だ，もちろん，それらの分子プローブを機能評価し有効性を証明するために，動物実験についても，マウス，ラット，ウサギ，マーモセット，マカク属のサルを用いて，麻酔下・無麻酔下の両方でマイクロPET研究を行える技術基盤を有する世界でもユニークな研究機関である．さらに，臨床研究へと展開していくために，先端医療センター，大阪市立大学，国立がんセンター，大阪大学，京都大学，兵庫医科大学，岡山大学，徳島大学，大分大学等々と，臨床研究ネットワーク作りを行っている．

当センターでは，Fig. 1のようなミッションと様々な共同研究先をも包含した研究テーマを推進するために，現在6チームと3ユニットの組織で総勢約260名の登録メンバーで研究を行っている．化学と動物実験に秀でている研究機関であり，世界有数のユニークな研究センターである．本センターでは，多くの類縁構造化合物に同様に標識できる方法[1]や2段階の連続反応システム[2]を開発しており，その波及効果は単なる数字に反映されない大きな力である．

これらの新規開発分子プローブを用いて，1）抗体やペプチドの標識[3]により，がん種別イメージング，動脈硬化プラークの早期イメージング，膵臓インシュリン産生細胞のイメージングに成功し，これらの一部は，既に臨床研究にあがっている．2）分子イメージングによる再生医療の高精細モニタリングができることをパーキンソン病モデルサルで実証．3）遺伝子治療の効果モニタリングにも利用．4）情動に係わる女性ステロイド生合成酵素aromataseの分子イメージングによりよいプローブを開発．[4-6]扁桃体，視床下部に加え，報酬系の脳内側座核への局在証明．これも今年から臨床研究を始めており，情動障害の脳科学研究に向かっている．5）薬物動態では，動物からヒトまで，経口投与からの腸管吸収，標的臓器・細胞や薬物代謝の中心である肝細胞への取り込み過程，胆汁排泄・尿中排泄過程が定量的に追え，係わるトランスポーターの同定により，同様な構造を有する新規薬物候補物質の体内動態が予測できること．[7-12]当然，DDS研究も直截にヒト・動物で行うことができ，インシュリン等の経口製剤開発研究，[13]核酸医薬のDDS研究[14]等を主軸にしている．6）遺伝子改変マウスなどの小動物PETにおいても，計測の高精度化により定量的な計測が可能であること．[15] 7）認知症，[16,17]緑内障，[18]慢性疲労症候群などの症状進行の鍵となる物質や神経炎症，神経伝達系のイメージングによる早期診断・鑑別診断．介入による先制医療へ進めている．8）コンプトンカメラを用いたgamma-ray emission imaging（GREI）の方法論を進め複数分子同時イメージングの理論的実装化ができたこと．[19]などの成果が次々に論文になっており，いくつかは論文誌の表紙[6,13,15,20]として取り上げられるなど，非常に注目されている．

2. 創薬における分子イメージング研究の寄与

2-1. 医療応用・創薬研究への分子イメージングの役割　医療応用，とくに，創薬部分では，動物・ヒトで同一個体での薬物動態を研究できること，ヒトにおける薬物送達システム（drug delivery system; DDS）の開発，標的分子以外への集積を知ることによる副作用情報，治療効果の標的分子に対する定量的把握，ゲノム・SNPs情報との適合と乖離，薬物相互作用のメカニズム解明等々，創薬に果たす分子イメージングの役割は多大である．

PETの手法は，超高比放射能の標識分子を作製して用いる（1–10 Ci/μmol）ので，通常のヒトPETでは，吸収線量を減らし安全域を広く行うために，1–10 nmol，すなわち，分子量が400の化合物であれば，0.4–4.0 μgが一人に投与される絶対量である．当然100 μg以下であるし，薬効量の100分の1以下でもある．ここに，マイクロドーズ臨床試験の範疇で，ヒトでの標的臓器での薬物動態やDDS，薬効指標評価試験が成立する．

ただ，研究の現状をみると，どのような研究体制で最適な分子プローブのレパートリーを作るか，どのように創薬の早い時期で分子イメージングの導入を図るか，日本では300を超える臨床PET施設のほとんどが［^{18}F］FDGを用いたがん診断・検診を行っているだけなので，環境整備をしていかないと，実は，PETマイクロドーズ臨床試験をどんどんやっていけるわけではない．レパートリー充実には，とくに，医療応用・創薬への分子標的を研究しているグループ，標的を定量し制御する化合物を持つグループのメンバーに分子イメージングの有用性とそのためにどのような開発が必要なのかをよく理解してもらう必要があり，いくつかのコンソーシアム，そして，それを統合するような仕組みを考えて

いくことが必須である．

2-2. 薬物動態予測研究とマイクロドーズ・早期探索的臨床試験 薬物動態は，規程因子である薬物トランスポーターの種別・活性と細胞内での代謝酵素（主にはP450）の種別・活性により，より確実な予測が立てられる．トランスポーターには，吸収や細胞内取り込み，細胞外への排出ポンプ，胆汁や尿中排泄の大きく分けて3分類が存在し，これまでに10数種類がクローニングされ，多くの遺伝子多型も知られている．この研究領域で世界をリードしてきており創薬研究の雄である東京大学大学院薬学系研究科の杉山雄一教授を始めとする薬物動態・DDS研究者と共同で，既知の薬物をPET用に標識しトランスポーター機能を追跡する研究を推進している．その中で，われわれが開発してきた中枢神経系プロスタサイクリン受容体を定量化できたアゴニストであり強い神経細胞保護作用を有する15*R*-[^{11}C]TIC-メチルエステルを用いて，マウス，ラット，サル，ヒトでの肝細胞取り込み，胆汁排泄過程のトランスポーター研究[7,12]（ヒト事例をFig. 2に示す[12]）が進展し，また，高血圧治療薬であるテルミサルタンを［^{11}C］標識して，動物研究[9]からマイクロドーズ臨床試験（New Energy and Industrial Technology Development Organization（NEDO）の研究費による，杉山雄一教授が代表研究者）を行っている．一方，摂南大学薬学部山下伸二教授の研究室，大阪市立大学医学研究科核医学教室とともに，経口投与の腸管吸収PET研究も進めている．[10,11] また，多くの医薬品企業とも連携し，様々な薬物動態研究を推進している（Fig. 3）．

2-3. 薬効評価と早期診断，細胞種別診断，theranostics 多くのイメージングバイオマーカーが開発されて，それらを用いた新機軸の創薬研究が行われている．受容体やトランスポーター，酵素が薬物標的である場合は，受容体・トランスポーター・酵素活性部位制御ポケット占有率から有効投与量が

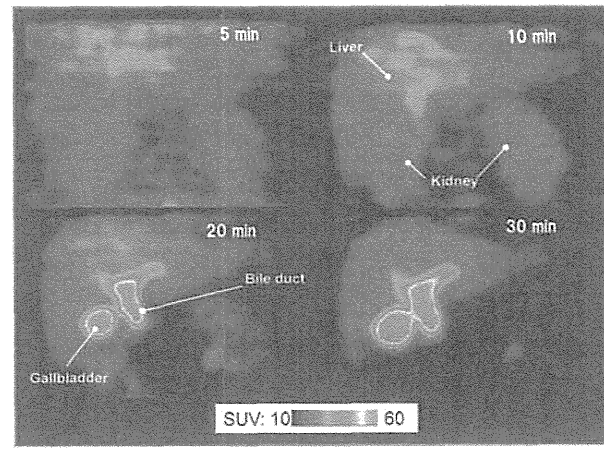


Fig. 2. Biliary Excretion Kinetics in Human: PET Study with *i.v.* Injection of 15*R*-[^{11}C] TIC-Methyl Ester

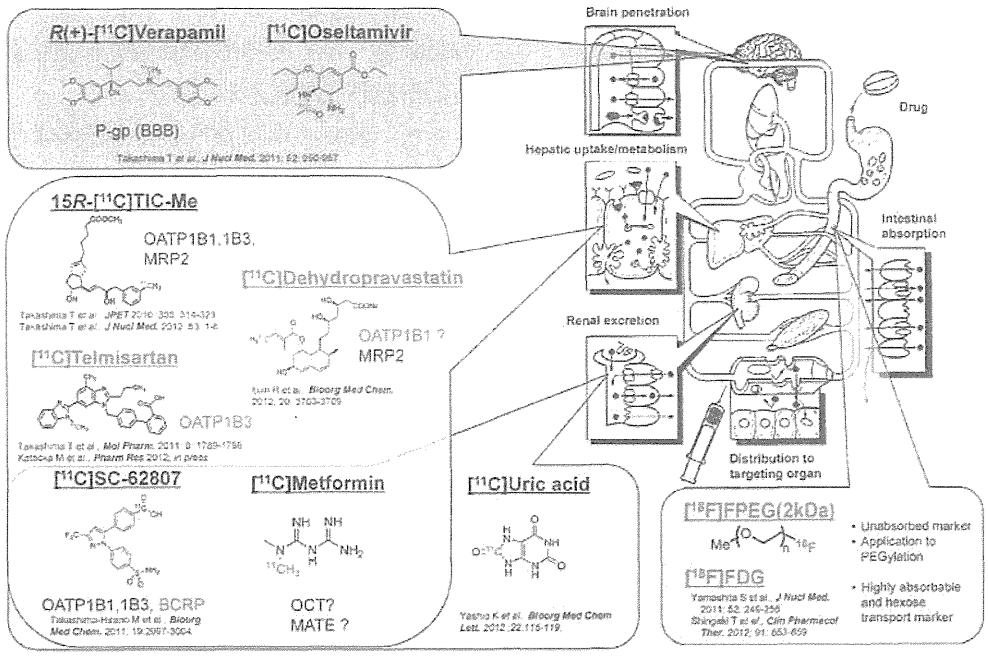


Fig. 3. Transporter Molecular Probes Used in PK-PET Studies

計算され，これに基づき投与計画が作成され，SNPs の研究等との情報を統合し，個別医療に対する有効な取り組みが行われ，この分子イメージングを活用した薬効評価は，多数の実例をもって，本手段の定着化に向かうと考えられる．

最初は，抗がん剤やがん治療における［^{18}F］FDG をバイオマーカーとした開発が試みられた．一定の成果は認められるものの，治療早期には，必然的に炎症が起き，マクロファージや浸潤リンパ球が［^{18}F］FDG を多く取り込むために，治療早期の効果を知ることが困難で，投与開始後，1-2 ヵ月での［^{18}F］FDG-PET での判定が行われてきた．[21] 最近の研究では，［^{18}F］FDG-PET シグナルが何を実際に表現しているかという質的な研究も進められている[22,23]が，このことは，それぞれのがん細胞に特異性の高いイメージングバイオマーカーの開発と炎症に特異的なイメージングバイオマーカー開発研究を進展させてきた．

