Antidepressant-Induced Increase in High-Affinity Rolipram Binding Sites in Rat Brain: Dependence on Noradrenergic and Serotonergic Function

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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. [3H]Rolipram and [3H]piclamilast were used to assess the high-affinity rolipram binding sites (HARBS) and low-affinity rolipram binding sites (LARBS) on PDE4 in the hippocampus and cerebral cortex. Repeated, but not acute, treatment with the antidepressants increased [3H]rolipram binding to membrane fractions in a dose-dependent manner; the HARBS component of [3H]piclamilast binding also was increased by these treatments. By contrast, the LARBS component of [3H]piclamilast binding was not altered. [3H]Rolipram and [3H]piclamilast binding to the cytosolic fractions of rat cerebral cortex and hippocampus was not altered by the antidepressant treatments. 6-Hydroxydopamine (6-OHDA; 300 μg i.c.v.) and 5,7-dihydroxytryptamine (5,7-DHT; 200 μg i.c.v.) were used to lesion noradrenergic and serotonergic neurons, respectively. The effects of desipramine, but not fluoxetine, on [3H]rolipram and [3H]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 β-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 coworkers 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 β-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

ABBREVIATIONS: CREB, cyclic AMP response element-binding protein; PDE4, type 4 cyclic AMP phosphodiesterase; HARBS, high-affinity rolipram binding site; LARBS, low-affinity rolipram binding site; 6-OHDA, 6-hydroxydopamine; 5,7-dihydroxytryptamine; DMI, desipramine; FLU, fluoxetine; 5,7-DHT, PKA, protein kinase A.
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) (Snieder et al., 1986; Jacobitz et al., 1996); 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 (Schmichen et al., 1990; Barnette et al., 1995; Duplantier et al., 1996). 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 antidepressants 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 [3H]piclamilast and [3H]rolipram (Zhao et al., 2003). 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) were housed in a temperature- (22–24°C) and light- (on 6:00 AM–6:00 PM) controlled room and were allowed free access 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. Cannulas were implanted bilaterally into the cerebral ventricle (0.5 mm posterior to bregma, ±1.6 mm lateral to the midline, and 3.9 mm ventral to the dura; O’Donnell et al., 1994). Rats were allowed at least 5 days to recover before use in experiments.

**Central Noradrenergic Lesions.** Noradrenergic lesions were produced by bilateral i.c.v. 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 [3H]nisoxetine binding (Tejani-Butt, 1992).

**Central Serotonergic Lesions.** Serotonergic lesions were produced by bilateral i.c.v. 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 i.c.v. infusion of 5,7-DHT (Breeze and Traylor, 1971). The serotonergic lesions were verified by measurement of serotonin uptake sites using [3H]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 i.c.v. infusion of 6-OHDA or 5,7-DHT.

**Radioligand Binding Assays.** Rats were killed by decapitation 24 h after the last injection. The cerebral cortex and hippocampus were dissected on ice and homogenized in incubation buffer (50 mM Tris, 120 mM NaCl, 5 mM KCl, pH 7.4) using a Polytron homogenizer. Samples were centrifuged at 15,000g for 15 min; the pellets were resuspended in incubation buffer.

[3H]Rolipram and [3H]piclamilast binding were measured as described previously (Zhao et al., 2003). Membrane or cytosolic preparations containing 200 to 300 μg of protein were incubated at 30°C in 250 μl of incubation buffer containing 2 nM [3H]rolipram or [3H]piclamilast. Nonspecific binding was determined in the presence of 10 μM unlabeled Ro 20-1724 [4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone] for [3H]rolipram binding or 1 mM unlabeled rolipram for [3H]piclamilast binding. For the saturation binding studies, different concentrations of [3H]rolipram (0.5–50 nM) and [3H]piclamilast (0.01–20 nM) were used. The saturation curves were determined by using only preparations of cerebral cortex. For calculating the percentage of the HARBS and the LARBS fractions, rolipram competition of [3H]piclamilast binding was determined. For the competition assay, a 2 nM concentration of [3H]piclamilast was used in the presence of different concentrations of unlabeled rolipram (Zhao et al., 2003).

For [3H]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 of protein) were incubated in 250 μl of incubation buffer (50 mM Tris, 300 mM NaCl, 5 mM KCl, pH 7.4) containing 5 nM [3H]nisoxetine for 4 h at 0°C. Nonspecific binding was determined using 25 μM desipramine.

For [3H]citalopram binding, which provides 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 of protein) were incubated in 250 μl of the same buffer containing 1 nM [3H]citalopram for 60 min 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 $B = B_{max}'(1 + C/I_{C50}') + B_{max}''(1 + C/I_{C50}'')$, where $B$ is equal to the amount of radioligand bound; $B_{max}'$ and $B_{max}''$ are the percentage of competitor binding to high- and low-affinity sites, respectively; $C$ is the competitor concentration; and $I_{C50}'$ and $I_{C50}''$ are the $IC_{50}$ values for the high- and low-affinity sites, respectively. For the one-site model, $B_{max}$ equaled zero and the equation reduced to $B = B_{max}'(1 + C/I_{C50}')$. The percentages of the HARBS and the LARBS were calculated from rolipram competition curves for inhibition of $[^3H]$piclamilast binding by nonlinear regression using a two-site model. All values are expressed as means $\pm$ S.E.M. from at least four independent experiments carried out in duplicate. Differences between the treatment and control groups were analyzed using one-way analysis of variance followed by Dunnett’s test.

