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Antidepressant effects of inhibitors of cAMP phosphodiesterase (PDE4)

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Despite initial promise, the development of type 4 phosphodiesterase (PDE4) inhibitors as antidepressants has not advanced significantly. This is due to an incomplete understanding of the functional importance of PDE4 subtypes and high-affinity and low-affinity inhibitor-binding conformers. However, recent developments have rekindled interest in the therapeutic potential of PDE4 inhibitors. First, PDE4 has been shown to be involved in cAMP signaling pathways that are affected by antidepressants. Second, data obtained using mouse knockout lines indicate that PDE4D and PDE4B mediate antidepressant effects. Third, it appears that the interaction of inhibitors with the high-affinity binding conformer of PDE4 is particularly important for antidepressant efficacy. These developments highlight the difficulties of dissociating the actions of PDE4 inhibitors and provide a guide for future research.

Research by Helmut Wachtel at Schering AG Pharmaceuticals over 20 years ago demonstrated that the then-novel compound rolipram, as well as other inhibitors of cAMP phosphodiesterase (PDE), have CNS activity [1]. Subsequently, it was demonstrated that its pattern of effects is indicative of antidepressant efficacy [2], which was borne out later in a clinical trial [3]. Examination of the neuropharmacological actions of rolipram showed that it is a potent, highly selective inhibitor of type 4 PDE (PDE4), one of the 11-member superfamily of cyclic nucleotide PDEs [4]. These findings led to the suggestion that inhibitors of PDE4 might represent a novel class of antidepressant drugs, the potential of which is also being investigated for the treatment of inflammatory and immunological disorders [5,6]. Several factors have prevented the realization of the initial promise of therapeutic utility in depression. These include the lack of highly selective inhibitors of the four PDE4 subtypes, little knowledge of the involvement of each subtype in the signaling pathways that are involved in mediating antidepressant effects, and an incomplete understanding of the nature and consequences of two distinct PDE4 conformers with which inhibitors interact, termed the high-affinity and low-affinity rolipram-binding sites (HARBS and LARBS, respectively) [7]. However, recent developments have rekindled interest in the potential of PDE4 inhibitors as antidepressant agents.

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The PDE superfamily and PDE4

PDE enzymes comprise an eleven-family group (PDE1–PDE11); there are multiple isoforms in each family, caused by multiple genes and alternative splicing [8,9]. The PDE families differ in their primary structure, ability to hydrolyze cAMP and cGMP, tissue and intracellular distribution, and sensitivity to modulators (e.g. Ca^{2+}, calmodulin and cGMP) and pharmacological inhibitors.

The PDE4 enzyme family, which also is referred to as the low K_m, cAMP-selective PDE and the rolipram-sensitive PDE, consists of four, independently coded subtypes (PDE4A–PDE4D) [10]. Of these, PDE4A, PDE4B and PDE4D are widely, but differentially, expressed throughout the brain, whereas the PDE4C subtype is expressed only minimally. Importantly, PDE4 is present in brain regions that are thought to be involved in reward and affect [11,12].

The PDE4 enzymes have been divided into three groups: the long form, short form and super-short form (Figure 1) [10]. The long-form PDE4s, consist of a subtype-specific C-terminus, a catalytic region that is highly conserved across subtypes and two conserved regions in the N-terminus, termed the upstream conserved region 1 (UCR1) and UCR2. Differential splicing results in distinct N-termini for the PDE4 enzymes; for example, the N-terminal region of PDE4D4 is distinct from that of PDE4D5. The N-terminal region of long-form PDE4s contains a conserved phosphorylation site for protein kinase A, which is involved in regulation of hydrolytic activity, as well as domains involved in protein-protein interactions. The short-form PDE4s and super-short-form PDE4s lack the UCR1 and the UCR1 plus a portion of the UCR2, respectively. The catalytic region of several PDE4 enzymes contains a phosphorylation site for extracellular-signal-regulated kinase that regulates hydrolytic activity in a variant-specific manner [13].