また，一方で，病態生理・病理と結びついた組織内蓄積物を分子イメージングにより定量化し，それらをバイオマーカーとして早期診断等に応用し，また，薬物の薬効解析を行うという研究も盛んである．とくに，軽度認知障害からアルツハイマー型認知症へは，アミロイドイメージング PET 臨床研究が行われ，アミロイドβ抗体，ワクチン，セクレターゼ阻害剤などの薬効効果判定指標として用いている．本路線では，同じく認知症における神経原線維変化のリン酸化タウタンパク蓄積のイメージング，パーキンソン病やレビー小体型認知症のα-シヌクレイン蓄積のイメージング，ハンチントン舞踏病や脊髄小脳変性症などのポリグルタミン蓄積のイメージングなどが進められている．

また，中枢神経関連疾患では，神経変性や脱随に付帯して起こるマイクログリアやアストログリアの活性化を見る末梢性ベンゾジアゼピン受容体（最近ではトランスロケータータンパクと考えられている）発現のイメージングが進められて，われわれも，緑内障[18]や片頭痛[24]に適用する研究を行ってきた．脳内の免疫情報シグナルは，多くの精神神経疾患や神経好性ウイルスの潜伏・再活性化などの病態で大いなる情報をもたらすことが期待されている．炎症，発熱，痛みや睡眠に係わるプロスタグランジンやロイコトリエン等のアラキドン酸カスケード系物質の生合成酵素シクロオキシゲナーゼ cyclo-oxygenase（COX-1, COX-2）のイメージングにも成功し，[25] 現在，認知症・軽度認知障害，緑内障，慢性疲労症候群及び類縁疾患（機能性身体化症候群），虚血性疾患後神経障害の脳内炎症についても臨床研究を進めている．

分子イメージングの方法論をさらに汎用化・網羅化するためには，高分子物質にも標識が行われ，本来の機能・生理活性・結合能などを損なわない方法論の開発が重要である．

タンパク質では，主には，幹細胞認識のための抗体，細胞障害性ヘルパー T 細胞の認識抗体，扁平上皮がんに対する抗 EGFR 抗体，抗ハーセプチン抗体などを標識して細胞種別 PET 研究を行っている．国立がん研究センター中央病院（藤原康弘副院長，田村研治医長，栗原宏明先生ら）と共同研究を行い，抗ハーセプチン抗体による乳がんのイメージングには成功している（Fig. 4）．治療薬を標識して，同時に診断薬（この場合は，がん細胞種別判別薬）としても利用するものについては，近年，theranostics という造語がある．

ペプチドに関しては，サイトカイン・ケモカイン，神経内分泌ペプチドを始め多くの活性ペプチドが標的であり，ソマトスタチンやその代謝耐性型のオクトレオタイド，セクレチン，グレリン，インシュリンなどの ^{68}Ga 標識を進めた．さらに，核酸配列や糖鎖への標識には，核酸化学，糖鎖工学，ケミカルバイオロジーの最先端化学者と共同して，新規標識法を開拓し PET 研究を進めている．[14,26,27] また，同様な方法論は，細胞自身の短時間・穏和な条件での標識により，細胞の動態そのものを追跡することも可能にした．[28,29]

3. NEDO プロジェクトによるカセットドーズ・マイクロドーズ臨床治験

NEDO による研究費「NEDO 橋渡し促進技術開発/マイクロドース（MD）臨床試験を活用した革新的創薬技術の開発」プロジェクト（杉山雄一教授代表，山下伸二教授副代表）により，わが国初の新規化合物を包含したカセットドーズ・マイクロドーズ（micro-dose; MD）臨床治験を行った．医薬品医療機器総合機構（Pharmacenticals and Medical Devices Agency; PMDA）の新規に始まった事前面談・薬事戦略相談対面助言第 1 号でもあった．アロマ

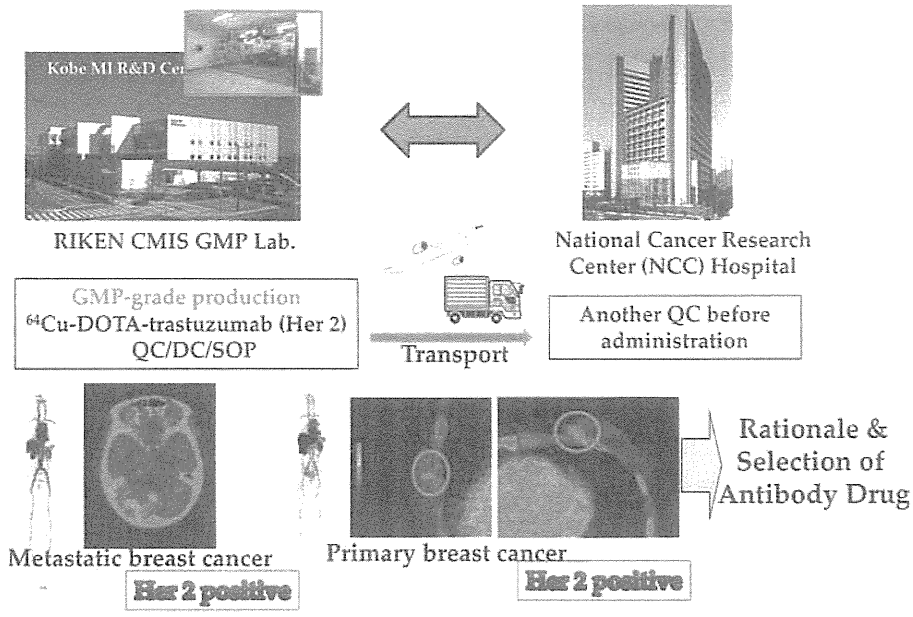


Fig. 4. Clinical Trials between RIKEN CMIS and NCC Tokyo

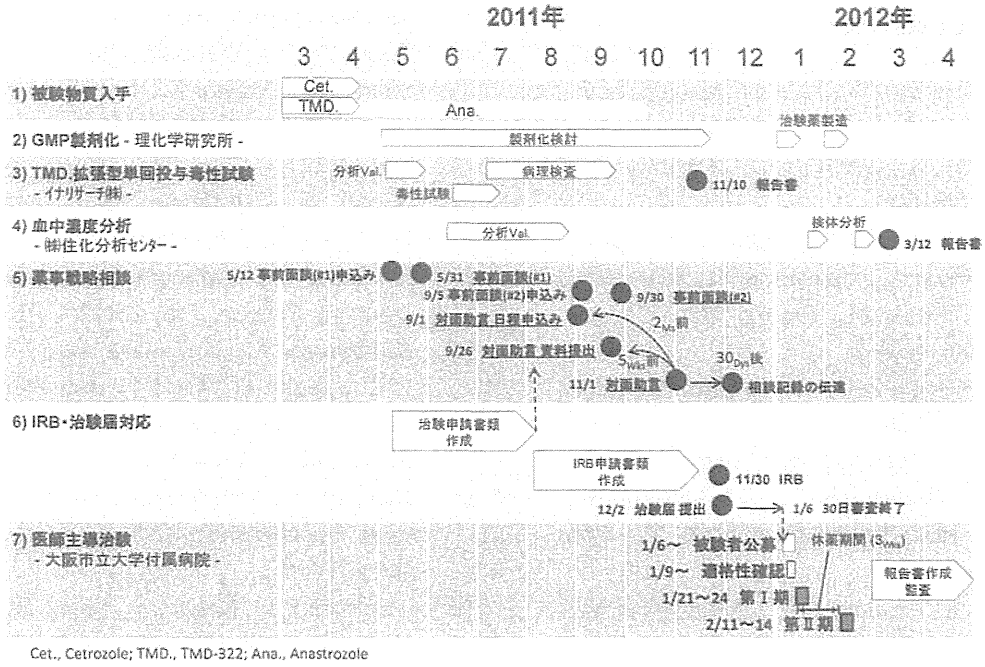
ターゼ阻害剤の MD カセット投与臨床試験（医師主導治験）として，被験物質のヒトにおける薬物動態に関する情報を医薬品開発の初期段階に得ることを目的とした．

アロマターゼは，男性ホルモン（テストステロン等）を女性ホルモン（エストロジェン等）に転化する性ステロイド生合成の最後の部分の酵素であり，P450 の一種である．当酵素は，ホルモン感受性乳がん細胞に高い発現を示し，産生するエストロジェン等により autocrine, paracrine 作用で乳がん細胞の増殖を促進する．そのため，アロマターゼ阻害剤はホルモン感受性乳がん治療薬として多数の化合物が開発されている．特に，閉経後のホルモン感受性乳がんの第一選択薬剤である．ただ，その副作用として，抑うつ状態や情動不安定が挙げられ，脳内移行性が問題となっていた．また，乳がんの好発転移臓器の 1 つに脳が知られ，この際は，脳へ移行するアロマターゼ阻害剤が必要になるという相反する薬物選択が必須となる．われわれは，前述のように，脳内にアロマターゼの高発現部位を発見し，情動障害等における PET 研究を目指して研究を行ってきた．[4-6] 最近，本研究は，MD 臨床試験ガイダンスに則った拡張型単回投与試験による安全性評価を経て，倫理委員会の同意を得て，ヒト研究へ進み，健常人において，被曝線量評価（ドージメトリー）等の安全性評価も終了し，脳内アロマターゼ研究に歩を進めている．

今回の試験では，われわれが新規に開発した PET 分子プローブであるセトロゾールと TMD-322 を候補化合物とした．これらに，既存の乳がん治療薬で脳内移行性が低いと考えられるアナストロゾールを参照薬として加えた．セトロゾールと TMD-322 は，既にサルの PET 研究では，これまでの化合物に比較し，脳内移行性がよく，脳内の視床下部・扁桃体・側座核など，情動や自律神経系，神経–免疫–内分泌調節機構に係わる重要な部分に多く存在するアロマターゼの量についての情報を与えてくれる優秀な分子プローブであることを明らかにしている．[4-6] 今回は，この 3 剤をカセットドーズで経口投与と静脈内投与試験の双方で，腸管吸収や血中動態等を明らかにすることを目的とした．6 例の健常被験者（20–40 歳の男性）に対し，無作為割り当てで 2 群に分け，経口投与から静脈内投与の群と静脈内投与から経口投与の群で 20 日のインターバルを置いた．

