(see above) and approaching the "nonspecific" binding level found in rat heart.

The liver was regularly visualized on images made with $^{123}$I-ICYP (Fig. 7A-D). The radiopharmaceutical is concentrated by hepatocytes and excreted through the biliary system, and the gall bladder can be seen on images of the abdomen. Excretion as %ID was 5%/3 hr and 12%/24 hr in the urine and 15%/24 hr in the feces. Absorbed radia-

by small doses of ICI-118,551 or of timolol. After this selective inhibition of ICYP in the dog lung, binding of ICYP in the heart was inhibitable by either propranolol or timolol; the latter beta antagonist reduced the heart concentration to 18% of total binding, a level comparable to that attained in the rat. These results indicated that the ICYP portrayed in the heart was largely bound to beta receptors. The distribution of ICYP, which reflected the sites of beta receptors in the left ventricle, appeared to be diffuse.

The highly selective inhibition of lung binding of ICYP should not impair future measurements aimed at quantifying the receptors in the myocardium. The method of producing tomographic images can be adopted in any Nuclear Medicine laboratory with a scintillation camera. Moreover, an instrument capable of acquiring single photon emission data in dynamic and tomographic modes,

of the β-adrenergic receptor pathway in the intact failing human heart: progressive receptor down-regulation and subsensitivity to agonist response. *Circulation* 1986;74:1290–1302.

6. Bristow MR, Ginsburg R, Umans V, et al. β₁- and β₂-adrenergic-receptor subpopulations in nonfailing and failing human ventricular myocardium: coupling of both receptor subtypes to muscle contraction and selective β₁-receptor down-regulation in heart failure. *Circ Res* 1986;59:297–309.

7. Homcy CJ, Strauss HW, Kopiwoda S. Beta receptor occupancy: assessment in the intact animal. *J Clin Invest* 1980;65:1111–1118.

8. Hughes B, Marshall DR, Sobel BE, Bergmann SR. Characterization of beta-adrenoreceptors in vivo with iodine-131-pindolol and gamma scintigraphy. *J Nucl Med* 1986;27:660–667.

9. Van Dort ME, Gildersleeve DL, Wieland DM. A rapid high yield synthesis of no-carrier-added (−) [I-123]iodocyanopindolol. *Int J Appl Radiat Isotop* 1991;42:309–311.

10. Hoyer D, Engel G. Binding of $^{125}$I-cyanopindolol to beta-1-adrenoceptors in a high and low affinity state. *J Recept Res* 1983;3:45–59.

11. Freed BR, Gelbard AS. Distribution of $^{13}$N following intravenous injection of [$^{13}$N]ammonia in the rat. *Can J Physiol Pharmacol* 1982;60:60–67.

12. Ferrone RA, Walsh GM, Tsuchiya M, Frohlich ED. Comparison of hemodynamics in conscious spontaneous and renal hypertensive rats. *Am J Physiol* 1979;236:H403–408.

13. Sisson JC, Lynch JJ, Johnson J, et al. Scintigraphic detection of regional

# Postinjection L-Phenylalanine Increases Basal Ganglia Contrast in PET Scans of 6-$^{18}$F-DOPA

Doris J. Doudet, Catherine A. McLellan, Thomas G. Aigner, Richard Wyatt, H. Richard Adams, Hitoshi Miyake, Ronald T. Finn, and Robert M. Cohen

*Section on Clinical Brain Imaging, LCM, IRP, Laboratory of Neuropsychology and Neuropsychiatry Branch, National Institutes of Mental Health, Bethesda, Maryland; and Radiopharmaceutical Chemistry, NMD, CC, National Institutes of Health, Bethesda, Maryland*