Antidepressant effects of PDE4 inhibitors

Rolipram and other PDE4 inhibitors [e.g. Ro201724 (see Chemical names) and ICI63197] produce antidepressant-like effects in several preclinical models. They reduce the

Chemical names

ICI63197: 2-amino-6-methyl-4-propyl-[1,2,4]triazolo[1,5-*a*]pyrimidin-5(4*H*)-one
Ro201724: 4-[(3-butoxy-4-methoxyphenyl)-methyl]-2-imidazolidinone

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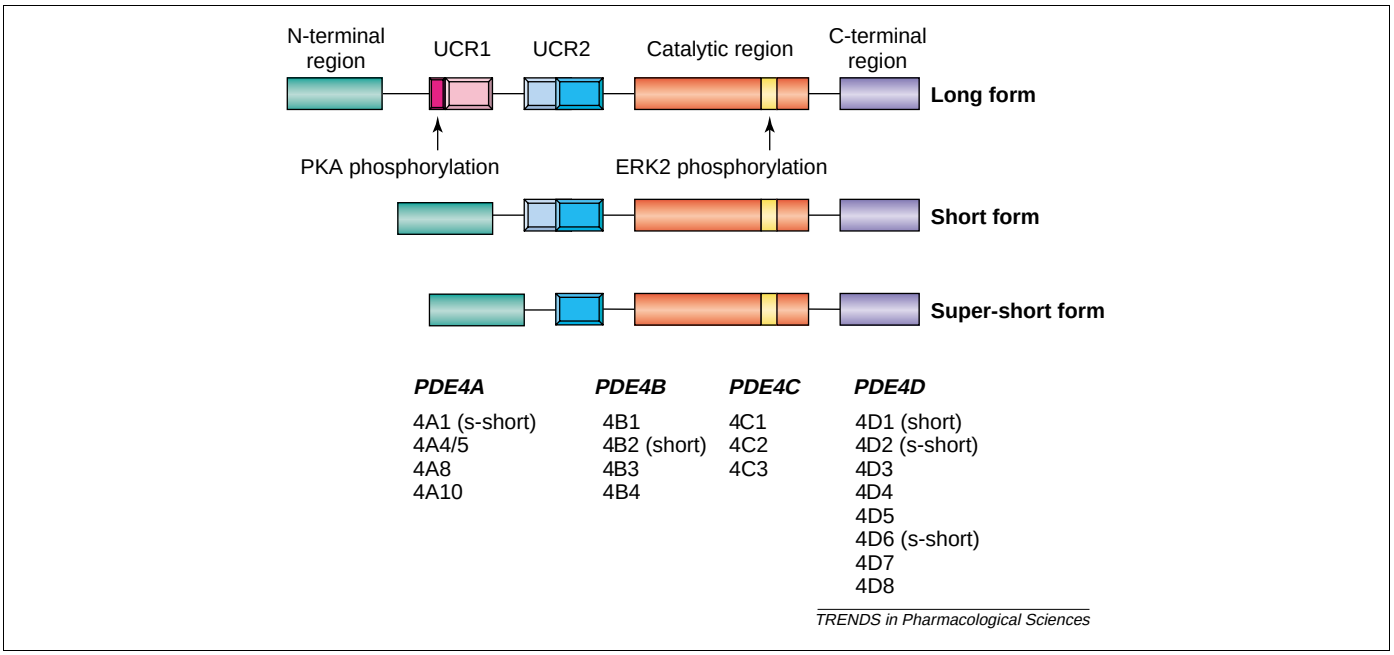

Figure 1. Type 4 phosphodiesterase (PDE4) enzymes are encoded by four genes, *PDE4A, PDE4B, PDE4C* and *PDE4D*, 19 variants of which are expressed by alternative splicing. Based on their primary structure, these variants are classified as long-form, short-form and super-short-form (s-short) PDE4s. Each PDE4 subtype has a unique C-terminus. Alternative splicing at the N-terminus results in unique N-terminal regions for each splice variant of PDE4. The N-terminus contains the upstream conserved region 1 (UCR1) and UCR2, and sites for phosphorylation and interactions with other proteins. The C-terminus contains an extracellular signal-regulated kinase (ERK2) phosphorylation site, which is located in the catalytic C-terminal regions.

time of immobility in the forced-swim test, decrease response rate and increase reinforcement rate under a differential-reinforcement-of-low-rate schedule, reverse the effects of chronic, mild stress, normalize the behavioral deficits observed in Flinders sensitive-line and olfactory-bulbecomized rats, antagonize the effects of reserpine, and potentiate yohimbine-induced toxicity [2,14–17]. These effects are typical of antidepressant drugs. PDE4 also appears to be involved in the CREB (cAMP response element-binding protein)-mediated induction of neurogenesis in the hippocampus [18] and the ability of antidepressant drugs to induce neurogenesis appears to be important in mediating late-developing effects on behavior [19].

