Downregulation of Brain Phosphodiesterase Type IV Measured with ^{11}C-(*R*)-Rolipram Positron Emission Tomography in Major Depressive Disorder

Masahiro Fujita, Christina S. Hines, Sami S. Zoghbi, Alan G. Mallinger, Leah P. Dickstein, Jeih-San Liow, Yi Zhang, Victor W. Pike, Wayne C. Drevets, Robert B. Innis, and Carlos A. Zarate Jr.

Background: Phosphodiesterase type IV (PDE4), an important component of the cyclic adenosine monophosphate (cAMP) cascade, selectively metabolizes cAMP in the brain to the inactive monophosphate. Basic studies suggest that PDE4 mediates the effects of several antidepressants. This study sought to quantify the binding of ^{11}C-(*R*)-rolipram, a PDE4 inhibitor, as an indirect measure of this enzyme's activity in the brain of individuals with major depressive disorder (MDD) compared with healthy control subjects.

Methods: ^{11}C-(*R*)-Rolipram brain positron emission tomography scans were performed in 28 unmedicated MDD subjects and 25 age- and gender-matched healthy control subjects. Patients were moderately depressed and about one half were treatment-naive. ^{11}C-(*R*)-Rolipram binding in the brain was measured using arterial ^{11}C-(*R*)-rolipram levels to correct for the influence of cerebral blood flow.

Results: Major depressive disorder subjects showed a widespread, approximately 20% reduction in ^{11}C-(*R*)-rolipram binding ($p = .002$), which was not caused by different volumes of gray matter. Decreased rolipram binding of similar magnitudes was observed in most brain areas. Rolipram binding did not correlate with the severity of depressive or anxiety symptoms.

Conclusions: This study is the first to demonstrate that brain levels of PDE4, a critical enzyme that regulates cAMP, are decreased in unmedicated individuals with MDD in vivo. These results are in line with human postmortem and rodent studies demonstrating downregulation of the cAMP cascade in MDD and support the hypothesis that agents such as PDE4 inhibitors, which increase activity within the cAMP cascade, may have antidepressant effects.

The second messenger cyclic adenosine monophosphate (cAMP) has been implicated in both the pathophysiology and treatment of major depressive disorder (MDD). This hypothesis posits that decreased signaling through the cAMP cascade is associated with depression (1–5) and that diverse antidepressant treatments increase signal transduction of the cAMP cascade as a common mechanism of action (6). Cyclic nucleotide phosphodiesterase type IV (PDE4) selectively metabolizes cAMP in the brain to the inactive monophosphate (7) and is, therefore, an important component of the cAMP cascade. Phosphodiesterase type IV may also be useful as an in vivo biomarker to assess the link between cAMP and MDD, given that PDE4 can be imaged using positron emission tomography (PET), and is also a potential target of antidepressants.

Several weeks of use are typically necessary before antidepressants manifest their therapeutic effects. This time lag is in line with decreased signaling through the cAMP cascade reported in human postmortem studies (1–5) and with antidepressant-induced increases in gene expression downstream of the cAMP cascade in

rodents (6) (e.g., increases in both brain-derived neurotrophic factor [BDNF] and a transcription factor regulating the expression of BDNF, cAMP response element binding protein [CREB]). Several postmortem brain studies in patients with MDD or depressed suicide victims found that multiple markers of the cAMP cascade were decreased, including adenylyl cyclase (1,2), cAMP-dependent protein kinase A (PKA) (3), CREB (4), and BDNF (5). In contrast, rodent experiments have consistently found that chronic, but not acute, administration of various pharmacologic classes of antidepressants—including selective serotonin reuptake inhibitors, monoamine oxidase inhibitors, and tricyclic antidepressants—upregulate those components of the cAMP cascade including PDE4 (6). One postmortem brain study found that unmedicated patients with MDD had lower CREB levels than control subjects, while medicated MDD patients had CREB levels similar to those of control subjects (4).