PMDA による薬事戦略相談対面助言では，本臨床治験の進め方全般，とくに，用量設定に関する見解に重要な助言を得て，また，非常に円滑に治験届を受理して頂き，企画から治験終了まで 1 年以内という速さで医師主導治験を行うことができた（Fig. 5）．

カセットドーズで MD 臨床試験を行う場合，と



Cet., Cetrozole; TMD., TMD-322; Ana., Anastrozole

Fig. 5. Schedule of Cassette-dose & Micro-dose Clinical Trials with Aromatase Inhibitors (in Japanese)

くに，経口投与からの腸管吸収量が少ないと血液中での化合物量を有意に定量できるかどうかの検出感度が問題になるが，今回の株式会社住化分析センターの卓抜した LC-MS-MS 技術により，各経時点でのデータも精確に検出できた．複数の新規候補化合物のヒトにおける比較試験としては，大成功と言える結果が得られ，現在，原著論文としてまとめている．

4. 分子イメージングを活用できるトランスレーショナル研究・臨床試験の環境整備

医療・創薬イノベーションに果たす分子イメージングの役割は多大であることを述べてきた．ただ，現状をみると，いかに（どのような研究体制で）最適な分子プローブのレパートリーを作るか，どのように医療や創薬の早い時期で分子イメージングの導入を図るか，とくに臨床の場を整備していくことが重要である．

日本には既に PET や PET-CT を有する臨床施設が 300 を超えるほど存在するが，多くは，[^{18}F]FDG を用いたがん診断や検診を行っているのみの機関である．また，この中で，治験を受け入れることのできる病院も少ない．ここに，マイクロドーズ・早期探索的臨床試験を始め，分子イメージング活用先制医療や分子イメージング活用創薬のための施設作りをしていくことは，国策として推進する以外には将来の展望はない．創薬ビジネスで負け続けのわが国が先行できるラストチャンスである．厚生労働省では，既に，医薬品や医療機器開発に対する早期探索的臨床試験拠点を選び，その方向に動いている．

われわれは，そのような訴えを行っていくとともに，いくつかの連携を模索していくことを構想している．その連携には，2 通りのやり方がある．われわれのような分子プローブ合成のできる施設に臨床施設を加えて集中的な施設を作るやり方と，既に存在する先行的臨床施設に連携施設を作り出張所的にものづくりや実際の研究・試験・治験を動かしていくやり方の 2 通りであり，現在の臨床の場の実情を鑑みると，この双方が必要であるという考えに至る．先端医療振興財団・先端医療センター，大阪市立大学，国立がん研究センター，東京大学，摂南大学，兵庫医科大学，岡山大学，京都大学，大阪大学，神戸大学，神戸中央市民病院，大分大学，徳島大学等々と，臨床研究ネットワーク作りについて協議している．

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Special Issue Review

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Endogenous compounds labeled with radionuclides of short half-life—some perspectives†

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In the article, the strategy and synthesis of some endogenous compounds labeled mainly with ^{11}C are presented. There are some examples illustrating how endogenous labeled compounds in connection with positron emission tomography have unique properties to describe various biological processes, and a few examples of the use of tracers labeled with ^{13}N and ^{15}O are also discussed.

Labeled endogenous compounds may be an important asset to describe the conditions and the status of biological systems and might therefore be a key for the future search of individualized medicine.

Keywords: endogenous compounds; ^{11}C-amino acids; carbohydrates; fatty acids; ketone bodies; neurotransmitters

Introduction

A statement by the French physiologist Claude Bernard (1813–1878) is enforcing why the development and use of endogenous tracers are important for the future of the positron emission tomography (PET) technology. 'Un jour nous saurons la physiologie lorsque nous pourrons suivre pas à pas une molécule de carbone ou d'azote, faire son histoire, raconter son voyage dans le corps d'un chien, depuis son entrée jusqu'à sa sortie' which in free translation is 'One day we will know physiology when we are able to follow step by step a molecule of carbon or nitrogen, to make its history, by describing its trip in the body of a dog, from its entrance to its exit'.

The development of PET has been very substantial in the last decades. PET is a noninvasive molecular imaging technique mainly used for study and visualization of biological process by using molecules, which have been labeled with short-lived positron emitters.[1] The use of ^{18}F with its 110-min half-life has been successful and mainly with applications using 2-[^{18}F]-fluorodeoxyglucose (FDG), which is a modified glucose analog.[2,3] There are a number of other positron emitting radionuclides that have been utilized such as ^{64}Cu and ^{68}Ga in a number of clinical applications.[4]

Returning back to Claude Bernard, most of the endogenous compounds consist of the elements carbon, oxygen, and nitrogen and thus the positron emitting radionuclides ^{11}C, ^{13}N, and ^{15}O with their short half-lives (Table 1) are ideal for radiolabeling endogenous substances found in organisms, tissues, or cells and bio-systems. This combined with the very high sensitivity that PET has for measuring tissue concentrations of molecules *in vivo* makes this technology unique.

The PET technique with these radionuclides might be used not only to track molecules but also even to track the fate of atoms in a molecule in order to provide metabolic and mechanistic information. The use of specific position labeled endogenous tracer is unique in its ability to give *in vivo* information of various processes within the time window imposed by the half-lives.[5]

A limitation of the technology at present is *how to get easy and reliable access to these labeled molecules, the tracers*, a sentence that might be true for the whole field of PET. One big challenge for PET is thus development of rapid and robust synthetic methods allowing an efficient and reproducible incorporation under good manufacturing conditions of the short half-lived radionuclides into the endogenous molecules of interest and allowing the validation of such tracers.

Herein, some examples will be presented where the use of labeled endogenous PET tracers serves to illustrate the ability to visualize and quantify metabolic pathways allowing mechanistic studies to explore *in vivo* biochemistry and utilizing the strengths of the PET technique. Although CNS is the focus of this special issue, the fact is that most endogenous compounds are applicable in biological systems, in general, and this is why some examples outside the CNS will be discussed.

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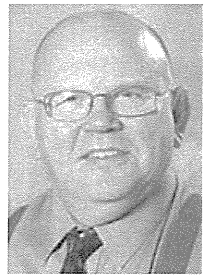
Biography

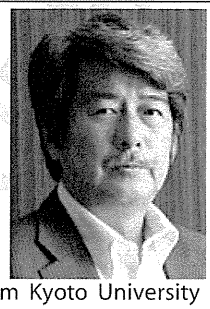


Bengt Långström was born in Boden, Sweden, in 1943. He has been active in the field of Molecular Imaging for around 40 years and is currently a Professor Emeritus at the Uppsala University and a guest professor at the University Southern Denmark, Odense. He has served science as a Professor of the Department of Organic Chemistry, Uppsala University building up Uppsala University PET-Centre and was the director until the PET center went into a joint venture with Amersham Health that later became GE Healthcare. He has over 800 peer-reviewed publications and more than 60 patents. After leaving as a CSO, he also became guest professor at the Imperial College, London (UK). He is the founder and the Chairman of the Board of Directors of Bencar AB (Sweden) working on new concepts for tracer production. He has received several awards for his research, and in 2010, for example, the Society of Nuclear Medicine's Georg Charles de Hevesy Nuclear Pioneer Award for his contributions to the nuclear medicine profession.

Biography

Farhad Karimi was born in Tehran, Iran, in 1965. He is currently a guest researcher at the Uppsala University. He received his PhD in 2002, and he then worked as senior scientist at the GE Healthcare/Uppsala Imanet (Sweden), Siemens Molecular Imaging Biomarker Research division/ University of California (Culver City, CA, USA) and Brigham and Women's Hospital/ Harvard Medical School (Irvine, MA, USA). He joined Bencar AB (Sweden) in 2012. He has over 40 publications and patents in the field of PET radiochemistry.

Biography



Yasuyoshi Watanabe was born in Kanazawa, Japan, in 1951. He is currently the director of RIKEN Center for Molecular Imaging Science and, concurrently, the professor of the Department of Physiology, Osaka City University Graduate School of Medicine. His background is biochemistry, neuroscience, and PET molecular imaging. After graduation from Kyoto University Faculty of Medicine in 1976, he defended his PhD thesis from Kyoto University Faculty of Medicine in 1980. He started to organize his research group at Kyoto University and then Osaka since 1984 as an Assistant Professor in Osaka Medical College. Since 1985, he started to collaborate with Prof. Bengt Långström at Uppsala University and has continued the mutual collaboration since then. From 1993 to 1998, the collaboration project on Molecular Imaging between Watanabe's team in Osaka Bioscience Institute and Långström's team in Uppsala University PET Centrum was selected and supported by Japan Science and Technology Agency as 'Subfemtomole Biorecognition Project'. His contribution to this field is shown by 341 original articles and 80 reviews and proceedings and more than 60 patents.

Some examples of biological applications in *in vivo* biochemistry/biology using endogenous compounds

Carbon-11

The carbon isotope ^{11}C has been used in many *in vivo* pharmacological and biochemical studies. There have been attempts to incorporate of the radionuclide in selected positions within tracer molecules in various man-made compounds targeting receptors, enzymes, and various other types of protein binding sites.[6] We will focus on the preparation of endogenous compounds such as glucose, fatty acids (acetate), lactate, pyruvate, ketone bodies, and urea in order to explore and quantify metabolic pathways. The concept of position specific labeling has been applied to track not only the molecule but actually even to follow the fate of the labeled atom itself, the radionuclide, as Bernard said. One example is the position-specific labeling of [1-^{11}C or 2-^{11}C]-acetate and its use in a study of heart energy metabolism.[5]

It is clear that there is a significant difference in the fate of radioactivity regarding acetate degradation in the metabolic pathway depending on the position of the label. As shown in Figure 1, there is no significant difference in the summation images of either ACE ([1-^{11}C]-acetate) or ACT ([2-^{11}C]-acetate) uptake in the myocardium of a pig. However, if one compares ACE and ACT time activity curves, there is a significant difference between the two molecules related to the different metabolic fate of the two carbons in the acetate molecule in the tricarboxylic acid cycle.