The results of clinical studies also indicate that PDE4 inhibitors have antidepressant efficacy, although only a small number of compounds have been evaluated [20–22]. It is clear from these studies that the side-effect profile of rolipram, notably its emetic and sedative actions, limits its clinical utility. This has led to attempts to dissociate the antidepressant effects of rolipram from the more troublesome side-effects, which appear to be, at least in part, mediated centrally [23,24]. This effort has focused on the subtypes (PDE4A, PDE4B and PDE4D) and the binding conformers of PDE4 (i.e. the HARBS and LARBS).

PDE4 in antidepressant-sensitive signaling pathways

In general, clinically used antidepressants enhance noradrenaline-mediated and serotonin (5-HT)-mediated neurotransmission, either by inhibiting reuptake catabolism or by blocking inhibitory, presynaptic α-adrenoceptors (either autoreceptors or heteroreceptors) [25]. Thus, it was of interest to determine whether PDE4 is involved in signaling mechanisms that are associated with these two neurotransmitters (Figure 2). PDE4 was found to be either the predominant or exclusive PDE that mediates the hydrolysis of cAMP formed by stimulation of β-adrenoceptors in rat cerebral cortex [26]. Furthermore, it appears that PDE4 is regulated via changes in noradrenaline-mediated activity. Using 6-hydroxydopamine to induce noradrenergic lesions reduces both PDE4 activity and the expression of PDE4A and PDE4B subtypes. By contrast, enhancing noradrenaline-mediated activity by repeated administration of the reuptake inhibitor desipramine markedly increases the expression of these two PDE4 subtypes [27]. In addition, PDE4D regulates phosphorylation of the β-adrenoceptor by interacting with β arrestin [28,29]. Overall, these data indicate that PDE4 is a regulated component of cAMP signaling mediated by β-adrenoceptors. Inhibition of PDE4 might produce antidepressant effects in part by altering noradrenaline-mediated neurotransmission.

Less is known of the involvement of PDE4 in 5-HT-mediated neurotransmission. However, some 5-HT-receptor subtypes are coupled positively to adenylyl cyclase, and so it is possible that PDE4 inhibitors enhance aspects of 5-HT-mediated neurotransmission that involve cAMP. Consistent with this suggestion, repeated treatment with an inhibitor of 5-HT reuptake such as fluoxetine increases the expression of PDE4A and PDE4B in rat cerebral cortex and hippocampus [30–32]. It is unclear whether the antidepressant-induced increase in the expression of PDE4A and PDE4B, but not PDE4D, indicates that the two former subtypes are more involved in the signaling pathways that mediate the effects of antidepressants or whether the expression of PDE4D in the brain is less susceptible to regulation. PDE4D activity is particularly affected by phosphorylation, which indicates that this, rather than altered expression, might be its primary mode