The enzymatic activity of PDE4 is regulated by PKA via a feedback mechanism. That is, high concentrations of cAMP stimulate PKA to phosphorylate PDE4, thereby increasing its enzymatic activity and returning the concentration of cAMP to steady state (7). Consistent with this feedback mechanism, both in vitro and in vivo studies indicated that PDE4 levels or its enzyme activity parallel the activity of the cAMP cascade (8–10). In particular, chronically enhanced or diminished noradrenergic neurotransmission altered PDE4 levels to higher and lower levels, respectively, in the same direction as the corresponding activity of noradrenergic neurotransmission (8).

This study sought to use ^{11}C-(*R*)-rolipram PET imaging to compare the density and enzymatic activity of PDE4 between unmedicated patients with MDD and healthy control subjects. Phosphorylation of PDE4 not only increases its enzymatic activity but also increases the potency (affinity) of rolipram to inhibit PDE4 (11). Previous studies from our laboratory confirmed that, in rats, the in vivo binding of ^{11}C-(*R*)-rolipram reflected the phosphorylation sta-

From the Molecular Imaging Branch (MF, CSH, SSZ, LPD, J-SL, YZ, VWP, RBI), and Experimental Therapeutics & Pathophysiology Branch (AGM, CAZ), National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland; and Laureate Institute for Brain Research and Department of Psychiatry (WCD), The University of Oklahoma College of Medicine, Tulsa, Oklahoma.

Address correspondence to Masahiro Fujita, M.D., Ph.D., National Institute of Mental Health, Molecular Imaging Branch, National Institutes of Health, Building 10, Room B1D43, 10 Center Drive, MSC-1026, Bethesda, MD 20892-1026; E-mail: fujitam@mail.nih.gov.

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Table 1. Demographic and Clinical Characteristics of the Study Samples

	Control Subjects ($n = 25$)	MDD ($n = 28$)
Proportion Female (n)	36% (9)	32% (9)
Age	37 ± 11	36 ± 11
Depression and Anxiety Ratings		
MADRS	.8 ± 1.6	30 ± 6
HDRS-17	.6 ± 1.0	20 ± 6
HAM-A	.6 ± 1.0	18 ± 7
Age of Onset	NA	17 ± 9
Duration of Current Episode (Months)	NA	76 ± 123
Treatment Naive	NA	13
Length of Time Medication Free (Months) [Range]	NA	28 ± 40 [.8–120]
Current Comorbid Anxiety Disorders	0	13
Subjects with Lifetime History of Suicide Attempts (n)	0	3
Prior Exposure to Antipsychotic Agent (n)	0	1
Lifetime History of Substance Abuse (n)	0	2
Proportion of Current Cigarette Smokers (n)	24% (6)	32% (9)[a]

Values are mean ± SD.

HAM-A, Hamilton Rating Scale for Anxiety; HDRS-17, Hamilton Rating Scale for Depression (17 item); MADRS, Montgomery-Åsberg Depression Rating Scale; MDD, major depressive disorder.

[a]Includes three social smokers. Six of the nine subjects smoked cigarettes daily.

tus of PDE4 (12). In that study, activation and deactivation of PKA, which regulates the activity of PDE4, increased and decreased rolipram binding, respectively. Therefore, in vivo binding of ^{11}C-(*R*)-rolipram measures both the density of the enzyme and its activity, as reflected by the affinity of rolipram to bind to PDE4. Within the framework of the cAMP hypothesis of MDD, we predicted that unmedicated individuals with MDD would have decreased cAMP cascade activity and therefore decreased binding of ^{11}C-(*R*)-rolipram in the brain.