The sensitivity of $^{18}$F-DOPA positron emission tomography for imaging presynaptic dopamine systems is limited by the amount of specific-to-nonspecific accumulation of radioactivity in brain. In rhesus monkeys, we have been able to increase this ratio by taking advantage of the lag time between $^{18}$F-DOPA injection and the formation of its main metabolite, the amino acid $^{18}$F-fluoromethoxydopa, the entrance of which into brain is responsible for most of the brain's nonspecific radioactivity. By infusing an unlabeled amino acid, L-phenylalanine, starting 15 min after $^{18}$F-DOPA administration, we preferentially blocked the accumulation of $^{18}$F-fluoromethoxydopa by preventing its entrance into brain through competition at the large neutral amino acid transport system of the blood-brain barrier. This method appears as reliable as the original and more sensitive, as demonstrated by the comparison of normal and MPTP-treated animals under both conditions

ine ($^{18}$F-3-OM-DOPA), one of the major metabolites of $^{18}$F-DOPA. This amino acid, primarily produced in the liver, can readily cross the blood-brain barrier (BBB), probably using the same large neutral amino acid (LNAA) transport system as L-DOPA. 3-OM-DOPA appears to have a uniform distribution throughout the brain in rodents (7) and in primates (8). Administration of $^{14}$C- or $^{3}$H-labeled L-DOPA or $^{18}$F-DOPA to rats or primates leads to significant concentrations of 3-OM-DOPA in plasma and a substantial background of 3-OM-DOPA in brain (9–11). The other metabolites of L-DOPA produced in the periphery and found in plasma, mainly dopamine (DA), homovanillic acid, 3,4-dihydroxyphenylacetic acid, and their sulfated conjugates, are not likely to cross the BBB to any significant degree.

iments, the effect on input into the heart produced little change in the estimates of the binding parameters of the mathematical model. In particular, the relative amounts of specifically and nonspecifically bound ICYP were largely unaffected. Within the myocardium the total concentration of ICYP is relatively stable over hours and the percent of deiodinated metabolite low.

The model appears to be valid even in the presence of a substantial extra-cardiac metabolism of ICYP. At sufficient concentrations, other antagonists might be expected to displace ICYP from the receptors. Yet, timolol, given at 34,000 nmole/kg and 30 min after the ICYP, did not appreciably displace ICYP from the heart over the next 75 min. On the other hand, when given simultaneously with or 15 min before the ICYP, timolol prevented binding of ICYP. This phenomenon of differing interactions between ICYP and timolol can be explained by the concept that once ICYP becomes bound to the receptor, the bond is very firm. For example, in vitro assays of binding and dissociation of binding were carried out in the presence of propranolol; the results showed that ICYP could be prevented from binding to beta receptors from the heart (to give the nonspecific binding of the assay), but, once bound, ICYP dissociated slowly from the receptors (5,9).

When increasing doses of ICYP were given to rats, the pattern of binding of ICYP indicated that saturation of receptors could be approached in vivo. The Scatchard analysis of the data pointed to two affinity sites for binding; in the higher affinity site, the Kd was 22 n$M$. Two affinity sites have been described for the in vitro binding of ICYP to beta receptors in membranes derived from guinea pig hearts (9,10), but, because the in vivo binding was largely attained from the first pass of blood and the in vitro binding occurs at an equilibrium in the presence of a given concentration of ICYP, we did not expect the measurements of in vivo binding to be the same as those obtained by in vitro assay in the heart of any species. The Kd for the high affinity sites has been calculated to be 9–23 p$M$ by in vitro assay of human (4–6,29), guinea pig (9), dog (24), and rat (23) heart membranes. However, if it is assumed that ICYP concentration available to the heart was less than that in the arterial whole blood, then the in vivo Kd would be less. For instance, if only the non-protein bound ICYP in plasma were available to the receptors, the Kd would be 88 p$M$. Nevertheless, even though the in vivo binding values may differ from the in vitro values, the changes in binding produced by effects of physiology and disease should be similar.

We confronted the question of whether, ICYP, a lipophilic and therefore potentially internalized antagonist, was binding to beta receptors within cells as well as surface receptors; the internalized receptors are of low affinity and presumed to be non-functional. Timolol, too, is lipophilic and could inhibit binding of ICYP to internalized low affinity receptors as well as to surface receptors, but CGP-12177 is a hydrophilic antagonist that is not internalized

(19,20) and would be expected to inhibit binding only to the surface receptors. Because the slope of the inhibition curve produced by timolol was steep, it appeared that timolol was inhibiting the binding of ICYP only to one class of high affinity receptors. The data do not suggest inhibition of binding to low affinity internalized receptors. CGP-12177 which should inhibit binding only to the surface receptors of cells produced a curve that was significantly less steep than that of timolol, but the reason for the difference is not clear.