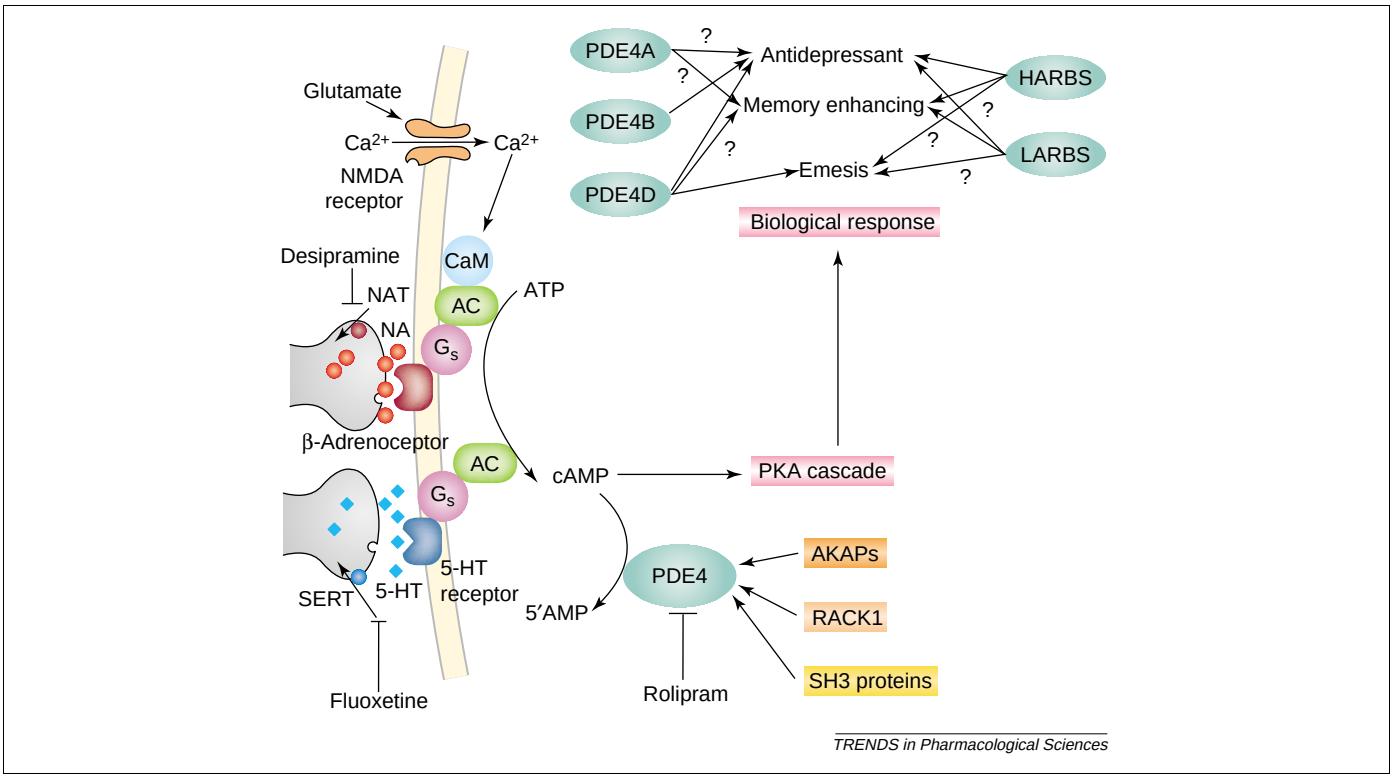

Figure 2. Type 4 phosphodiesterase (PDE4) is a component of β-adrenoceptor-mediated and NMDA-receptor-mediated signaling, and might also be involved in 5-HT-receptor-mediated signaling. Both the intracellular localization and the function of PDE4 are affected by its interaction with other proteins, including receptors for activated C kinases 1 (RACK1) and A-kinase-anchoring proteins (AKAPs), and proteins that contain SH3 domains. Inhibition of PDE4 increases cAMP-mediated signaling in these pathways, an effect that is similar to that expected following administration of the antidepressants desipramine and fluoxetine, which inhibit noradrenaline transporters (NATs) and 5-HT transporters (SERTs), respectively, and following stimulation of NMDA receptors. Some actions of PDE4 inhibitors, such as antidepressant and memory-enhancing effects, might be mediated via these signaling pathways and involve specific PDE4 subtypes and binding conformers, such as the high-affinity rolipram-binding sites (HARBS) and low-affinity rolipram-binding sites (LARBS). Abbreviations: AC, adenylyl cyclase; CaM, calmodulin; PKA, protein kinase A.

of regulation in the brain [33,34]. However, it should be noted that PDE4D has been shown to be regulated at the expression level in several cell types [35,36].

A third pathway that is implicated in antidepressant actions and involves PDE4 is NMDA-receptor-mediated signaling (Figure 2). The role of NMDA receptors in mediating antidepressant effects is not well understood. NMDA antagonists such as MK801 (dizocilpine) are reported to produce antidepressant-like effects on behavior [37,38]; however, this interpretation has been questioned [39]. Also, rolipram has been shown to reverse memory deficits that result from dizocilpine treatment, which indicates that the role of PDE4 in NMDA-receptor-mediated signaling might be related more to cognitive deficits that occur in depression [40]. Regardless of the exact nature of the functional role of PDE4 in NMDA receptor-mediated signaling, it does seem to be an important factor. Stimulation of NMDA receptors in primary cultures of rat cerebral cortical neurons increases intracellular concentrations of cAMP and cGMP. These neurons express enzymes from different PDE families, but the cAMP formed by NMDA-receptor stimulation is hydrolyzed exclusively by PDE4; the cGMP that forms is hydrolyzed by PDE2 only [41]. At present, it is not known which PDE4 subtypes are components of NMDA-receptor-mediated signaling in neurons.