Methods and Materials

Participants

Two groups were studied: 1) healthy control subjects with no personal history of a major psychiatric or neurological disorder and no first-degree relative with a mood or psychotic disorder ($n = 25$), and 2) patients who met DSM-IV criteria for MDD, in a current major depressive episode, without psychotic features ($n = 28$) (Table 1). Subjects were between 18 and 55 years of age and in good physical health, as determined by medical history, physical examination, blood labs, electrocardiogram, and urinalysis. Volunteers were excluded if they had a history of alcohol or substance abuse within the past year or a lifetime history of alcohol or substance dependence. All participants underwent a urine drug screen for amphetamines, benzodiazepines, cocaine metabolites, opiates, and cannabinoids on the day of the ^{11}C-(*R*)-rolipram PET scan. Participants were not allowed to ingest caffeine after midnight on the day before the PET scan. In addition, herbal remedies or use of over-the-counter medications with known central nervous system effects were not permitted during the study.

Diagnoses for MDD patients were established by an unstructured interview with a psychiatrist and the Structured Clinical Interview for DSM-IV (13). Major depressive disorder patients with serious suicidal ideation or psychosis were excluded from the study, but those with certain secondary anxiety disorders (generalized anxiety disorder, panic disorder, social phobia, anxiety disorder not otherwise specified, agoraphobia without panic disorder) were allowed to participate. In addition, we included patients with a remote history of posttraumatic stress disorder or obsessive-compulsive disorder but excluded subjects if these disorders were active at the time of enrollment. Patients with MDD were required to have a score of ≥20 on the Montgomery-Åsberg Depression Rating Scale (MADRS) (14) at the time of the ^{11}C-(*R*)-rolipram PET scan. Major depressive disorder patients were required to be free from psychotropic medications for at least 2 weeks (6 weeks for fluoxetine) before the PET scan.

Data Acquisition

Evaluation of Symptom Severity. Severity of depressive and anxiety symptoms was assessed for both control subjects and MDD patients using the MADRS, the 17-item Hamilton Rating Scale for Depression (HDRS-17) (15), and the Hamilton Rating Scale for Anxiety (HAM-A) (16).

Brain Imaging. Positron emission tomography, magnetic resonance imaging, and data processing were conducted as previously described (17). Please see the Supplemental Methods in Supplement 1 for further details.

After intravenously administering ^{11}C-(*R*)-rolipram, PET images were acquired for 90 minutes. To calculate ^{11}C-(*R*)-rolipram binding in the brain, which is not influenced by cerebral blood flow or peripheral clearance, unmetabolized arterial ^{11}C-(*R*)-rolipram levels were measured for 90 minutes. Because only free ^{11}C-(*R*)-rolipram enters the brain, plasma free fraction (f_P) of ^{11}C-(*R*)-rolipram was measured using arterial plasma in each scan. High-resolution anatomical magnetic resonance imaging scans were performed for each subject, except one MDD patient, to analyze PET data after transforming into the single standard space (Montreal Neurological Institute space) to eliminate intersubject variability in shape and size of the brain (see Supplemental Methods in Supplement 1 for details).

Calculation of ^{11}C-(*R*)-Rolipram Binding in Brain

^{11}C-(*R*)-Rolipram binding levels were measured by compartmental modeling as total distribution volume (V_T/f_P) (18) in 10 large preselected regions covering most brain areas: frontal, parietal, lateral temporal, occipital, medial temporal, and anterior cingulate cortices; caudate; putamen; thalamus; and cerebellum. Right- and left-side data were combined for each region. Two additional supplementary analyses were performed. First, to investigate possible changes in rolipram binding in small regions, V_T/f_P was also calculated in each volume element (i.e., voxel) of the images by Logan plot (19), and parametric images were created where each voxel value was V_T/f_P. These parametric images were analyzed using Statistical Parametric Mapping (SPM) version 2005 (SPM5; Wellcome Trust Centre for Neuroimaging, London, United Kingdom). Second, to eliminate the influence of individual differences in gray matter volume, partial volume correction (20) was applied to the parametric images using magnetic resonance images segmented to gray and white matter. Rolipram binding levels in the 10 regions were subsequently measured (see Supplemental Methods in Supplement 1 for details).