Isoproterenol, a beta agonist, also inhibited ICYP binding to the heart. Although isoproterenol differs from the antagonists in pharmacologic action and in the interaction with beta receptors, it did not produce an inhibition curve with a slope that was different from that of timolol. Of importance is that, with sufficient levels of inhibition, the in vivo method is capable of defining slopes of inhibition curves.

The patterns of inhibition of binding by several beta antagonists gave further support to the concept that ICYP is bound to beta receptors in vivo. The EC-50 of timolol was about one-eighth that of propranolol giving relative potencies for the two agents in this model of receptor measurement that were similar to those described for their pharmacologic activities (18). As a non-selective beta antagonist, ICYP should bind to both beta-1 and beta-2 receptors, but inhibition of binding to these subgroups of receptors will differ if antagonists with selective subgroup activities are employed. In fact, ICYP binding to the heart where beta-1 receptors predominate (22–24) was inhibited significantly more by the beta-1 antagonist, atenolol, than by the beta-2 antagonist, ICI-118,551, and the reverse relationship in potencies was observed in the lung where beta-2 receptors predominate (25,26).

Also, stereospecificity tests were consistent with ICYP binding to the receptor. It is possible that some of the (+) ICYP in the racemic (±) ICYP bound to low affinity receptors (9,10) so that the measured "specific" binding of (±) was somewhat greater than 50% of the binding of (−) ICYP. Nevertheless, the slope of the inhibition curve of (±) ICYP was not different from that of (−) ICYP.

A correlation was shown between the beta receptor density in human atrial myocardium and circulating lymphocytes (29), but it seems unlikely that such a relationship would hold for all diseases and, in any case, the beta receptor density in lymphocytes could not reflect changes in distribution of the receptors within a heart. Employment of a $^{11}$C-labeled antagonist and positron emission tomography to depict the beta receptors in the heart has been reported only in abstract form thus far (30–33), and, even if successful, such an approach will be limited to centers with the capability of positron emission tomography. We attained the goal of scintigraphic portrayal of the beta receptors in the heart of living animals by giving 5 mCi of $^{123}$I-ICYP to dogs. To obtain well-defined images of the heart, it was necessary to suppress lung binding of ICYP

by small doses of ICI-118,551 or of timolol. After this selective inhibition of ICYP in the dog lung, binding of ICYP in the heart was inhibitable by either propranolol or timolol; the latter beta antagonist reduced the heart concentration to 18% of total binding, a level comparable to that attained in the rat. These results indicated that the ICYP portrayed in the heart was largely bound to beta receptors. The distribution of ICYP, which reflected the sites of beta receptors in the left ventricle, appeared to be diffuse.

The highly selective inhibition of lung binding of ICYP should not impair future measurements aimed at quantifying the receptors in the myocardium. The method of producing tomographic images can be adopted in any Nuclear Medicine laboratory with a scintillation camera. Moreover, an instrument capable of acquiring single photon emission data in dynamic and tomographic modes, such as SPRINT (*34*), may be able to give estimates of blood flow and of Bmax and Kd of binding following bolus injections of two doses of $^{123}$I-ICYP, one with high- and one with low-specific activity (*35–37*). Finally, the absorbed dose of radiation from the procedure should be acceptable in patients.

Ultimately, scintigraphy of the post-synaptic beta receptors may be combined with scintigraphy of the pre-synaptic neuron obtained by using $^{123}$I-MIBG (*38*) or $^{11}$C-hydroxyephedrine (*39*) to give a more complete functional picture of adrenergic neurons in the heart.

In summary, the non-selective beta antagonist ICYP appears to bind to beta receptors in vivo. The concentrations of the bound ICYP are sufficient to enable scintigraphic portrayal of the presumed beta receptors of the heart when the ICYP is labeled with $^{123}$I. The method has the potential to reveal changes in the distribution of beta receptors within the heart and changes in global concentrations of receptors, and thereby give new knowledge of the role of the adrenergic nervous system in health and disease.

