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# PET of (*R*)-<sup>11</sup>C-Rolipram Binding to Phosphodiesterase-4 Is Reproducible and Sensitive to Increased Norepinephrine in the Rat Heart

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Phosphodiesterase-4 (PDE4) plays a critical role in the regulation of  $\beta$ -adrenergic receptor-stimulated cyclic adenosine monophosphate cell signaling in the heart. (*R*)-rolipram, a PDE4-selective inhibitor, has been studied previously as a radiotracer for the quantification of PDE4 levels. The aim of this study was to characterize (*R*)-<sup>11</sup>C-rolipram binding in the rat myocardium in vivo, using small-animal PET. **Methods:** Male Sprague-Dawley rats ( $n = 30$ ) were administered (*R*)-<sup>11</sup>C-rolipram and imaged for 60 min to evaluate tracer binding and reproducibility, quantified using Logan slope analysis of the distribution volume. Dynamic <sup>13</sup>N-ammonia imaging was performed to quantify myocardial blood flow and assist in cardiac regional analysis. Saturation studies evaluated the sensitivity of (*R*)-<sup>11</sup>C-rolipram to PDE4 blocking by unlabeled cold (*R*)-rolipram (0.0001–1.0 mg/kg), for estimation of the median effective dose (ED<sub>50</sub>) in the heart. (*R*)-<sup>11</sup>C-rolipram response to enhanced norepinephrine stimulation of the  $\beta$ -adrenergic receptor with desipramine (20 mg/kg, intravenous) was also studied. Intrarat variability studies ( $n = 5$ ) were conducted with test-retest imaging at  $16 \pm 7$  d. **Results:** A reduction of Logan slope was observed with increasing cold mass coadministered with the tracer, with an ED<sub>50</sub> of 0.0019 mg/kg (95% confidence interval, 0.0014–0.0052) estimated from the saturation studies. This ED<sub>50</sub> predicted less than 10% enzyme occupancy at 0.0002 mg of cold (*R*)-rolipram per kilogram (mass/body weight). Low-occupancy imaging at  $0.00018 \pm 0.00002$  mg/kg produced a mean Logan slope of  $5.5 \pm 0.85$  mL/cm<sup>3</sup>. Enzyme saturation of more than 90%, compared with low-occupancy conditions, occurred at more than 0.02 mg/kg, with a complete blocking dose ( $>1$  mg of (*R*)-rolipram per kilogram) resulting in a Logan slope of  $3.3 \pm 0.1$  mL/cm<sup>3</sup>, representing a 40% reduction. Compared with baseline, a Logan slope of  $6.8 \pm 0.7$  mL/cm<sup>3</sup> in desipramine-challenged animals was observed, representing a 30% increase due to acute norepinephrine stimulation, despite a reduction in myocardial blood flow. Intrarat and intra-operator variability was less than 5% between repeated measures. **Conclusion:** (*R*)-<sup>11</sup>C-rolipram shows the ability to

monitor increases and decreases in PDE4 availability in the rat myocardium, with good reproducibility.

**Key Words:** (*R*)-rolipram; PDE4; desipramine; test-retest repeatability

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**P**hosphodiesterase (PDE) enzymes are responsible for the hydrolysis of secondary messengers cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate to their inactive 5' forms, terminating intracellular signaling. In the heart, the primary phosphodiesterase isozymes are phosphodiesterase-3 and phosphodiesterase-4 (PDE4) (1). Importantly, PDE4 is regulated by cAMP levels through downstream effectors, increasing PDE4 activity in acutely altered signaling states and increasing PDE4 expression with chronically altered states (1).

Rolipram ((*R/S*)-4-(3-cyclopentyloxy-4-methoxyphenyl)-2-pyrrolidone), a PDE4-selective inhibitor (2), has been labeled with <sup>11</sup>C (3) and applied as a radiotracer for in vivo imaging with PET in rat (4–7), pig (8), and human (9) studies. Quantifying tissue distribution of <sup>11</sup>C-labeled (*R*)-rolipram may provide an in vivo measure of PDE4 levels as an indirect measure of cAMP intracellular signaling.

Our previous in vitro work demonstrated specific and selective PDE4 binding in the rat heart (10–12). Thus, the aim of the present study was to characterize the in vivo binding specificity, responsiveness to acute norepinephrine treatment, and reproducibility of (*R*)-<sup>11</sup>C-rolipram imaging in the rat myocardium with small-animal PET.