Similar studies have been performed using 1-^{11}C-labeled and 3-^{11}C-labeled L-lactate[7,8] and pyruvate.[9] Labeling not only acetate specifically with ^{11}C but also long-chain fatty acids offers another possibility to explore and understand *in vivo* energy metabolism using PET.[10]

The use of ^{11}C-labeled position-specific endogenous glucose molecules as well as ketone bodies gives another important angle on energy metabolism, emphasizing the possibility to track the fate of an atom in line with Bernard.[11]

Normally, the brain's fuel is glucose, but during fasting, it increasingly relies on ketone bodies (β-hydroxybutyrate, acetoacetate, and acetone) produced in the liver mitochondria from fatty acid β-oxidation. [^{11}C]Acetoacetate metabolism was measured in the brain, heart, and tissues in the head and neck area. The results indicate that [^{11}C]acetoacetate utilization by rat brain increases approximately sevenfold to eightfold under dietary conditions raising plasma ketones. Combining PET methodology and dietary approaches to manipulate ketone production provides a minimally invasive approach to studying brain energy metabolism and has potential application in studies of brain ketone metabolism in human health and disease.[12]

The elegant concept of metabolic trapping explored by Sokoloff using 2-^{14}C-2-deoxyglucose serving as the lead for [^{18}F]FDG has conquered the PET field giving a beautiful example of the autoradiography/contrast media technology, as a way of making snapshots of metabolic processes and applications *in vivo*.[13,14] There is however an increasing awareness that maybe FDG does not reflect all aspects of our understanding of energy metabolic processes.[15]

Another interesting example in this perspective of energy metabolism and reflecting various pathways is the work using position-specific labeled acetylcarnitines to explore *in vivo* biochemistry. In this study, three different ^{11}C-labeled acetylcarnitines

Table 1. Radionuclides suitable to label endogenous compounds

Radionuclides	Half-life (min)	Target	Nuclear reaction	Product	Maximum positron energy (MeV)	Decay product
^{11}C	20.4	$\frac{N_2(O_2)}{N_2(H_2)}$	^{14}N (p, α) ^{11}C	$\frac{[^{11}C]CO_2}{[^{11}C]CH_4}$	0.96	^{11}B
^{13}N	9.96	$\frac{H_2O}{H_2O\ (EtOH)}$	^{16}O (p,α) ^{13}N	$\frac{[^{13}N]NO_x}{[^{13}N]NH_3}$	1.19	^{13}C
^{15}O	2.07	N_2 (O_2)	^{14}N (d,n) ^{15}O	$[^{15}O]O_2$	1.72	^{15}N

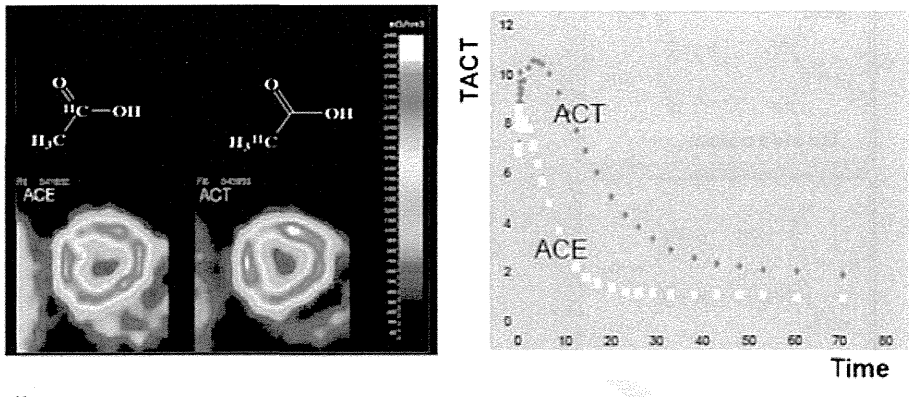


Figure 1. Position-specific ^{11}C-labeled acetates are illustrating different metabolic fate of the label in the myocardium of the pig heart. A presentation of the time activity curves (right) and the summation image (left). This figure is available in colour online at www.interscience.wiley.com/journal/jlcr

were synthesized, ACC (N-^{11}C-methyl), ACN (2-position of the acetyl group), and finally, ACM (1-position of the acetyl group). In Figure 2, the summation images of these three tracers in the same monkey are presented.[16,17]

Later, human studies were performed comparing age-matched healthy volunteers and patients with chronic fatigue syndrome using the ACM molecule that resulted in interesting differences between patients and healthy controls.[18] The study was followed up by using ^{14}C-labeled ACM in a mouse demonstrating that the ^{14}C-label ended up in the excitatory amino acid pool.[19] So, the question is what is the pathway differentiating the healthy and the patients with chronic fatigue syndrome. Scheme 1 illustrates the potential interactions between different metabolic pathways interacting with the tricarboxylic acid cycle. These data may indicate that it will be difficult to use only one tracer each time in order to understand and describe the fate of the atoms as Bernard stated.[20]

Amino acids as precursors to track neurotransmitter synthesis, the use of position labeling to probe metabolic pathways

The main route to study neurotransmitter functions in the CNS has been explored by specific labeled compounds used for receptor and enzymatic studies. This strategy is not discussed; instead, the focus will be on the use of amino acids as precursors for neurotransmitters and using that information in order to obtain ways to have dose and biological information. Using multi-enzymatic synthetic procedures, a series of ^{11}C-labeled amino acids were produced. This approach combined with the position-specific concept led to the preparation of 1-^{11}C-labeled and 3-^{11}C-labeled, L-phenylalanine, L-tyrosine, L-DOPA, L-tryptophan, and 5-hydroxy-L-tryptophan (Scheme 2). These molecules were then used to illustrate the metabolic fate of the position specific atom.[21]

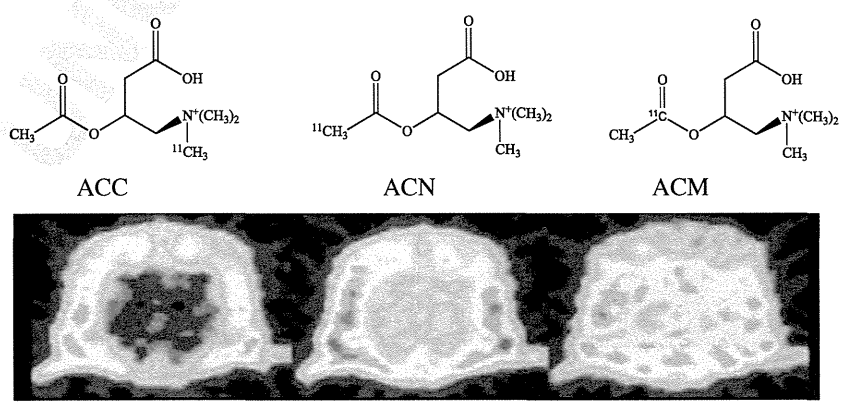
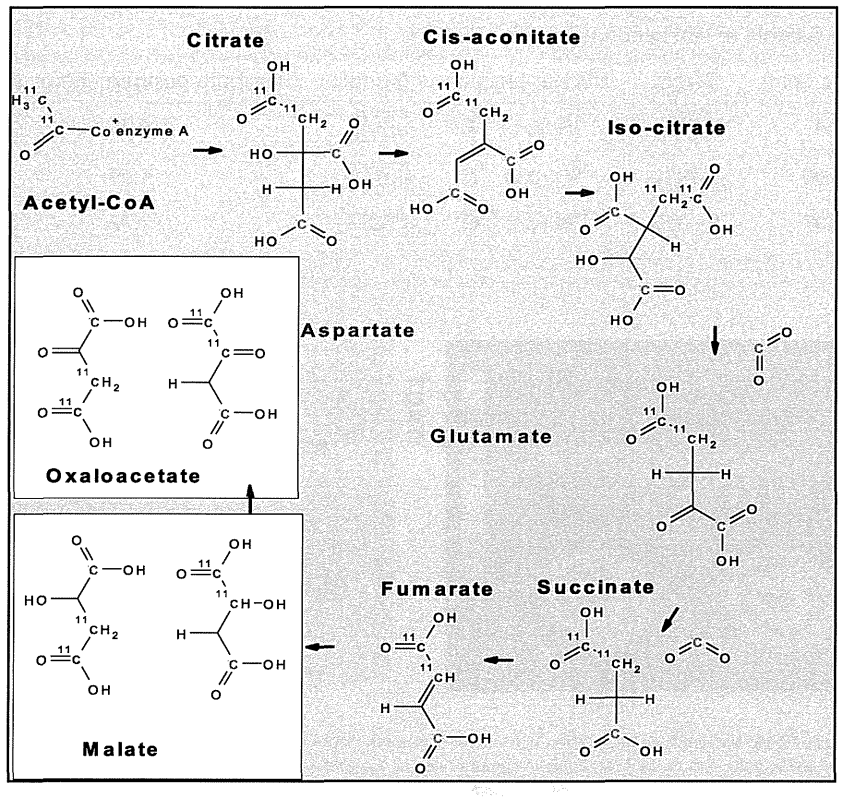
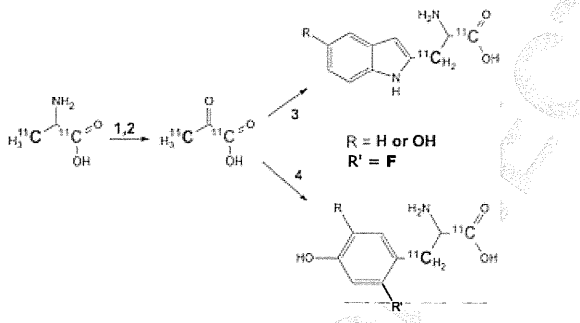


Figure 2. The structures (top) and summation images (bottom) of three different [^{11}C]acetylcarnitines ACC, ACN, and ACM in a monkey brain are shown. This figure is available in colour online at www.interscience.wiley.com/journal/jlcr



Scheme 1. The tricarboxylic acid cycle indicates the fate of the 1-^{11}C-labeled or 2-^{11}C-labeled acetate from acetylcarnitine, which may leave and enter into the excitatory amino acid pool. This figure is available in colour online at www.interscience.wiley.com/journal/jlcr



Scheme 2. Multi enzymatic synthetic route to produce aromatic ^{11}C-labeled amino acids in two different positions.1, d-AAO/catalase; 2, glutamic-pyruvic transaminase; 3, tryptophanase (indole or 5-hydroxy indole); and 4, β-tyrosinase (phenol, catechol, 4-fluoro-2-hydroxyphenol). This figure is available in colour online at www.interscience.wiley.com/journal/jlcr

The utility of this strategy is illustrated in the following examples. In Figure 3, the results of a study showing how the dopamine (DA) biosynthesis could be followed and verified by using 3-[^{11}C]-L-DOPA and 1-[^{11}C]-L-DOPA demonstrating either the formation and trapping of DA or the release of labeled carbon dioxide all depend on the position of the label.[22]