## ACKNOWLEDGMENTS

## REFERENCES

1. Vatner DE, Lavallee M, Amano J, Finizola A, Homcy CJ, Vatner SF. Mechanisms of supersensitivity to sympathomimetic amines in the chronically denervated heart of the conscious dog. *Circ Res* 1985;57:55–64.
2. Kammerling JJ, Green FJ, Watanabe AM, et al. Denervation supersensitivity of refractoriness in noninfarcted areas apical to transmural myocardial infarction. *Circulation* 1987;76:383–393.
3. Inoue H, Zipes DP. Results of sympathetic denervation in the canine heart: supersensitivity that may be arrhythmogenic. *Circulation* 1987;75:877–887.
4. Gilbert EM, Eiswirth CC, Mealey PC, Larrabee P, Herrick CM, Bristow MR. β-adrenergic supersensitivity of the transplanted human heart is presynaptic in origin. *Circulation* 1989;79:344–349.
5. Fowler MB, Laser JA, Hopkins GL, Minobe W, Bristow MR. Assessment of the β-adrenergic receptor pathway in the intact failing human heart: progressive receptor down-regulation and subsensitivity to agonist response. *Circulation* 1986;74:1290–1302.
6. Bristow MR, Ginsburg R, Umans V, et al. $\beta_1$- and $\beta_2$-adrenergic-receptor subpopulations in nonfailing and failing human ventricular myocardium: coupling of both receptor subtypes to muscle contraction and selective $\beta_1$-receptor down-regulation in heart failure. *Circ Res* 1986;59:297–309.
7. Homcy CJ, Strauss HW, Kopiwoda S. Beta receptor occupancy: assessment in the intact animal. *J Clin Invest* 1980;65:1111–1118.
8. Hughes B, Marshall DR, Sobel BE, Bergmann SR. Characterization of beta-adrenoreceptors in vivo with iodine-131-pindolol and gamma scintigraphy. *J Nucl Med* 1986;27:660–667.
9. Van Dort ME, Gildersleeve DL, Wieland DM. A rapid high yield synthesis of no-carrier-added (−) [I-123]iodocyanopindolol. *Int J Appl Radiat Isotop* 1991;42:309–311.
10. Hoyer D, Engel G. Binding of $^{125}$I-cyanopindolol to beta-1-adrenoceptors in a high and low affinity state. *J Recept Res* 1983;3:45–59.
11. Freed BR, Gelbard AS. Distribution of $^{13}$N following intravenous injection of [$^{13}$N]ammonia in the rat. *Can J Physiol Pharmacol* 1982;60:60–67.
12. Ferrone RA, Walsh GM, Tsuchiya M, Frohlich ED. Comparison of hemodynamics in conscious spontaneous and renal hypertensive rats. *Am J Physiol* 1979;236:H403–408.
13. Sisson JC, Lynch JJ, Johnson J, et al. Scintigraphic detection of regional disruption of adrenergic neurons in the heart. *Am Heart J* 1988;116:67–76.
14. Keukens HJ, Aerts MM. Determination of residues of carazolol and a number of tranquilizers in swine kidney by high-performance liquid chromatography with ultraviolet and fluorescence detection. *J Chromatogr* 1989;464:149–61.
15. Munson PJ, Rodbard D. Ligand. A versatile computerized approach for characterization of ligand-binding systems. *Anal Biochem* 1980;107:220–239.
16. DeLean A, Munson PJ, Rodbard D. Simultaneous assay of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose-response curves. *Am J Physiol* 1978;235:E97–E102.
17. Laduron PM. Stereospecificity in binding studies: a useful criterion though insufficient to prove the presence of receptors. *Biochem Pharmacol* 1988;37:37–40.
18. Weiner N. Drugs that inhibit adrenergic nerves and block adrenergic receptors. In: Gilman AG, Goodman LS, Rall TW, Murad F, eds. *The pharmacological basis of therapeutics*. New York: Macmillan Publishing Co.; 1985:194.
19. Staehelin M, Simons P, Jaeggi K, Wigger N. CGP-12177: a hydrophilic β-adrenergic receptor radioligand reveals high affinity binding of agonists to intact cells. *J Biol Chem* 1983;258:3496–3502.
20. Hertel C, Müller P, Portenier M, Staehelin M. Determination of the desensitization of β-adrenergic receptors by [$^3$H]CGP-12177. *Biochem J* 1983;216:669–674.
21. Bilski AJ, Halliday SE, Fitzgerald JD, Wale JL. The pharmacology of a $\beta_2$-selective adrenoceptor antagonist (ICI 118,551). *J Cardiovasc Pharmacol* 1983;5:430–437.
22. Minneman KP, Hegstrand LR, Molinoff PB. Simultaneous determination of beta-1 and beta-2-adrenergic receptors in tissues containing both receptor subtypes. *Mol Pharmacol* 1979;16:34–46.
23. Buxton ILO, Brunton LL. Direct analysis of β-adrenergic receptor subtypes on intact adult ventricular myocytes of the rat. *Circ Res* 1985;56:126–132.
24. Murphree SS, Saffitz JE. Delineation of the distribution of β-adrenergic receptor subtypes in canine myocardium. *Circ Res* 1988;63:117–125.
25. Rugg EL, Barnett DB, Nahorski SR. Coexistence of beta$_1$ and beta$_2$ adrenoceptors in mammalian lung: evidence from direct binding studies. *Mol Pharmacol* 1978;14:996–1005.
26. Dickinson K, Richardson A, Nahorski SR. Homogeneity of beta$_2$-adrenoceptors on rat erythrocytes and reticulocytes. *Mol Pharmacol* 1981;19:194–204.
27. Loevinger R, Berman M. A revised schema for calculating the absorbed dose from biologically distributed radionuclides. *MIRD pamphlet no. 1, revised*. New York, NY: Society of Nuclear Medicine, 1976:10.
28. Hammond HK, White FC, Buxton ILO, Saltzstein P, Brunton LL, Longhurst JC. Increased myocardial β-receptors and adrenregic responses in hyperthyroid pigs. *Am J Physiol* 1987;252:H283–290.
29. Brodde O-E, Kretsch R, Ikezono K, Zerkowski H-R, Reidemeister JC. Human β-adrenoceptors: relation of myocardial and lymphocyte β-adrenoceptor density. *Science* 1986;231:1584–1585.
30. Delforge J, Nakajima K, Syrota A, et al. PET investigation of β-adrenergic receptors using CGP 12177. *J Nucl Med* 1989;30:825.