## MATERIALS AND METHODS

### Animals

Male Sprague-Dawley rats ( $n = 30$ ) weighing 225–515 g were maintained on a 12-h light-dark cycle with water and food ad libitum. All experiments were conducted with approval from the Animal Care Committee at the University of Ottawa.

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## Radiotracer Synthesis

(*R*)-<sup>11</sup>C-rolipram (7.4–315 GBq/μmol) was synthesized as described previously (3) or in higher specific activity using a recirculation gas-phase system from <sup>11</sup>C-methane and iodine (13) in high radiochemical purity (>95%). <sup>13</sup>N-ammonia was obtained from routine clinical synthesis. Unlabeled (*R*)-rolipram (Ascent Scientific) was dissolved in ethanol:propylene-glycol:0.9% saline (5:20:75, v/v/v). Desipramine hydrochloride (Sigma-Aldrich) was dissolved in 0.9% saline.

## Arterial Input Function Corrections

**Plasma-to-Whole-Blood Ratio.** Activity in whole blood and plasma was measured at serial time points to determine the proportion in plasma to whole blood. Rats (*n* = 9) were treated with buprenorphine analgesic (0.04 mg/kg, intraperitoneally) and anesthetized with 1%–2% isoflurane. The carotid artery was cannulated for serial blood collection. (*R*)-<sup>11</sup>C-rolipram (11.1–85.5 MBq) was injected via the tail vein, and arterial blood (150–300 μL) was collected at 0.5, 1, 5, 20, 30, 45, and 60 min. Blood samples were weighed and counted for activity (Cobra II γ-Counter; Packard). Samples were centrifuged for 5 min at 4,000g, and the supernatant was collected, weighed, and counted to determine the plasma activity per gram.

**<sup>11</sup>C-Labeled Metabolites in Plasma.** Studies were performed to determine the proportions of unchanged (*R*)-<sup>11</sup>C-rolipram and <sup>11</sup>C-labeled metabolites in plasma. Rats received a 300- to 370-MBq dose of (*R*)-<sup>11</sup>C-rolipram and were sacrificed at 5, 15, and 60 min (*n* = 3 for each) for collection of trunk blood. Blood samples were centrifuged for 5 min at 4,000g, and plasma was collected and double-filtered (0.22 μm) to remove excess plasma proteins for analysis with our high-performance liquid chromatography column-switch method (12). Previous data (12) were used to complete the plasma metabolite time course at 30 and 45 min.

## PET

Animals were anesthetized with 1%–2% isoflurane, with heart rate, respiratory rate, and temperature monitored during imaging with a Siemens Inveon scanner. A 30-min dynamic <sup>13</sup>N-ammonia scan (52–126 MBq) was obtained to quantify myocardial blood flow (MBF) and assist with region-of-interest placement in coregistered (*R*)-<sup>11</sup>C-rolipram images. Thirty minutes later, a 60-min (*R*)-<sup>11</sup>C-rolipram (7.4–92.5 MBq) dynamic scan (9 × 10 s, 3 × 30 s, 2 × 60 s, and 5 × 300 s) was acquired. A 10-min <sup>57</sup>Co transmission scan was acquired for attenuation correction. PET images (Bq/cm<sup>3</sup>) were reconstructed using the vendor-provided 3-dimensional ordered-subset expectation maximization maximum a posteriori algorithm (β = 1), with all corrections enabled.

## PET Image Analysis

FlowQuant (uOHI) software was used to generate time–activity curves of tracer concentration in arterial blood and polar maps of tracer uptake and clearance in the left-ventricular myocardium. Quantitative MBF values (mL/min/g) were derived from <sup>13</sup>N-ammonia curves using a 1-tissue-compartment model (14).

Logan slopes (mL/cm<sup>3</sup>) were derived graphically from the (*R*)-<sup>11</sup>C-rolipram curves at 20–60 min after injection, using a partial-volume recovery coefficient of 0.6. The Logan slope is a function of free receptor or enzyme concentration, providing an index of in vivo binding potential for reversibly bound tracers (15). Some labeled metabolites are present in rat cardiac tissue, although these do not bind specifically to PDE4 (12). Therefore, a component of the Logan slope can represent labeled metabolites;

however, the method still provides a useful index of tracer binding potential in lieu of full compartmental modeling. <sup>11</sup>C-rolipram image analysis was repeated twice to assess operator variability. Intraoperator variability was evaluated using 2 measurements obtained by operator A. Interoperator variability was assessed by comparing values between operator A and a second operator B.