Significant increase in striatal L-[3-^{11}C]DOPA retention was observed in the adult female rhesus monkeys with PET following administration of drugs that increase cerebral L-DOPA concentrations. The monkeys were repeatedly scanned within a few hours during continuous administration of L-DOPA: at baseline using tracer substance, L-DOPA, or 6-R-L-erythro-4,5,6,7,8-tetrahydrobiopterin (6R-BH4). In all studies, the specific striatal L-[^{11}C]DOPA influx rate increased by an average of 17–20%. These increases were significantly higher than the retest variability

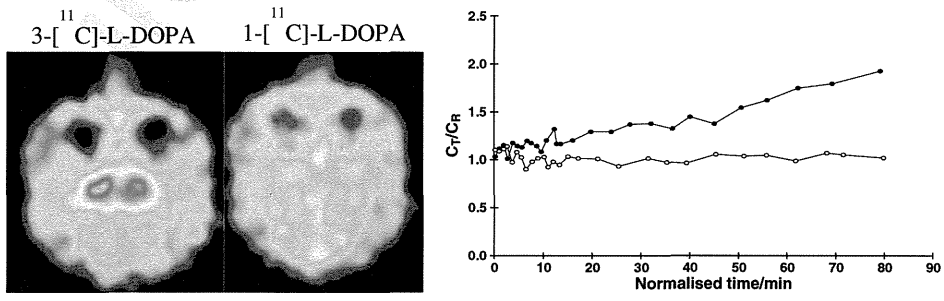


Figure 3. The summation images (left) and the time activity curves (right) of 3-^{11}C-L-DOPA (filled dots) and 1-^{11}C-L-DOPA (open circles) in the brain of the same monkey during a 4 h experiment with two consecutive administrations of the two-^{11}C-labeled L-DOPA molecules. The data was interpreted as follows: with the label in the 3-position, the radioactivity is trapped as [^{11}C]dopamine in the neuronal tissue; however, with the label in the carboxylic position, the same enzyme cleaves off labeled carbon dioxide that is just going into the carbonate pool. This figure is available in colour online at www.interscience.wiley.com/journal/jlcr

obtained with saline infusions under identical experimental conditions. The magnitude of increase in the striatal L-[^{11}C]DOPA influx rate varied in individual monkeys from no effect of the drug infusion to a 45% increase. The results of this study demonstrate that L-DOPA itself can affect dopaminergic neurotransmission *in vivo* and adds evidence that the neuromodulatory effects of the amino acid are predominantly auto-receptor antagonist-like. The findings may have importance for the understanding of the dopaminergic system in neurodegenerative and psychiatric disorders.[23] A similar approach using the 1-labeled or 3-labeled L-DOPA or 5-hydroxy-L-tryptophan has been applied in the study of certain neuroendocrine tumors as illustrated for L-DOPA in Figure 4.

Endogenous tracers v/s analogs: L-DOPA/F-DOPA as an example

In addition to the development of receptor and enzyme specific tracers, there has been an on-going research in utilizing ^{18}F-analogs of which 6-[^{18}F]fluoro-L-DOPA is one example. The behavior of small and especially endogenous molecules is certainly modified if one substitutes any atom or a small group such as a methyl moiety. Therefore, the concept of making 'analogs' by fluoro substitution has to be explored and validated. In a study using the multi enzymatic approach to label these amino acids, 6-fluoro-L-DOPA was labeled with ^{11}C in the same way as L-DOPA by replacing the catechol with the corresponding 6-fluorocatechol. A comparison between the L-[β-^{11}C]DOPA and 6-fluoro-[β-^{11}C]-L-DOPA was made in a monkey before and after adding the endogenous co-factor of tyrosine hydroxylase tetrahydrobiopterin.[24] The result showed the difference between these two monoamine precursor amino acids (Figure 5).

Drug effects on neurotransmitter biosynthesis

Receptor or enzyme-specific PET tracers have been used extensively in the field of drug development in studies to explore dose/occupancy, dose/inhibition relations, and also to gain insight into dosing regimes based on occupancy. There are still interesting scientific questions to be addressed in these types of studies related to the competitive experimental design.

We would like to make the point that there is a unique opportunity for PET to utilize endogenous tracers as a way of characterizing the individual status of subjects, and this will be illustrated in the following.

In Figure 6, data from a meta-analysis of 10 monkeys followed over 9 years using [3-^{11}C]-L-DOPA is presented illustrating the value of using endogenous tracers to track biology. There was a significant difference between the individual monkeys; however, the standard deviations on the individual level were small, and furthermore, over the 9-year time span in which these monkeys were followed, there was a reduction in the individual monkeys' DA biosynthesis as measured with L-[3-^{11}C]DOPA. The K_1 decreased with 1–2% and was in the same range as the assumed degeneration in humans.[25,26]

With this background, a study was designed using [^{11}C]-L-DOPA at a baseline and after dosing with OSU6162, a potential DA modulator.[27,28] Figure 7 shows that monkeys with a high striatal K_1 downregulated and monkeys with low K_1 upregulated the DA biosynthesis as measured with PET.

[3-^{11}C]L-DOPA and impact of neuroleptics

In another clinical study, two groups of patients with Parkinson's disease (PD) (advanced PD or early on-set PD) were compared with regard to the impact of L-DOPA and apomorphine on the

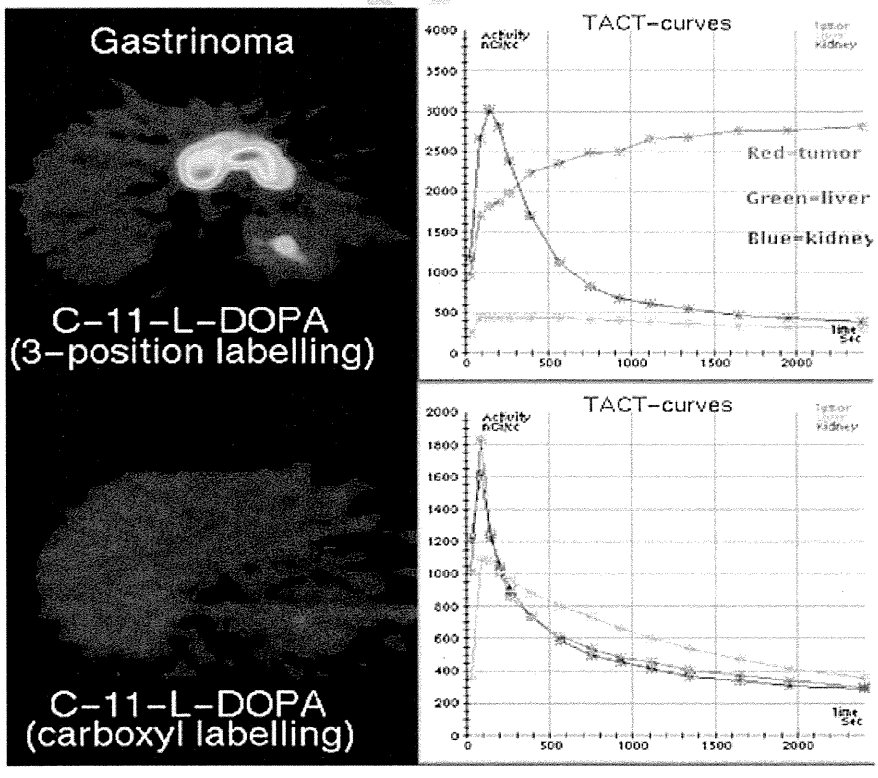
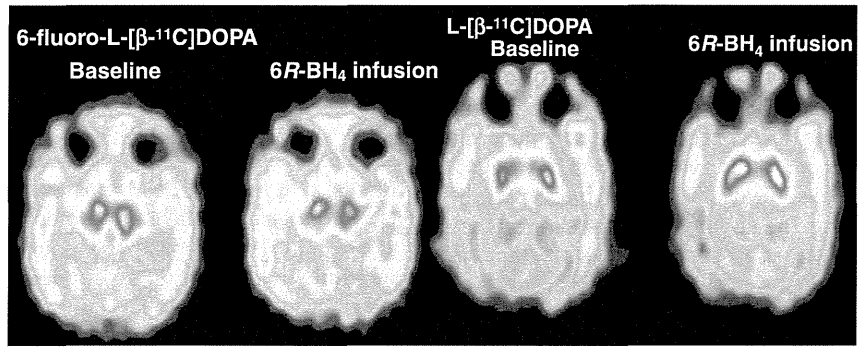


Figure 4. Summation images and time activity curves of 3-^{11}C-L-DOPA and 1-^{11}C-L-DOPA in tumor, kidney, and liver in a patient with a gastrinoma. This figure is available in colour online at www.interscience.wiley.com/journal/jlcr



Tracer	Baseline	6*R*-BH4 infusion
6-Fluoro-L-[^{11}C]DOPA (FDO)	0.0130 ± 0.0023 min-1	0.0134 ± 0.0020 min-1
L-[^{11}C]DOPA (DOP)	0.0129 ± 0.0015 min-1	0.0145 ± 0.0019 min-1

Figure 5. 3-^{11}C-Labeled 3,4-dihydroxy-L-phenylalanine (L DOPA) and [3-^{11}C]6-fluoro-L-DOPA were used to measure the functional state of the presynaptic dopamine system in anesthetized monkeys with PET. The radiotracer disposition in brain tissue and plasma was studied, and the effects induced by a challenge of 6R-Lerythro-5,6,7,8-tetrahydrobiopterin (6R-BH4) (a cofactor for the hydroxylation enzyme producing tyrosine and L-DOPA intra neuronal) increased the striatal influx rate constant, for example, striatal K_1 for L-[3-^{11}C]-DOPA but induced no effect on the K_1-value of [3-^{11}C]6-fluoro-L-DOPA. This figure is available in colour online at www.interscience.wiley.com/journal/jlcr

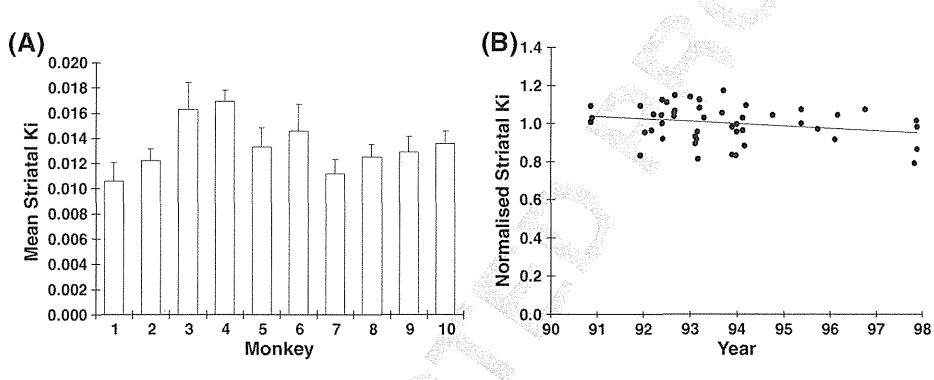


Figure 6. Data from 10 monkeys studied from 1990 to 1998 showing the estimated K_1 of [3-^{11}C]L-DOPA. The values for each monkey with the standard deviation (A) and the degradation of the K_1 over time (B) are presented.