31. Seto M, Syrota A, Crouzel C, et al. Beta adrenergic receptors in the dog heart characterized by ¹¹C-CGP 12177 and PET. *J Nucl Med* 1986;27:949.

32. Syrota A, Marty J, Seto M, et al. Halothane-induced decrease of ¹¹C-CGP 12177 binding to myocardial beta adrenergic receptor demonstrated by PET in the dog. *J Nucl Med* 1988;29:940.

33. Law MP, Burgin J. Evaluation of CGP-12177 for characterization of beta-adrenergic receptors by PET: in vivo studies in rat. *J Nucl Med* 1989;30:766–767.

34. Rogers WL, Clinthorne NH, Shao L, et al. SPRINT II: a second generation single photon ring tomograph. *IEEE Trans Med Imaging* 1988;291:297.

35. Frey KA, Hichwa RD, Ehrenkaufer RLE, Agranoff BW. Quantitative in vivo receptor binding. III. Tracer kinetic modeling of muscarinic cholinergic receptor binding. *Proc Natl Acad Sci USA* 1985;82:6711–6715.

36. Mintun MA, Raichle ME, Kilbourn MR, Wooten GF, Welch MJ. A quantitative model for the in vivo assessment of drug binding sites with positron emission tomography. *Ann Neurol* 1984;15:217–227.

37. Huang S-C, Bahn MM, Barrio JR, et al. A double-injection tech-nique for in vivo measurement of dopamine D2-receptor density in monkeys with 3-(2'-[¹⁸F]fluoroethyl)spiperone and dynamic positron emission tomography. *J Cereb Blood Flow Metab* 1989;9:850–858.

38. Sisson JC, Shapiro L, Meyers L, et al. Metaiodobenzylguanidine to map scintigraphically the adrenergic nervous system in man. *J Nucl Med* 1987;28:1625–1636.

39. Schwaiger M, Kalff V, Rosenspire K, et al. Noninvasive evaluation of the sympathetic nervous system in the human heart by positron emission tomography. *Circulation* 1990;82:457–464.