## PDE4 Enzyme Saturation Studies

To determine the effect of an injected mass (0.0001–1.0 mg/kg) of unlabeled cold compound on (*R*)-<sup>11</sup>C-rolipram binding, studies were conducted to estimate a median effective dose (ED<sub>50</sub>) in the rat heart.

Full PDE4 blockade studies (*n* = 3) were conducted in rats preinjected with a saturating dose of (*R*)-rolipram (1 mg/kg, intravenously) (11) to establish the nondisplaceable proportion of binding. In the range of 0.003–0.35 mg/kg, rats (*n* = 5) received (*R*)-<sup>11</sup>C-rolipram coinjected with added unlabeled (*R*)-rolipram. Below 0.03 mg/kg (*n* = 22), the unlabeled component of the tracer formulation was calculated from the specific activity; radiochemistry limitations permitted an injection of 0.00016 mg/kg as the lowest mass dose. PET camera sensitivity also prevented the injection of lower masses while still maintaining activity above 7.4 MBq.

The ED<sub>50</sub> for myocardial (*R*)-rolipram binding was estimated by fitting the following function to all (*n* = 30) Logan slope values:

$$\text{Logan slope} = [V_S / (1 + \text{IM}/\text{ED}_{50})] + V_{\text{ND}}, \quad \text{Eq. 1}$$

where IM is the injected mass dose, *V<sub>S</sub>* is the specific binding distribution volume, and *V<sub>ND</sub>* is the nondisplaceable distribution volume of the tracer (16). Using these data, we established a low receptor occupancy (<10%) target to perform the following studies.

## Response to Acute Norepinephrine Elevation

Rats (*n* = 5) were imaged with (*R*)-<sup>11</sup>C-rolipram first at baseline and then 2–7 d later with acute desipramine challenge (20 mg/kg, intraperitoneally, at 3 h before tracer administration). Because desipramine can cause depressed hemodynamics at high doses (17), <sup>13</sup>N-ammonia MBF was used to evaluate potential changes in the tracer delivery of (*R*)-<sup>11</sup>C-rolipram.

## (*R*)-<sup>11</sup>C-Rolipram Reproducibility Studies

Interrater variability was assessed at low-occupancy conditions (<10%; 0.00018 ± 0.00002 mg/kg) in 13 animals. The intrarater variability was assessed using test–retest imaging (*n* = 5) within 16.4 ± 6.7 d to yield repeated measures.

## Mathematic and Statistical Analysis

Curve fitting was performed using MATLAB (The MathWorks). Student *t* tests were completed using SPSS software (SPSS Inc.), and a *P* value less than 0.05 was considered statistically significant.

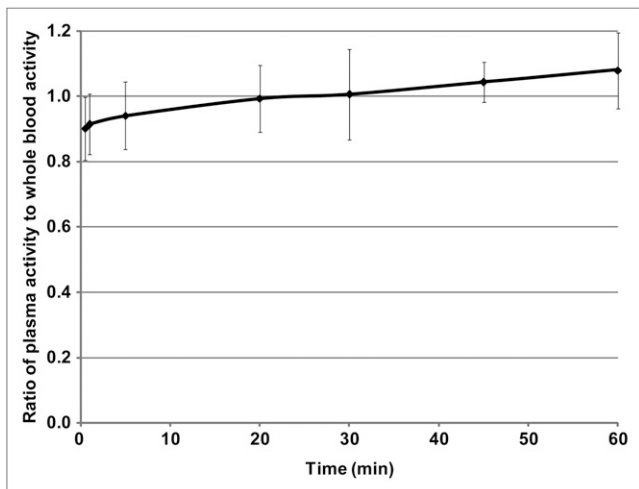
## RESULTS

### Arterial Input Function Corrections

**Plasma-to-Whole-Blood Ratio.** Plasma-to-whole-blood ratios varied from 0.8 initially to 1.2 at 0–60 min (Fig. 1). The following equation was fit to the ratio data (*r*<sup>2</sup> = 0.98):

$$F(t) = 1.15 - 0.24 e^{-0.02t}, \quad \text{Eq. 2}$$

providing a whole-blood correction for radioactivity in plasma over time.