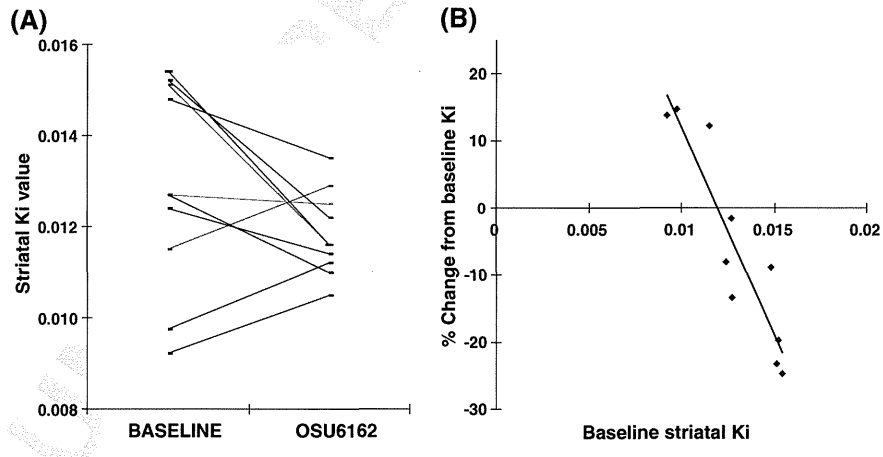


Figure 7. The impact on K_1 [3-^{11}C]L-DOPA in each monkey at base line and after a single dose of OSU 6162 (A). The change in % of K_1 from baseline after one single dose (B).

endogenous DA biosynthesis as measured with L-[3-^{11}C]DOPA, and the result indicated a significant impact when comparing patients with early on-set or advanced PD (Figure 8).[29]

Amino acid transport

The analog tracers [2-^{18}F]fluoro-L-phenylalanine[30,31] and [2-^{18}F] fluoro-L-tyrosine[43] have been used as radiotracers for amino acid transport; another example is *O*-([2-^{18}F]fluoroethyl)-L-tyrosine ([^{18}F]FET) for tumor imaging.[32] [Methyl-^{11}C]-L-methionine has been used for measuring amino acid transport across the placenta[33] and also for brain tumor imaging.

Schizophrenia is a mental illness causing debilitating symptoms. Although the causes of schizophrenia are not entirely clear, most probably, a combination of genetic, environmental, and chemical factors plays a role in its development. Brain abnormalities

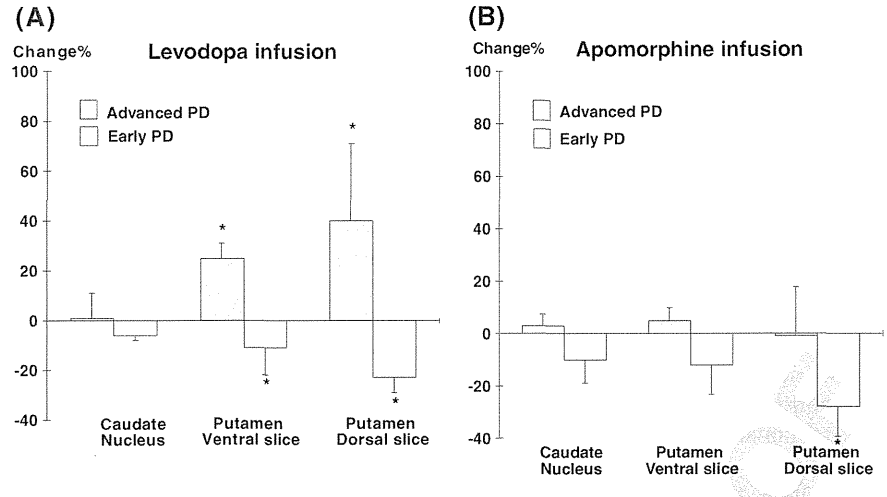


Figure 8. Results using [3-^{11}C]L-DOPA to measure dopamine synthesis in a study with Parkinson's patients in an advanced or early state before and after a Levodopa (A) or Apomorphine (B) infusion.

involving the transport of the amino acid tyrosine have been observed in schizophrenic patients. Research involving tyrosine supplementation on schizophrenics shows promising results using [1-^{11}C]-L-tyrosine. The transport rate dropped in the controls after tyrosine loading, which is consistent with the prevailing notion that the brain's transport system for neutral amino acids works close to saturation, whereas it was virtually unchanged in the schizophrenics. The results demonstrated that tyrosine transport was not saturated in the patients with schizophrenia and thus could lead to elevated brain concentrations of tyrosine.[34,35]

Nitrogen-13

This radionuclide has been used in the laboratory mostly for ^{13}N-labeled amino acids and amines as a synthon.[36,37] Ammonia is naturally occurring and is contained in many plants and animals and in humans.[38,39]

[^{13}N]Ammonia has been extensively used to measure myocardial blood flow even if there are questions about its value. This tracer moves from the vascular space to tissue by both active transport (sodium–potassium pump) and by passive diffusion. Once inside the cells, the glutamic acid–glutamine pathway metabolizes this tracer. [^{13}N]Ammonia has been valuable to measure regional myocardial perfusion in normal and diseased states. An advantage of [^{13}N]ammonia is that it allows repeat study designs. Clearance of [^{13}N]ammonia from blood is rapid, with high tissue retention fractions, resulting in high-contrast cross-sectional images of the myocardium.[40] [^{13}N]Ammonia studies are many times combined with [^{18}F]FDG to compare myocardial blood flow with glucose metabolism to detect 'mismatch', an index of viable but compromised tissue.[41–43]

Oxygen-15

The application of oxygen-15 for measuring the regional oxygen extraction and perfusion in the human brain was reported in 1969.[44] Oxygen gas has been employed to study oxygen-utilizing enzyme reactions such as metapyrrocatechase.[45] Myocardial oxygen extraction fraction was also measured using bolus inhalation of [^{15}O]oxygen gas and dynamic PET.[46] Carbon [^{15}O]monoxide and carbon [^{15}O]dioxide have been used for determination of the total blood volume and arterial blood volume, respectively.[47] ^{15}O-Labeled water has been used to quantify regional cerebral blood flow.[48] In general, oxygen-15 has been considered not only as a useful physiological PET tracer applied extensively in neurological and neuroscience studies[49,50] but also in cardiology.[51]

Oxygen-15 with a half-life of 2 min has severe restrictions where it concerns the labeling of compounds; however, it is possible to have access to essential endogenous molecules of life such as oxygen, water, and carbon dioxide. It must be emphasized that the short half-life oxygen-15 allows repeatable study designs and should be used much more than today. The field is nicely reviewed in an extensive article by Jones and Baron.[52]

There have been approaches to use ^{15}O to label molecules such as glucose and [6-^{15}O]2-deoxy-D-glucose ([^{15}O]DG)[53,54] The latter is interesting as an alternative for ^{18}F-FDG but outside of the scope of this article.

Preparation and labeling of endogenous compounds

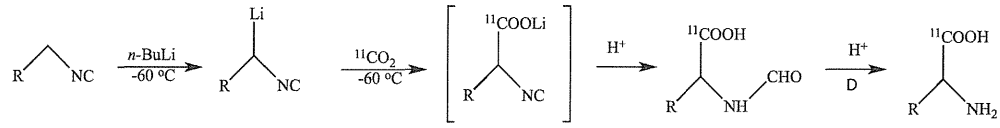
Carbon-11

Carbon-11 plays an important role in the synthesis of labeled endogenous substances because of a reasonable half-life and the fact that virtually all endogenous compounds contain carbon.

There are a number of endogenous compounds, which have been labeled with ^{11}C such as glucose, fatty acids, carbohydrates, amino acids, nucleosides, small peptides, neurotransmitters, and uric acid.

As illustrated in a few examples, some of these compounds can be labeled with ^{11}C in various positions and therefore be used in new quantitative approaches. Thus, the utilization of endogenous compounds will provide critical information in the study of normal and abnormal physiology and in biochemistry applied in *in vivo* biosystems.

The first syntheses of [^{11}C]amino acids and lactic acid were published in early 1940s.[55] The first ^{11}C-labeled L-amino acid was methionine using ^{11}C-methyl iodide as the labeling agent.[56,57] Carbon-11 labeled racemic phenylalanine, phenylglycine, and DOPA labeled in the carboxylic position via isocyanides (Scheme 3) were reported at the same time.[58,59]



Scheme 3. Synthesis of ^{11}C-amino acids via ioscyanides.

