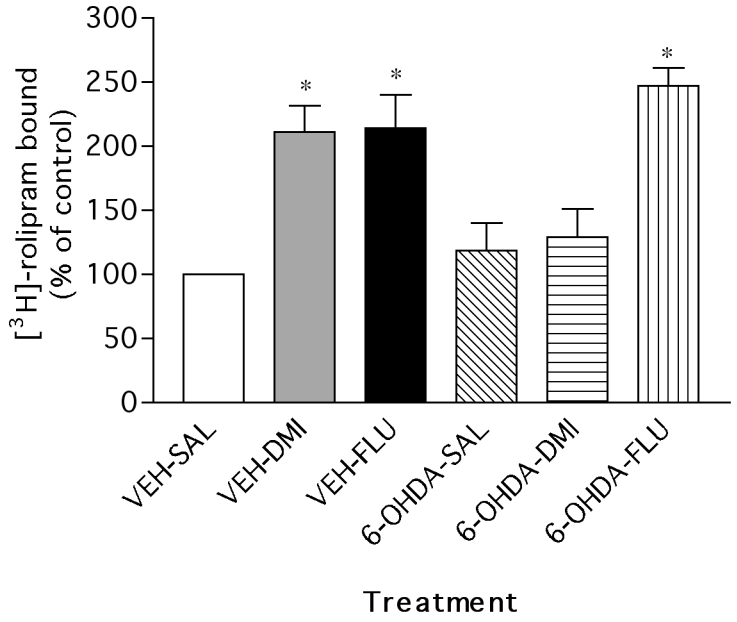
B. Cerebral cortex, 10 mg/kg dose, HARBS



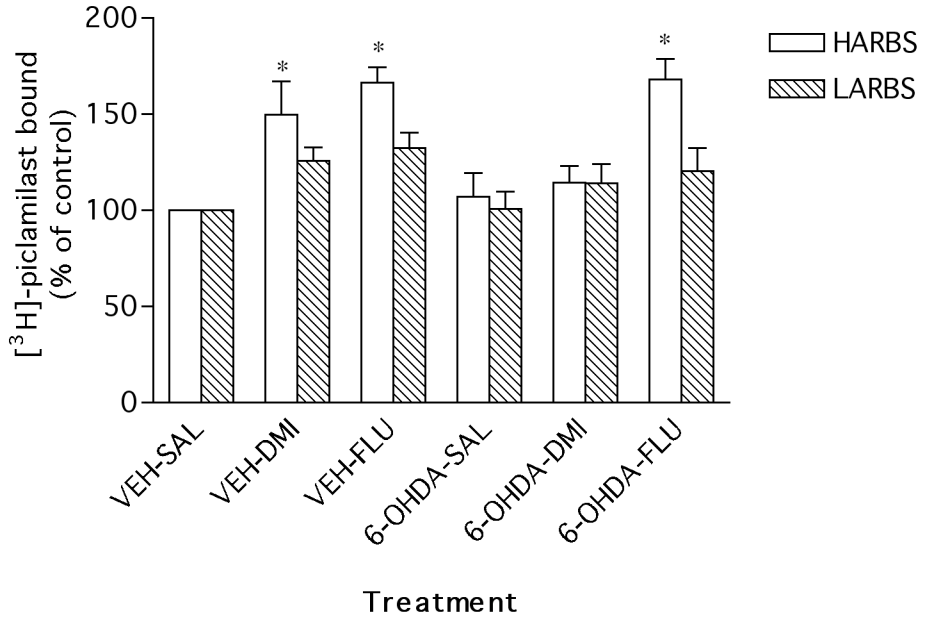
JPET Fast Forward. Published on September 3, 2003 as DOI: 10.1124/jpet.103.053215
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 6