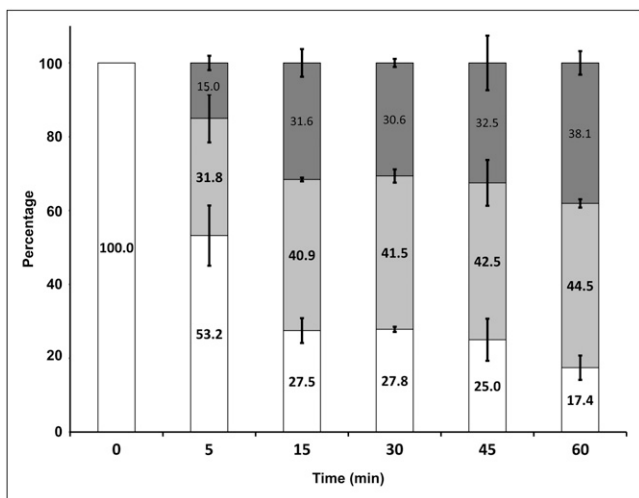


**FIGURE 1.** Ratio of plasma-to-whole-blood activity over time (min) in rat for (*R*)-<sup>11</sup>C-rolipram as measured by carotid artery blood sampling (150–300 μL). Data are presented as mean ± SD (*n* = 3 at each data point). Fitted whole-blood-to-plasma correction function is  $F(t) = 1.15 - 0.24 e^{-0.02t}$ .

**<sup>11</sup>C-Labeled Metabolites in Plasma.** (*R*)-<sup>11</sup>C-rolipram was rapidly metabolized. At 15 min, 27% of radioactivity in plasma was associated with the unaltered parent compound, whereas the remaining activity derived from labeled metabolites (Fig. 2). The following equation was fit to the metabolite data ( $r^2 = 0.95$ ):

$$F(t) = 0.7 e^{-0.23t} + 0.3 e^{-0.007t}, \quad \text{Eq. 3}$$

providing a metabolite correction for unaltered parent tracer in plasma.



**FIGURE 2.** Proportion of plasma activity associated with hydrophilic metabolites eluted from capture column (light shaded bars), hydrophilic metabolites eluted with solvent front (dark shaded bars), and unchanged parent tracer (white bars) at various time points (min). Derived plasma metabolite correction function is  $F(t) = 0.7 e^{-0.23t} + 0.3 e^{-0.007t}$ . Error bars represent ±SD.

## PET

The (*R*)-<sup>11</sup>C-rolipram small-animal PET images consistently show high uptake in the heart (Fig. 3) and liver (Fig. 4). The myocardium-to-blood ratio was  $1.4 \pm 0.23$  averaged across all imaging conditions.

## PDE4 Enzyme Occupancy Studies

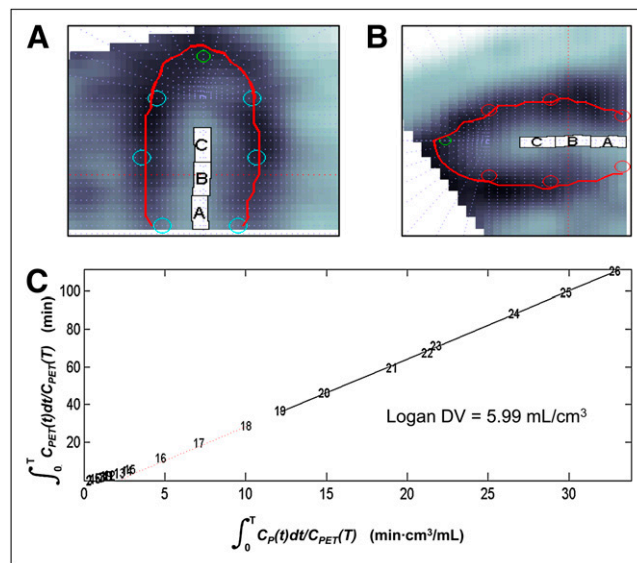
The  $ED_{50}$ , estimated using Equation 1, was 0.0019 mg/kg (95% confidence interval, 0.0014–0.0052;  $r^2 = 0.63$ ) (Fig. 5), and the specific binding was 2 mL/cm<sup>3</sup>.

Assuming this  $ED_{50}$ , less than 10% occupancy would occur at 0.0002 mg/kg. In the low-occupancy studies (*n* = 13;  $0.00018 \pm 0.00002$  mg/kg), the Logan slope was  $5.5 \pm 0.85$  mL/cm<sup>3</sup> (Table 1).