Using $^{11}CO_2$, D, L-α-alanine-1-^{11}C was synthesized involving several reaction steps within 70 min (Scheme 4).[60]

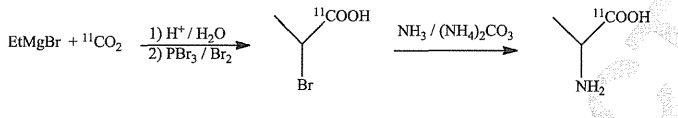
The aforementioned approach, starting with introduction of radioactivity, makes the procedure less attractive for routine production. Generally, the Strecker reaction is used for synthesis of racemic carboxyl-labeled amino acids, where [^{11}C]cyanide is reacted with the bisulfate adduct of an aldehyde or ketone and the product treated with ammonium hydroxide as shown in Scheme 5.[61]

Racemic carbon-11amino acids such as [3-^{11}C]alanine and [3-^{11}C]phenylalanine have been separated into enantiomers by means of chromatography on a chiral stationary phase[62] or enzymatically in the case of [3-^{11}C]valine preparation.[63]

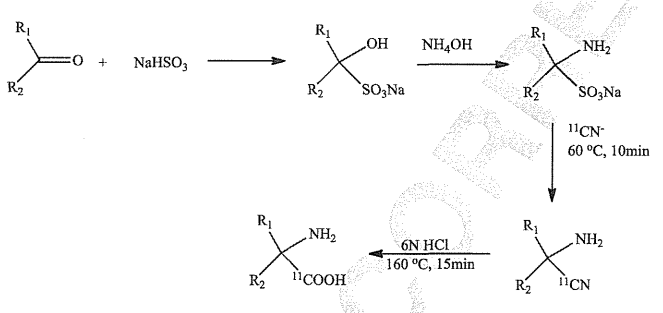
Using commercially available Oppolzer's synthon, that is, (2R)-*N*-[*N*-[bis(methylthio)methy-lidene]glycyl]bomane-10,2-sultam, provides asymmetric [^{11}C]alanine in high e.e. (Scheme 6).[64]

Asymmetric synthesis of [3-^{11}C]-L-phenylalanine using chiral diphosphines-rhodium as chiral hydrogenation catalysts was reported in 1984 (Scheme 7).[65]

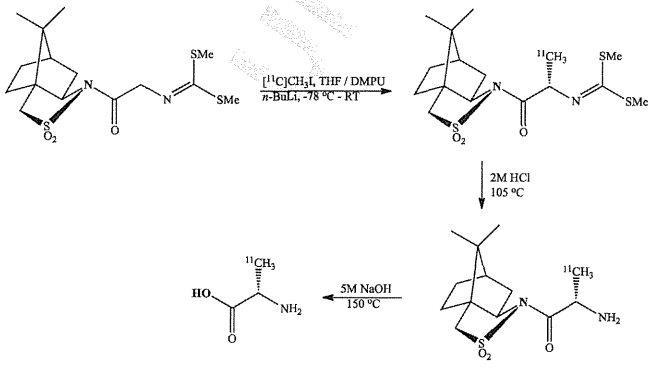
Labeling in the 3-position (or β in the case of aromatic amino acids) can be achieved by alkylation using aliphatic or aromatic alkyl halides.[63,66] This approach has also been applied in asymmetric syntheses of aliphatic [^{11}C]amino acids using different glycine derivatives such as [(+)-2-hydroxypinanyl-3-idene]glycine *tert*-butyl ester,[67,68] the nickel complex of the Schiff base of (S)-*O*-[(*N*-benzylpropyl)amino]benzophenone and glycine.[62,69] The most promising method, which gives nearly enantiomerically pure [^{11}C]amino acids, used an imidazolidinone derivative (Scheme 8).[70,71] These syntheses utilizing [^{11}C]alkyl halides in alkylation reaction on stabilized carbanions are efficient ways of forming C-^{11}C bonds.



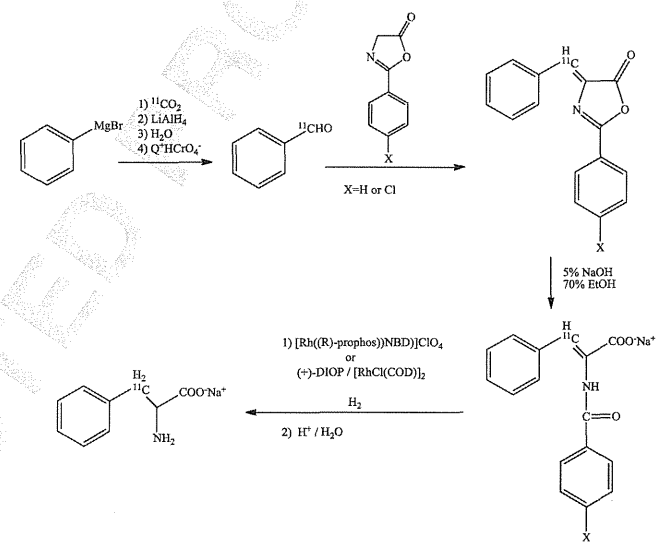
Scheme 4. Synthesis of ^{11}C-D, L-α-alanine.



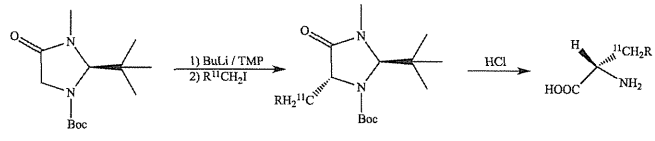
Scheme 5. Synthesis of 1-^{11}C-labeled amino acids.



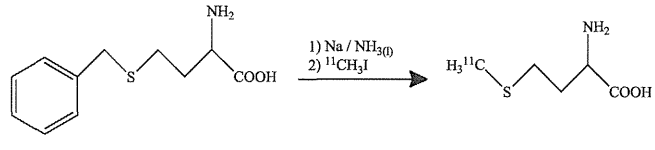
Scheme 6. Synthesis of L-[3-^{11}C]alanine.



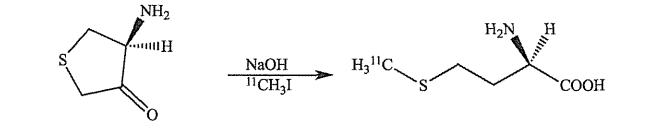
Scheme 7. Synthesis of L-[3-^{11}C]phenylalanine.



Scheme 8. Asymmetric synthesis of [^{11}C]amino acids by alkylations of imidazolidinone.



Scheme 9. Synthesis of D and L-[methyl-^{11}C]methionine.

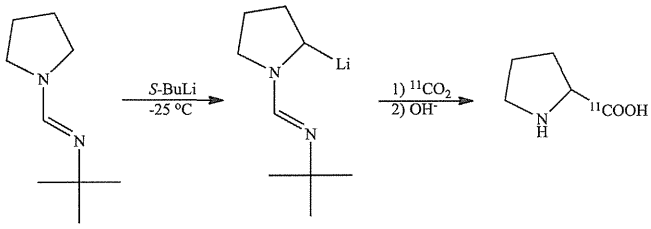


Scheme 10. Fast synthesis of L-[methyl-^{11}C]methionine.

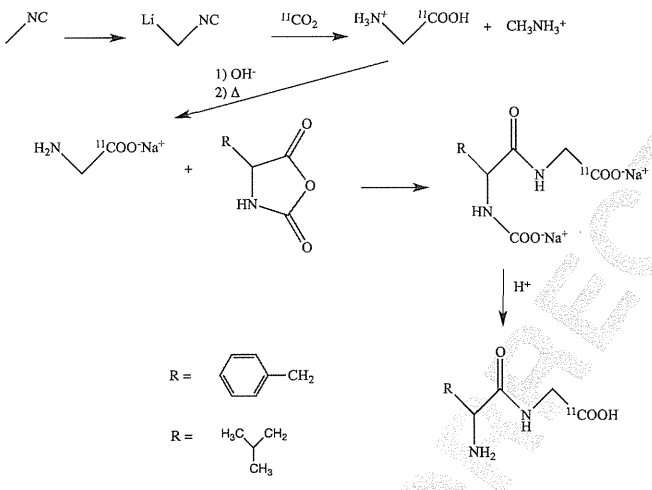
Both D and L-form of [methyl-^{11}C]methionine were prepared from the corresponding L or D-benzyl homocysteine, converted to their respective sodium salts of the sulfide anions by sodium in liquid ammonia (Scheme 9).[57,72]

A fast, clean, and reproducible method for the manufacture of the radiotracer L-[methyl-^{11}C]methionine using L-homocysteine thiolactone was reported (Scheme 10).[73]

The reaction at room temperature of the precursor with [^{11}C]CH_3I in an HPLC loop led to the formation of the desired radiotracer with a high radiochemical yield (38 ± 4% end of synthesis) in a short production time (12 min) with specific radioactivity in the range 11–45 GBq/μmol.[74]



Scheme 11. Synthesis of D, L-[1-^{11}C]proline.



Scheme 12. Synthesis of [1-^{11}C]glycine, L-phenylalanyl and L-leucyl-[1-^{11}C]glycine.

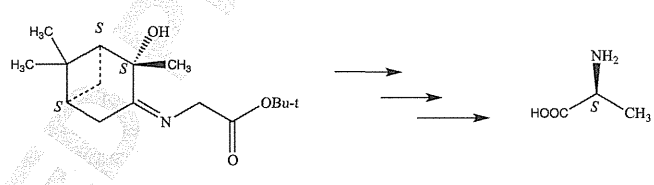
D, L-[1-^{11}C]Proline was synthesized by carboxylation of α-lithiopyrrolidyl-*N*-*tert*-butyl-formamidine with a radiochemical yield of up to 18% without correction for decay (Scheme 11).[75]

[1-^{11}C]Glycine was prepared in 30 min by carboxylation of α-lithiomethylisocyanide with a radiochemical yield of 10–15%. After coupling with L-phenylalanine-*N*-carboxy-anhydride or L-leucine-*N*-carboxyanhydride followed by HPLC purification, the corresponding dipeptides were obtained (Scheme 12).[76]

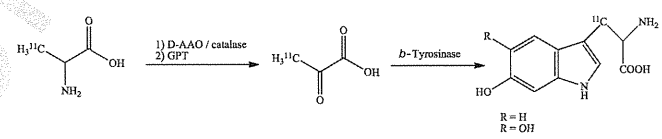
D, L-[3-^{11}C]Valine, was synthesized by phase-transfer alkylation of *N*-(diphenylmethylene)-glycine *t*-butyl ester with 2-[^{11}C]isopropyl iodide, followed by acidic hydrolysis (Scheme 13). Treatment with immobilized D-amino acid oxidase produced L-[3-^{11}C] valine in 90–99% enantiomeric excess.[77]

The synthesis of L-[3-^{11}C]alanine from [(+)-2-hydroxypinanyl-3-idene]glycine *t*-butyl ester and [^{11}C]methyl iodide was reported (Scheme 14).[67]

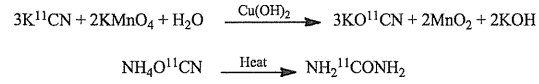
5-Hydroxy-L-tryptophan, the biosynthetic precursor of serotonin (5-HT), is decarboxylated to serotonin in the brain by the enzyme L-aromatic amino acid decarboxylase (L-AADC). Multi-enzymatic syntheses of L-[β-^{11}C]tryptophan and 5-hydroxy-L-[β-^{11}C]tryptophan from racemic [3-^{11}C]alanine were reported (Schemes 2 and



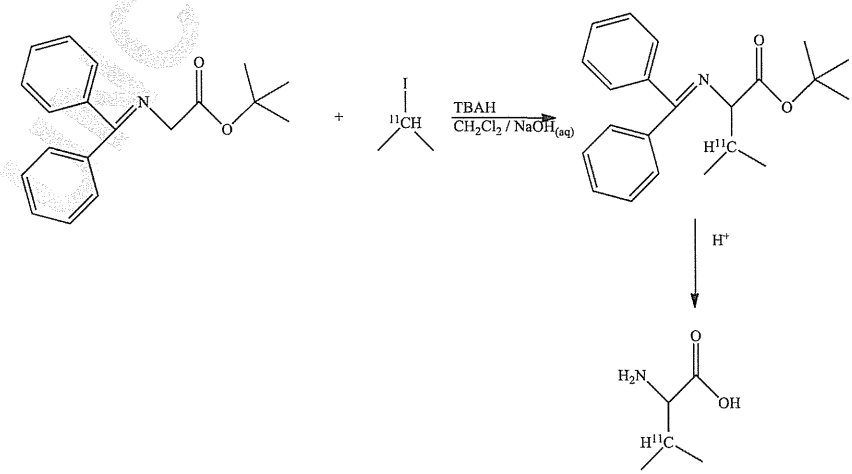
Scheme 14. Synthesis of L-[3-^{11}C]alanine.