PDE4 subtypes in the mediation of antidepressant effects

As yet, no highly selective inhibitors of the PDE4 subtypes have been developed; available compounds are only about 10-fold selective, which limits their utility for studies *in vivo*. The most-studied inhibitors, such as rolipram and Ro201724, are equipotent at inhibiting the four PDE4 subtypes. Thus, it is necessary to examine the behavioral phenotype and pharmacological sensitivity of mouse lines that are deficient in a particular subtype to assess the relative roles of the PDE4 subtypes in the mediation of antidepressant effects. At present, constitutive, PDE4B-knockout and PDE4D-knockout lines have been established [42].

PDE4D-deficient mice exhibit an antidepressant-like profile in the forced-swim and tail-suspension tests [43], evidenced by reduced immobility relative to wild-type controls. This reduced immobility is similar to that observed if the mice are administered proven antidepressants or the putative antidepressant rolipram. A further reduction in immobility in the forced-swim test is observed when the antidepressants desipramine and fluoxetine are administered to PDE4D-deficient mice. By contrast, rolipram causes, at best, a minimal, additional reduction in the time of immobility in PDE4D-knockout mice. This reduced effect of rolipram indicates that its actions are mediated to a significant degree by the PDE4D subtype.

Consistent with this, the ability of rolipram to enhance β-adrenoceptor-mediated cAMP formation in the cerebral cortex also is diminished in PDE4D-deficient mice [43]. Interpretation of the effect of desipramine and fluoxetine is less obvious. Although, it might mean that PDE4D is not involved in their actions, this might not be the case. If the monoamine-uptake inhibitors produce their effects by increasing receptor-mediated cAMP formation, then loss of the PDE4 subtype in the affected pathway would either enhance the effects of desipramine and fluoxetine or increase the potency of these drugs. At present, pharmacological analyses sufficient to detect a change in sensitivity have not been carried out.

Much less is known about the role of the PDE4B subtype in the mediation of antidepressant effects. Preliminary results indicate that PDE4B-deficient mice also exhibit an antidepressant-like profile in the forced-swim test and rolipram causes no significant, additional reduction in immobility (H-T. Zhang and J.M. O'Donnell, unpublished). However, unlike PDE4D-deficient mice, desipramine produces no further antidepressant-like effects in PDE4B-deficient mice. This indicates an important difference between the roles of PDE4B and PDE4D in signaling pathways affected by antidepressant drugs. It remains to be determined whether this difference is observed with 5-HT-reuptake inhibitors.

Although the subtype involved in mediating the antidepressant-like effects of PDE4 inhibitors is by no means resolved, particularly because PDE4A-deficent mice have not been examined, results obtained to date do indicate an important role for the PDE4D subtype. This raises two issues. First, it contrasts with data obtained in rats, which shows that repeated treatment with antidepressants from different pharmacological classes increases the expression of PDE4A and PDE4B but not PDE4D [30,31]. Second, it is important to know to what degree the side-effects of emesis and sedation are related to inhibition of PDE4D. The first issue indicates either a species difference (i.e. that rats and mice utilize different PDE4 subtypes in particular signaling pathways) or that PDE4D might be regulated differently to PDE4A and PDE4B (e.g. by phosphorylation rather than altered expression). The second issue is somewhat more troubling. PDE4D appears to be expressed highly in the area postrema, an emetic-trigger zone [44], but its relative contribution to overall hydrolysis of cAMP in this region is not known. However, data obtained using a surrogate model that appears to be related to emetic potential, also indicate a role for PDE4D [45]. Obviously, this issue needs to be clarified if we hope to dissociate the antidepressant and emetic effects of PDE4 inhibitors.

Inhibitor binding to conformers of PDE4

Another level of complexity in understanding the actions of PDE4 inhibitors concerns the high-affinity and low-affinity binding conformers (the HARBS and LARBS). The first indication that rolipram binding might be somewhat complex was the finding that whereas [^{3}H]-rolipram binds with high affinity to brain membranes ($K_i = 1–10$ nM), little high-affinity binding is detected in preparations of peripheral organs [46]. A systematic analysis of binding using a technique that assesses both high-affinity and low-affinity interactions to recombinant PDE4A, showed that [^{3}H]-rolipram binds with two distinct affinity states that differ ~500-fold in their apparent K_i values [7]. Both high-affinity and low-affinity interactions require the catalytic site; the N-terminal region of PDE4 stabilizes the high-affinity component of rolipram binding. Because both components of rolipram binding are to the catalytic site, they are described more accurately as distinct affinity states or conformers, rather than independent sites [47]. It should be noted that the HARBS and LARBS refer specifically to rolipram; some drugs exhibit high, equal affinity for both affinity states (e.g. piclamilast).