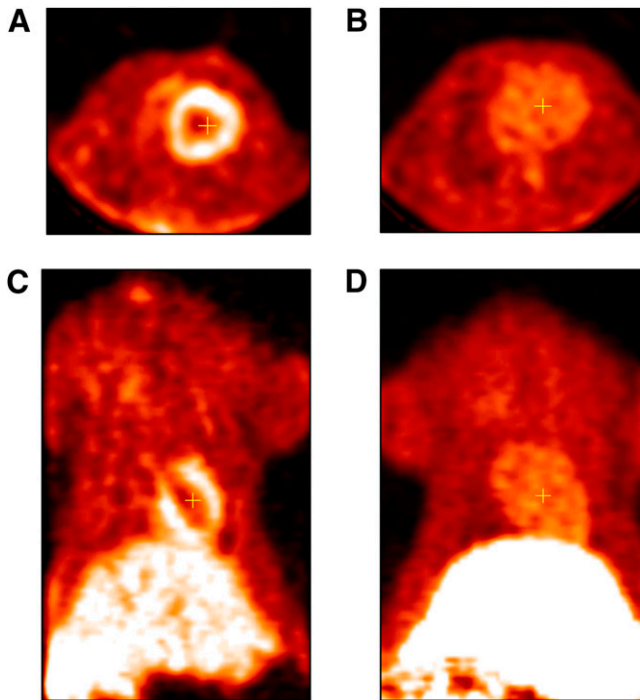
Images (>1 mg/kg, (*R*)-rolipram) in the PDE4-blocking animals, as compared with the low-occupancy rats ( $1.6 \pm 0.22$ ), were characterized by a low myocardium-to-blood ratio ( $1.1 \pm 0.06$ ) ( $P < 0.05$ ; Fig. 4). A mean Logan slope of  $3.3 \pm 0.1$  mL/cm<sup>3</sup> (Table 1) in these animals, compared with low-occupancy conditions, represented a significant 40% reduction ( $P < 0.01$ ).

## Response to Acute Norepinephrine Elevation

Acute desipramine challenge resulted in Logan slopes of  $6.8 \pm 0.70$  mL/cm<sup>3</sup> (Table 1). These same rats had baseline Logan slopes of  $5.2 \pm 0.77$  mL/cm<sup>3</sup>, representing a signifi-

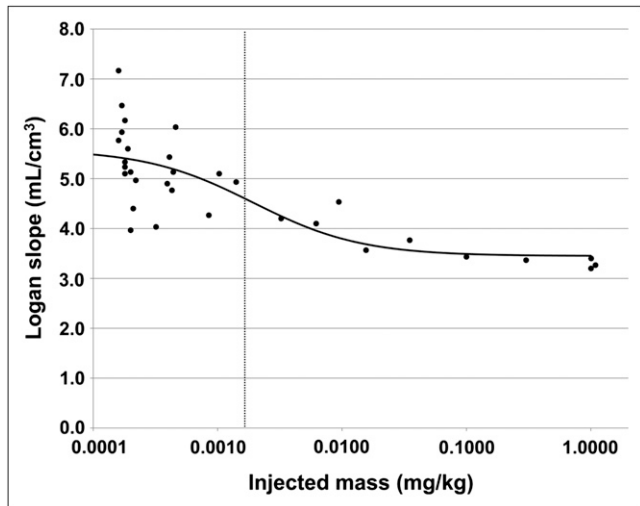


**FIGURE 3.** Reconstructed (*R*)-<sup>11</sup>C-rolipram images showing high tracer uptake in rat heart (frame 15, 4–5 min after tracer administration; 17 MBq injected activity, 0.04 μg injected mass [0.00016 mg/kg]). Myocardial (red) regions of interest placed using horizontal-long-axis (A) and vertical-long-axis (B) orientations with whole-blood regions taken at locations A, B, and C, representing left atrium, base, and cavity of heart, respectively. Representative Logan slope graphic analysis plot (C) showing linear relationship from 20 to 60 min (frames 19–26). In axis labels,  $C_P(t)$  = concentration of parent (*R*)-<sup>11</sup>C-rolipram in plasma and  $C_{PET}(t)$  = concentration of (*R*)-<sup>11</sup>C-rolipram in tissue after correction for blood volume and partial-volume loss.



**FIGURE 4.** Reconstructed (*R*)-<sup>11</sup>C-rolipram transverse and coronal images in rats comparing cardiac signal in representative baseline image (A and C) (frame 15, 4–5 min after tracer administration; 17 MBq of injected activity, 0.04 μg injected mass [0.00016 mg/kg]) vs. complete blocking image (B and D) with 1 mg of (*R*)-rolipram per kilogram injected at 2–5 min before (*R*)-<sup>11</sup>C-rolipram (frame 15, 4–5 min after tracer administration; 81 MBq of injected activity, 0.89 μg injected mass [1.003 mg/kg]).

cant 30% increase ( $P < 0.01$ , Fig. 6) and a significant 24% increase, compared with low-occupancy conditions ( $P < 0.01$ ).