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

**Results**

Dose- and Time-Dependent Effects of Antidepressant Treatments on $[^3H]$Rolipram Binding to Rat Hippocampus and Cerebral Cortex. $[^3H]$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. $[^3H]$Rolipram binding was increased for both hippocampal and cerebral cortical membranes after 14 days of treatment with either antidepressant (Figs. 1 and 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 $[^3H]$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 $[^3H]$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 $[^3H]$rolipram binding (Fig. 2); the $K_d$ values were not altered by the treatments. Antidepressant treatments did not alter $[^3H]$rolipram binding to cytosolic fractions of hippocampus or cerebral cortex (data not shown).

Dose- and Time-Dependent Effects of Antidepressant Treatments on $[^3H]$Piclamilast Binding to Rat Hippocampus and Cerebral Cortex. $[^3H]$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 $[^3H]$piclamilast binding was performed to calculate the percentage of the HARBS and the LARBS composing the total binding. Of the total $[^3H]$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 $[^3H]$rolipram binding, the effects of desipramine and fluoxetine treatment on $[^3H]$piclamilast binding were time-dependent. Repeated, but not acute, treatment with either antidepressant increased $[^3H]$piclamilast binding (Figs. 4 and 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 and 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 $[^3H]$rolipram binding, the antidepressant treatments did not alter $[^3H]$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 $[^3H]$Rolipram and $[^3H]$Piclamilast Binding to Rat Hippocampal Membranes. $[^3H]$Rolipram binding to hippocampal membranes from control rats was increased by desipramine or fluoxetine treatment (Fig. 6). For $[^3H]$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 $[^3H]$rolipram and the HARBS component of $[^3H]$piclamilast binding was not affected by the noradrenergic lesions. By contrast, desipramine treatment did not increase $[^3H]$rolipram binding or the HARBS component of $[^3H]$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 $[^3H]$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 $[^3H]$Rolipram and $[^3H]$Piclamilast Binding to Rat Hippocampal Membranes. $[^3H]$Rolipram binding to hippocampal membranes from control rats was increased by desipramine or fluoxetine treatment (Fig. 8). For $[^3H]$piclamilast binding, the HARBS component was increased by desipramine or fluoxetine treat-
Antidepressant Effects on Inhibitor Binding to PDE4

A. Hippocampus, 14 days treatment

B. Cerebral cortex, 14 days treatment

Fig. 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 h after the last injection. Saturation curves were generated using rat cerebral cortical membranes and \( B_{\text{max}} \) values were calculated. Data are means ± S.E.M. of \( ^{3}H \)rolipram binding expressed as a percentage of control [control values (fmol/mg protein): hippocampus, 31.2 ± 9.8; cerebral cortex, \( B_{\text{max}} = 166.8 ± 4.3 \) (n = 4 per group). Significantly different from the saline-treated group. *, p < 0.05; **, p < 0.01.

A. Hippocampus, 10 mg/kg dose

B. Cerebral cortex, 10 mg/kg dose

Fig. 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 h after the last injection. Saturation curves were generated using rat cerebral cortical membranes and \( B_{\text{max}} \) values were calculated. Data are means ± S.E.M. 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_{\text{max}} = 327.1 ± 63.3; 7 \text{ days, hippocampus, } 19.2 ± 1.9, \text{ cerebral cortex, } \text{ } B_{\text{max}} = 310.0 ± 36.1; 14 \text{ days, see legend to Fig. 2} \] (n = 4 per group). Significantly different from saline-treated group. *, p < 0.05; **, p < 0.01.

Antidepressants; 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; Ozawa et al., 1991; Nibuya et al., 1996; 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). Post-traumatic 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 coworkers (1998) reported a correlation between hippocampal atrophy and impaired verbal learning in depressed patients. The cerebral cortex, especially the 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 the 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 coworkers (Schmiechen et al., 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 $[^3H]$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 $[^3H]$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-
A. \( ^{3}H \)-Rolipram binding

![Graph showing \( ^{3}H \)-Rolipram binding](image)

Fig. 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 i.c.v. infusions of either vehicle or 200 \( \mu \)g of 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 h 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 ± S.E.M. 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.

B. \( ^{3}H \)-Piclamilast binding

![Graph showing \( ^{3}H \)-Piclamilast binding](image)

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 ecosinophil 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 (Sette and Conti, 1996; Hoffmann et al., 1998). 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 termini. 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 (Dalgarino 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 compose 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 leads to an alteration of PDE4 expression and an increase in...
Antidepressant Effects on Inhibitor Binding to PDE4


and may represent another component of cAMP-mediated 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.

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