The issue of high-affinity and low-affinity binding sites for rolipram is not just an esoteric aspect of the pharmacology of PDE4. It appears to have important functional consequences because binding to each affinity state mediates a unique range of pharmacological effects. The order of potency of PDE4 inhibitors for producing some effects, such as induction of head twitches and tremor in mice, and emesis in ferrets, correlates with affinity for the HARBS; other effects, including inhibition of mast cell degranulation and antigen-induced T-cell proliferation in guinea-pigs appear to be more closely related to the LARBS [16,48–51].

Interestingly, the HARBS appears to be present in brain, but not in peripheral tissues [46,52]. PDE4 is expressed throughout the body, and so it is likely that this observation results from differences in the intracellular environment rather than an intrinsic difference between PDE4 in brain and peripheral tissues. Several factors affect the affinity of inhibitors for PDE4. These include the phosphorylation state of PDE4 and its interaction with proteins such as A-kinase-anchoring proteins (AKAPs), receptors for activated C kinases 1 (RACK1) and proteins that contain SH3 domains [53–56]. Data obtained to date support the suggestion that the CNS effects of PDE4 inhibitors are mediated by the HARBS. Saccomano and co-workers [16] found that the relative potency of a series of inhibitors in reducing immobility of mice in the forced-swim test (an index of antidepressant efficacy) correlates with that of inhibition of [^{3}H]-rolipram binding (an index of interaction with the HARBS). Similar data have been obtained recently using the forced-swim test in rats (Y. Zhao *et al.*, unpublished).

In addition, repeated treatment with either desipramine or fluoxetine, antidepressants that exert their antidepressant effects primarily via noradrenaline-mediated and 5-HT-mediated actions, respectively, increases the HARBS but not the LARBS in rat cerebral cortex and hippocampus [57]. If this increase in binding is a simple reflection of the overall increase in PDE4 expression that is observed [30,31], then an increase in binding to both conformers would be expected. The selective increase in inhibitor binding to the HARBS, together with the finding that this increase occurs in membrane but not cytosolic fractions, indicates that the antidepressants alter components of signaling pathways that involve PDE4. It appears that these antidepressant effects are secondary to enhanced noradrenaline-mediated and 5-HT-mediated neurotransmission, because prior

lesion of these systems blocks the ability of desipramine and fluoxetine to increase binding of inhibitors to the HARBS [57].

Future directions

Much has been learned in the past several years about the mechanisms that mediate the antidepressant actions of PDE4 inhibitors. However, several areas need to be addressed more fully. First, the field would be advanced significantly by the development of highly subtype-selective PDE4 inhibitors. This has proved difficult because the catalytic site to which PDE4 inhibitors bind is highly conserved across subtypes [10]. However, as understanding of PDE4 structure and inhibitor binding increase, aided by the publication of the crystal structure of the core, catalytic region [58,59], some progress in this area might be realized.

Second, although the understanding of the involvement of PDE4 in signaling pathways that are affected by antidepressants has improved, it is essential that these systems are defined more fully. For example, it is known that PDE4 is the predominant PDE involved in the β-adrenoceptor-linked adenylyl cyclase pathway [26], but which subtype and splice variants are particularly important in individual brain areas is unknown. Less is known about the involvement of PDE4 in 5-HT-receptor-mediated signaling and other signaling pathways affected by antidepressants.

Last, the concept of high-affinity and low-affinity binding conformers for PDE4 inhibitors (i.e. the HARBS and LARBS) has to advance from phenomenology to a fuller mechanistic understanding. Given that the HARBS is present in brain but not peripheral tissues in appreciable numbers [52], it is likely that the intracellular milieu of CNS neurons (and, possibly, glia), results in a unique inhibitor-binding profile. Although many factors affect the binding of inhibitors to PDE4, including the phosphorylation state and several protein-protein interactions, as yet, none have been linked causally to the HARBS *in vivo*. Because factors that influence binding affinity of inhibitors often affect catalytic activity, they could provide a means to alter cAMP hydrolysis in specific signaling pathways independent of overt pharmacological inhibition. This might indicate novel ways to alter PDE4-related CNS function, including mediation of antidepressant activity.

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