**FIGURE 5.** Dose–response curve for injected mass of (*R*)-<sup>11</sup>C-rolipram vs. Logan slope over injected mass range of 0.00016–1.09 mg/kg. An  $ED_{50}$  of 0.0019 mg/kg (95% confidence interval of 0.0014–0.0052, dashed vertical line) was estimated by fitting the following equation to the data:  $LS = [V_S/(1 + IM/ED_{50})] + V_{ND}$ . IM = injected mass; LS = Logan slope;  $V_{ND}$  = nondisplaceable distribution volume;  $V_S$  = specific binding distribution volume.

**TABLE 1**  
Quantitative (*R*)-<sup>11</sup>C-Rolipram Logan Slope and <sup>13</sup>N-Ammonia MBF Values for Baseline, Blocked, and Desipramine Challenge Groups

Group	Logan slope (±SD)	MBF (±SD)
Baseline, low occupancy (0.00018 mg/kg)	5.5 (±0.85)	3.5 (±0.60)
Blocked, total PDE4 saturation (>1.0 mg/kg)	3.3* (±0.10)	3.9 (±0.61)
Desipramine challenge (0.00018 mg/kg)	6.8†± (±0.70)	3.0 (±0.55)

\* $P < 0.01$  blocked vs. baseline.

† $P < 0.001$  desipramine vs. baseline.

‡ $P < 0.001$  desipramine vs. blocked.

Parallel MBF measurements in these rats revealed a significant 23% reduction in flow with desipramine versus untreated rats ( $P < 0.001$ , Fig. 6). In absolute terms, MBF dropped from 3.9 to 3.0 mL/min/g at 3 h after desipramine administration. Therefore, the increased (*R*)-<sup>11</sup>C-rolipram binding observed with desipramine cannot be attributed to changes in tracer delivery.

#### (*R*)-<sup>11</sup>C-Rolipram Reproducibility Studies

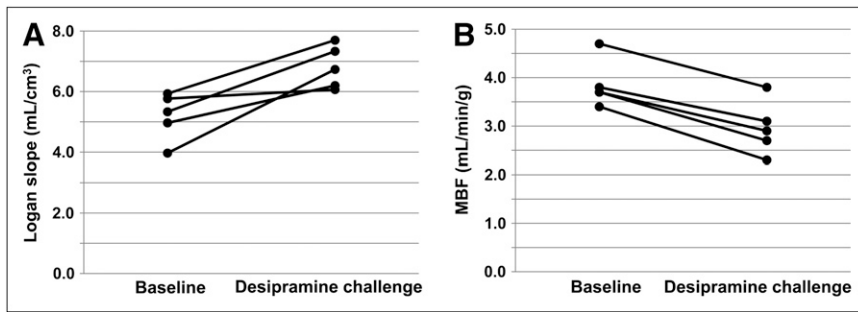
Logan slope values at less than 10% occupancy ranged from 4.0 to 7.2, with a mean of  $5.5 \pm 0.85$  mL/cm<sup>3</sup> in 13 studies (Table 1), representing a 15% coefficient of variation between rats.

Test–retest studies revealed small variability within rats; the difference between repeated measures ranged from 1.9% to 8.6%, with a mean of  $4.6\% \pm 2.8\%$ . Bland–Altman analysis showed a small 5% bias toward higher values in the second study relative to the first ( $P < 0.05$ , Fig. 7).

Operator variability studies showed that the interoperator difference in Logan slope values was  $4.7\% \pm 2.8\%$ , whereas intraoperator values differed by  $4.4\% \pm 4.3\%$  on average and always by less than 13%.

#### DISCUSSION

PDE enzymes, through their ability to hydrolyze cyclic nucleotides, play a critical role in limiting and attenuating cell signaling. cAMP-specific PDE4 is of particular functional importance because it hydrolyzes most cAMP produced in rats (*I*). Further, cAMP levels act to regulate PDE4 expression and activity through negative-feedback mechanisms (*I*). Given this relationship between PDE4 and cAMP, PDE4-specific (*R*)-<sup>11</sup>C-rolipram distribution volume is reflective of the cardiac signaling environment, and using this radioligand with small-animal PET can provide an in vivo window to monitor this intracellular signaling cascade. The in vivo characterization of (*R*)-<sup>11</sup>C-rolipram small-animal PET reported here demonstrates qualitative and quantitative features favorable for cardiac imaging in rats.