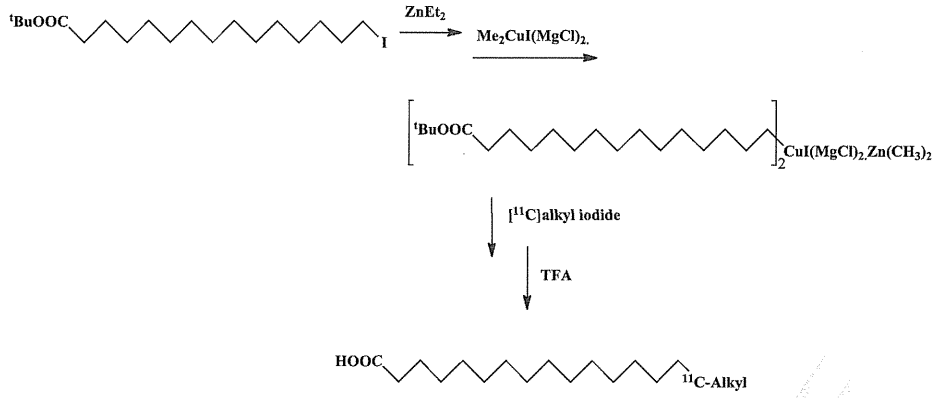
Scheme 15. Syntheses of L-[β-^{11}C]DOPA.



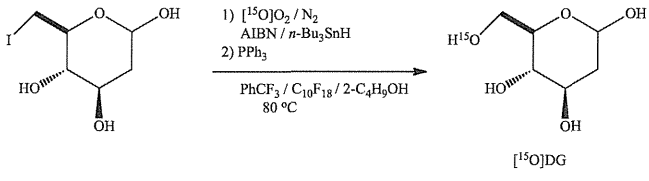
Scheme 16. Urea synthesis via cyanate.



Scheme 13. Synthesis of D, L-3-[^{11}C]valine.



Scheme 17. Synthesis of various ^{11}C-fatty acids.



Scheme 18. Rapid synthesis of [^{15}O]DG.

15). ^{11}C-Labeled alanine was prepared by an alkylation of a glycine derivative, *N*(diphenylmethylene)-glycine *t*-butyl ester, with [^{11}C] methyl iodide obtained from [^{11}C]carbon dioxide and subsequent hydrolysis.[57] The enzymatic syntheses were carried out in a one-pot reaction using D-amino acid oxidase/catalase (D-AAO), glutamic-pyruvic transaminase, and tryptophanase.[78]

The synthesis of L-[β-^{11}C]tyrosine and L-[β-^{11}C]DOPA (Scheme 2) from [^{11}C]carbon dioxide via D, L-3-[^{11}C]alanine using a combination of organic synthetic methods and a multi-enzymatic procedure were presented. The enzymatic syntheses were performed using a one-pot reaction procedure with D-amino acid oxidase (D-AAO)/catalase, glutamic-pyruvic transaminase, and β-tyrosinase.[79]

[1-^{11}C]Pyruvic acid was prepared by carboxylation of a masked acyl carbanion. The addition of [^{11}C]CO_2 to the lithium aldimine from methyllithium and 1.1.3.3-tetramethylbutylisocyanide yields the α-amino acid, which is then hydrolysed to the [l-^{11}C]pyruvic acid.[80]

L-Ornithine is one of the products of arginase action on L-arginine, creating urea. Ornithine is a central part of the urea cycle, which allows for the disposal of excess nitrogen processes. L-[4-^{11}C]Ornithine was prepared by the displacement reaction of potassium [^{11}C]cyanide with the functionally protected γ-bromohomoserine followed by selective reduction of the [^{11}C] nitrile with cobalt chloride–sodium borohydride complex, deprotection with 6 M HCl(aq), and purification by HPLC.[81]

[^{11}C]Urea was synthesized by thermal transformation of ^{11}C-labeled ammonium isocyanate. The [^{11}C]isocyanate was prepared by oxidation of [^{11}C]cyanide (Scheme 16).[82]

[^{11}C]Carbohydrates such as glucose are important tools for *in vivo* studies of energy metabolism. Several methods employing [^{11}C]cyanide, [^{11}C]methyl iodide, or [^{11}C]nitromethane have been developed for the specific labeling of glucose in positions 1 and 6.[83–88]

[^{11}C]Glucose was also made using a biosynthetic method.[89] In 1986, the synthesis of [2-^{11}C]-2-deoxyglucose was reported as a useful PET tracer for brain metabolism and regional activity.[90]

Among the other endogenous compounds, labeling of [^{11}C]acetate,[91,92] fatty acids,[93] and steroids such as [17α-^{11}C] methyltestosterone,[94] [17α-^{11}C]methylestradiol and 11β-ethyl-[17α-^{11}C]-methylestradiol have also been reported.[95]

As an example, fatty acids have been used for following the kinetics of biological processes, such as membrane transport, blood flow, and cardiac fatty acid metabolism.[96] [ω-^{11}C]Palmitate is a well-established PET tracer to probe myocardial fatty acid metabolism.[97] Synthesis of [ω-^{11}C]palmitate involves incorporation of ^{11}C-alkyl iodide employing Grignard reaction,[98] Suzuki cross coupling,[99] organocopper chemistry,[100] or functionalized copper–zinc reagent (Scheme 17).[101]

Nitrogen-13

In early 1980, the synthesis of L-[^{13}N]-amino acids was reported by utilizing glutamate dehydrogenase immobilized onto Sepharose. These amino acids (glutamate, α-aminobutyrate, valine, methionine, leucine, and alanine) were labeled with nitrogen-13 in high radiochemical yield and radiochemical purity.[102]

γ-Aminobutyric acid is an important neurotransmitter in the mammalian central nervous system and is biosynthesized in the brain from glutamate using the enzyme L-glutamic acid decarboxylase and pyridoxal phosphate. Later on, γ-[^{13}N]-aminobutyric acid was also synthesized.[103]

Oxygen-15

The cyclotron produced oxygen-15 can be used as such but also converted to [^{15}O]carbon monoxide, [^{15}O]carbon dioxide, or [^{15}O]water. Oxygen gas is an essential molecule not only utilized for cellular respiration but also for biosynthesis and metabolism of various biomolecules such as steroids, eicosanoids, and neuroactive substances. Because of the short half-life of oxygen-15, it has rarely been used in the synthesis of more complex molecules. A recent interesting example is a single-step synthesis of 2-deoxy-D-6-[^{15}O]-glucose ([^{15}O]DG) from the corresponding iodide as shown in Scheme 18.[50,51]

Conflict of Interest

The authors did not report any conflict of interest.

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4) HCV 粒子形成に関与する脂肪滴周辺膜蛋白の同定と機能解析

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はじめに

エンベロープウイルスは小胞体，ゴルジ体，形質膜など細胞の生体膜を被って出芽するため，細胞の生体膜は粒子形成に重要な役割を果たしていると考えられる[1,2]．近年，C 型肝炎ウイルス（HCV）粒子形成に細胞内脂肪滴（LD）が重要であることが示された[3]．JFH-1[4] 感染細胞を電顕で観察すると，LD と粗面小胞体が結合している部分に黒い点状のものが認められた．われわれは，この LD 周辺の膜構造が HCV 粒子産生の場と考え，LD 近傍膜のプロテオーム解析を行い，ウイルス粒子産生に関与する宿主因子の同定と機能解析を行った．

1．LD 周辺膜に含まれる蛋白の同定

はじめに感染細胞のライセートを遠心法により分画した．ショ糖濃度は LD を分けやすい 0%から，膜を分けやすい 8.5%までを用い，感染性は軽い分画に存在していた．そこで次にこの軽い分画に含まれている蛋白について解析した．この感染性の高い分画からは，コア蛋白，E2，NS5A などの HCV 蛋白，それに小胞体（ER），ゴルジ体，脂質ラフトなどに含まれる蛋白が検出された．一方，リソソーム，細胞質などに含まれる蛋白は検出されなかった．LD 由来蛋白が強く残っていることから，LD が強く濃縮されていたことが分かった．この分画から LD を精製したところ，膜蛋白も HCV 蛋白も検出できなくなった．以上のことから，HCV 粒子産生の場は LD そのものではなく，その周辺膜であると考えられた．

そこで，LD 周辺膜に含まれる宿主蛋白質同定のため，比較プロテオーム解析を行ったところ，LD および LD 周辺膜を含む蛋白質として 75 の蛋白が同定された．また，そこから精製した LD からは約 30 の蛋白が検出できた．そこで，これら 30 の蛋白を除く 45 の蛋白を LD 周辺膜蛋白と仮定して解析を行った．その中には，さまざまな機能の蛋白が含まれていたが，そのうち Apolipoprotein E（ApoE）等は既に HCV の生活環に加わっていることが報告されている[5]．これらの蛋白について siRNA でスクリーニングを行い，候補蛋白が HCV 粒子感染性および複製に与える影響について調べた．その結果，24 dehydrocholesterol reductase（DHCR24），17 beta hydroxysteroid dehydrogenase（HSD）11，protein disulfide isomerase（PDI），ApoE の SiRNA は培養上清中および細胞内の HCV 粒子の感染性を低下させることが分かった．さらに，レプリコン細胞を siRNA で処理したところ，