A. [^{3}H]-Rolipram binding



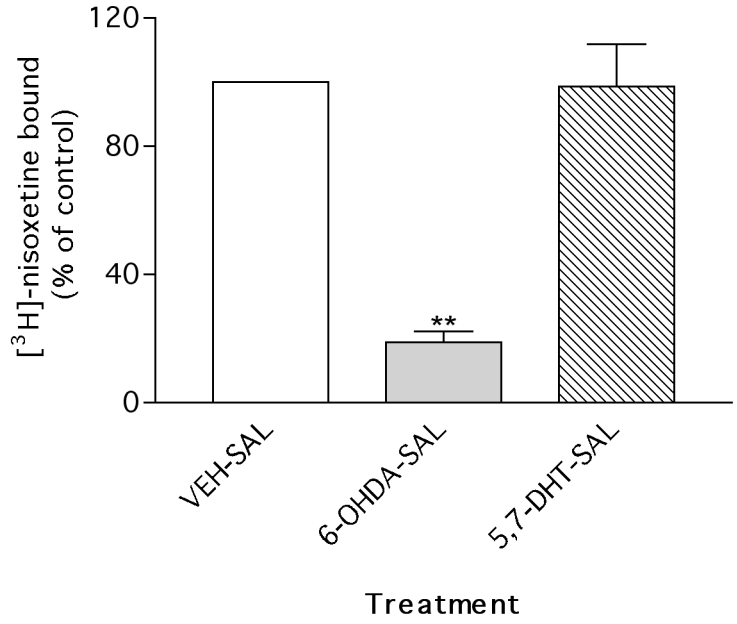
B. [^{3}H]-Piclamilast binding



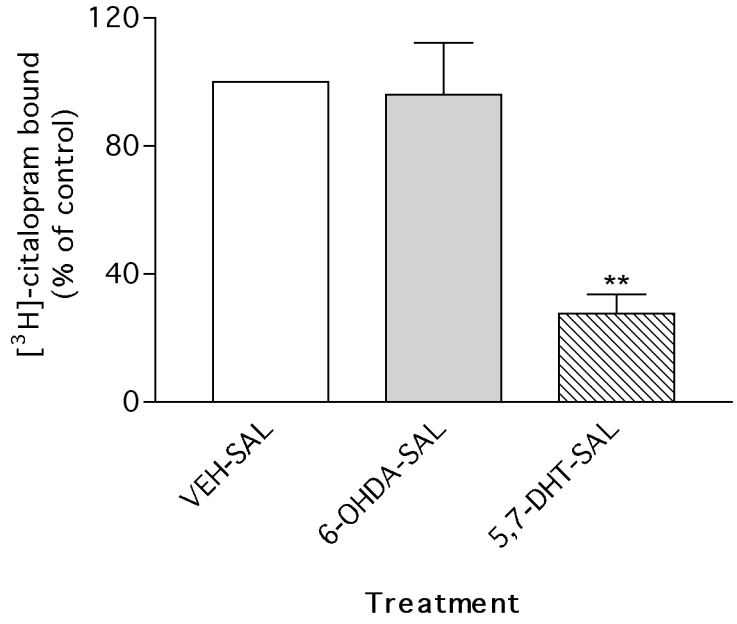
JPET Fast Forward. Published on September 3, 2003 as DOI: 10.1124/jpet.103.053215
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 7

A. [^{3}H]-Nisoxetine binding



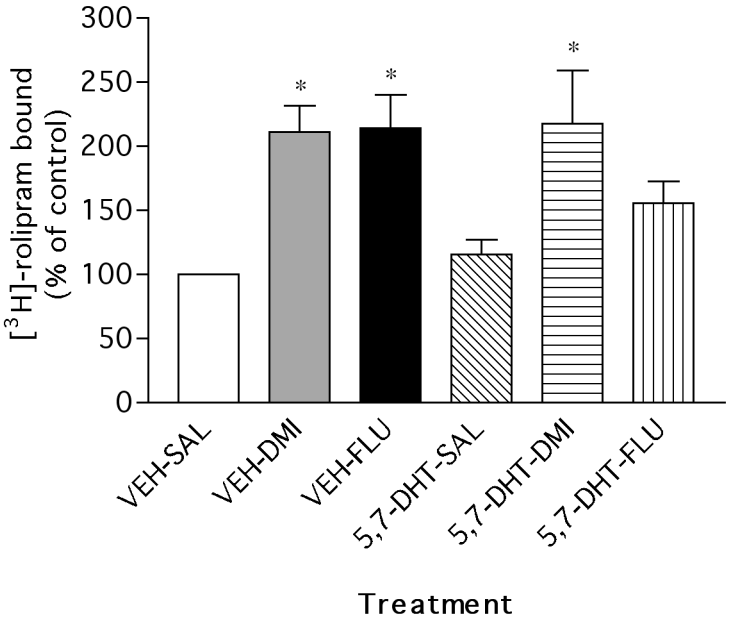
B. [^{3}H]-Citalopram binding



JPET Fast Forward. Published on September 3, 2003 as DOI: 10.1124/jpet.103.053215
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 8

A. [^{3}H]-Rolipram binding



B. [^{3}H]-Piclamilast binding