**FIGURE 6.** Baseline vs. desipramine challenge (20 mg/kg, 3 h before tracer administration, intraperitoneally) studies. (A) Increased Logan slope values after desipramine challenge ( $P < 0.01$ ). (B) Decreased MBF after desipramine challenge ( $P < 0.001$ ).

(*R*)-<sup>11</sup>C-rolipram metabolism results in radioactivity accumulating in whole blood at early time points, with more activity found in the plasma fraction at later time points (>30 min), consistent with our previous data (17). The shift in radioactivity from whole blood to plasma could be due to the appearance of labeled metabolites over time or the release of parent (*R*)-<sup>11</sup>C-rolipram through competitive displacement by unlabeled compound.

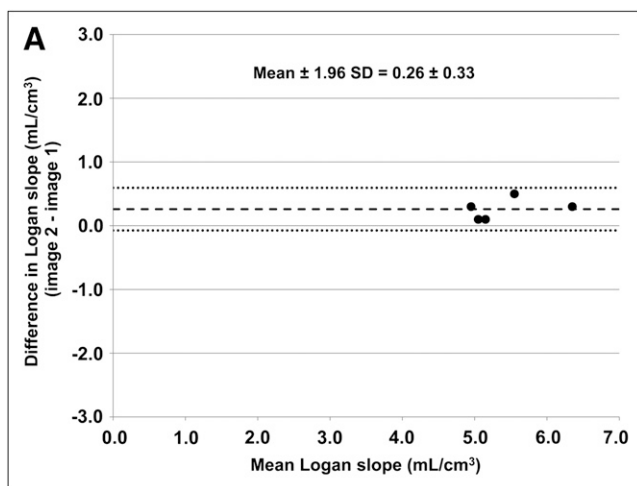
Rapid (*R*)-<sup>11</sup>C-rolipram metabolism in rats was observed, with about 50% of the parent compound in plasma at 5 min and less than 30% at 15 min after tracer administration. The metabolite correction derived from our high-dose metabolite studies is similar to the high-specific-activity (low-dose) data reported by Fujita et al. (4), suggesting a minimal effect of cold rolipram in the range encountered here. To our knowledge, there are no reports on the effect of desipramine on (*R*)-<sup>11</sup>C-rolipram metabolism. Despite this potential effect, the rolipram blocking and desipramine results are in good agreement with the percentage changes reported in our previous ex vivo biodistribution studies (10,11).

Small-animal PET with (*R*)-<sup>11</sup>C-rolipram in rats revealed the greatest tracer binding to the heart, brain, and liver. High liver activity is typical of radiotracers that undergo hepatic metabolism, and it has been demonstrated that liver activity associated with (*R*)-<sup>11</sup>C-rolipram does not represent specific binding (11). Conversely, the tracer accumulation in cardiac and brain tissues confirms previous in vivo biodistribution studies (10,11) and imaging experiments (4–7), reflecting the high PDE4 levels in these tissues (18).

Enzyme saturation studies at varying injected masses (0.00016–1.09 mg/kg) revealed an inverse relationship between increasing injected mass and reduced Logan slope, consistent with greater PDE4 enzyme saturation by non-labeled compound. This effect follows the expected shape of a sigmoid curve, indicating more than 90% saturation of the available enzyme with injected masses greater than 0.02 mg/kg. This study determined an in vivo cardiac ED<sub>50</sub> for (*R*)-rolipram of about 0.002 mg/kg, compared with previous estimates of 0.03 mg/kg in rat heart (11) and 0.002 mg/kg in pig brain (8). Our estimated in vivo ED<sub>50</sub> is approximately an order of magnitude smaller than the ex vivo-derived value reported by Kenk et al. (11) but virtually identical to that reported by Parker et al. (8).

Using Equation 1 with the ED<sub>50</sub> presented here, we estimate the in vivo enzyme occupancy of PDE4 by unlabeled rolipram to be less than 5% at mass doses of less than 0.0001 mg/kg and less than 10% at less than 0.0002 mg/kg in the rat heart. Unfortunately, radiochemistry limitations in achievable specific activity prevented us from investigating the less than 5% occupancy that is advised in radioligand binding studies (16). To achieve these small injected masses with a typical 325-g rat, routine radiochemistry tracer synthesis would have to yield specific activities of more than 166.5 GBq/μmol, to also allow injected activities of 18.5 MBq or more that are required for high-quality PET. Therefore, a value of less than 0.0002 mg/kg was targeted for imaging in the low-occupancy (<10%) condition. This value proved sufficient to show the presence of PDE4-specific binding in the rat heart that was reproducible and responsive to desipramine challenge.

Preadministration of a saturating dose of (*R*)-rolipram (>1 mg/kg) was sufficient to decrease (*R*)-<sup>11</sup>C-rolipram binding to nonspecific levels, with a Logan slope of about 3.3 mL/cm<sup>3</sup>; however, substantial blocking appears to occur



**FIGURE 7.** Bland-Altman plot comparing test-retest Logan slope values for (*R*)-<sup>11</sup>C-rolipram in left ventricular rat myocardium. Mean difference between Logan slope repeated measures ( $n = 5$ , image 2 – image 1) was 0.26, representing small but significant bias of 5% ( $P < 0.05$ ). Repeatability coefficient was 0.33 mL/cm<sup>3</sup>, or 6% of mean.

with doses as low as 0.02 mg/kg, or 90% occupancy. Qualitatively, this blocking results in a dramatic loss of contrast between myocardium and blood in the PET images. Quantitatively the reduction in Logan slope, compared with baseline, suggests about 40% specific binding of (*R*)-<sup>11</sup>C-rolipram, assuming that the bias from labeled metabolites is relatively small and constant across all imaging conditions. The reduction seen here closely parallels *ex vivo* findings: biodistribution studies in rats showed a 30% reduction in (*R*)-<sup>11</sup>C-rolipram LV binding when blocked with unlabeled rolipram (*11*), and (*R*)-<sup>11</sup>C-rolipram specific binding in the heart at 45 min after tracer administration was 41%, as determined by high-performance liquid chromatography (*12*).

Compared with baseline, desipramine challenge resulted in a 24%–30% increase in Logan slope. This follows the increase reported using *ex vivo* biodistribution, for which a 21% increase in (*R*)-<sup>11</sup>C-rolipram specific binding in the heart was attributed to desipramine treatment (*10*). Interestingly, a 23% reduction in MBF was observed in these studies using <sup>13</sup>N-ammonia imaging. Hemodynamic depression is noted at high doses of imiprine (*19*), the active metabolite of desipramine. Thus, the reduction of myocardial flow, opposite to the increase in (*R*)-<sup>11</sup>C-rolipram uptake, provides additional evidence of the tracer's specific and directed uptake related to PDE4 levels.

Quantitatively, the low-occupancy imaging condition revealed a mean Logan slope of about 5.5 mL/cm<sup>3</sup>, confirmed by test–retest studies within the same animals and in measurements of independent animals. Indeed, the reproducibility of tracer quantification was highly robust, as test–retest studies within the same animal resulted in quantitative differences of less than 5%. As expected, variability between interanimal studies at about 15% is higher than that seen in intraanimal studies. The larger difference seen in interrater studies may result from the application of population average arterial input function corrections. Indeed this observation of low intraanimal variability highlights the potential advantage of small-animal PET for repeated measures in the same animal, allowing the detection of *in vivo* differences more readily with smaller samples.

Itoh et al. (*6*) has demonstrated that isoflurane anesthesia reduces (*R*)-<sup>11</sup>C-rolipram brain uptake in rats, and a possible analogous effect on the heart should be explored to ensure reliable interpretation of the data. Future work should aim to correlate *in vivo* cardiac (*R*)-<sup>11</sup>C-rolipram binding with *in vitro* measures of PDE4 expression or activity. Differences in sympathetic nervous system signaling, tracer binding and metabolism will need to be evaluated in translating these results from rats to other species. The application of (*R*)-<sup>11</sup>C-rolipram in animal models of disease may also help facilitate the translation of this radiotracer to clinical practice. In particular, cardiac pathologies that are either the cause or a consequence of elevated sympathetic nervous system tone are potential imaging targets. Heart failure is a prime example, in which chronic sympathetic nervous system stimulation alters the β-adrenergic receptor

function. Given the pivotal importance of cAMP in regulating ventricular contractile function, an index of cAMP levels could help to ultimately guide therapeutic approaches on an individual basis or to evaluate the effects of new therapies in heart failure.

## CONCLUSION

(*R*)-<sup>11</sup>C-rolipram small-animal PET demonstrates PDE4 binding in the rat heart, which is sensitive to changes in PDE4 levels. Quantification of the tracer distribution volume by Logan graphical analysis is reproducible and minimally affected by operator and test–retest variability. (*R*)-<sup>11</sup>C-rolipram with small-animal PET has the ability to evaluate cardiac intracellular cAMP-mediated PDE4 signaling in an *in vivo* setting.

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