Dual Tracer Autoradiographic Study of β-Methyl-(1-^{14}C) Heptadecanoic Acid and 15-*p*-(^{131}I)-Iodophenyl-β-Methylpentadecanoic Acid in Normotensive and Hypertensive Rats

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The myocardial distribution of 15-p-[^{131}I]iodophenyl-3-(R,S)-methylpentadecanoic acid (BMPDA) and 1[^{14}C]-3-(R,S)-methylheptadecanoic acid (BMHDA) was compared in normotensive and hypertensive rats using quantitative dual tracer autoradiographic techniques. The myocardial distribution of carbon-14 [^{14}C] BMHDA and iodine-131 [^{131}I] BMPDA was nearly homogenous in the normotensive rats, while both tracers showed similar, though very heterogenous, distribution in hypertensive hearts with decreased uptake in the endocardial region. Our data demonstrate that myocardial distribution of [^{131}I]BMPDA was essentially the same as [^{14}C]BMHDA, and thus single photon emission computed tomographic imaging with ^{123}I-labeled BMPDA could be useful for the detection of regional changes of myocardial fatty acid uptake in patients with prolonged and severe hypertension.

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Myocardial utilization of free fatty acids as major substrates for energy production (*1,2*) has led to the development of fatty acid analogs labeled with radionuclides, such as carbon-11 (^{11}C) and iodine-123, for the noninvasive evaluation of regional fatty acid metabolism (*3–6*). Radioiodinated 15-(*p*-iodophenyl)-3-(R,S)-methylpentadecanoic acid (BMPDA) is a new fatty acid analog in which a methyl group has been introduced at the beta carbon to inhibit beta-oxidation, and a terminal iodophenyl group added to stabilize the iodine label (*7*). This agent was designed to provide a single photon labeled equivalent of its ^{11}C-labeled predecessor, 3-(R,S)-methylheptadecanoic acid (BMHDA). Previous studies with BMHDA indicated that it is transported into cells in proportion to palmitic acid, and temporarily trapped and retained in the myocardium (*8*). The prolonged retention is believed to result from inhibition of beta-oxidation because of the presence of the methyl group in the beta position (*8,9*). The addition of the phenyl group and the shorter alkyl chain length of BMPDA compared with BMHDA raised a question about the relative behavior of both compounds in vivo. The present study was performed to compare the regional myocardial distribution of iodine-131-(^{131}I) labeled BMPDA and [^{14}C]BMHDA in both hypertensive and normotensive rats using the quantitative dual tracer autoradiographic technique. This model was selected for comparison studies since previous autoradiographic studies showed a regional change in BMHDA uptake in the myocardium of severely hypertensive rats (*10*).

MATERIALS AND METHODS

Seven salt-sensitive hypertensive Dahl strain rats (blood pressure 214 ± 18 mmHg, body weight 203 ± 18 g) and seven normotensive rats (blood pressure 138 ± 6 mmHg, body weight 201 ± 12 g) of the same strain

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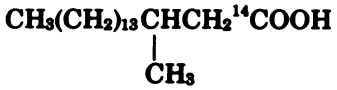
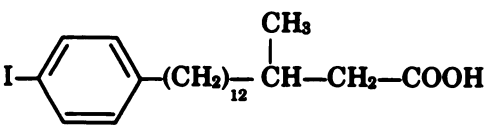
were used as controls. These rats were derived from common Sprague-Dawley ancestors and have the genetic predisposition to develop fatal hypertension if they are given extra salt in their diet (*11*). Hypertensive salt sensitive rats received 8% NaCl and normotensive rats received 0.4% NaCl by weight in the food for 5 wk. Food and water was given ad libitum until 2 hr before experiment. The [1-^{14}C]-3(R,S)-methylheptadecanoic acid ([^{14}C]BMHDA) was obtained commercially and the 15-(*p*-[^{131}I]-iodophenyl)-3,(R,S)-methylpentadecanoic acid ([^{131}I]BMPDA) was synthesized either through decomposition of the piperidyltriazene derivative of the corresponding *p*-amino analog or by thallation of 15-phenyl-3 (R,S)-methylpentadecanoic acid followed by treatment with potassium iodide as described earlier (*12,13*).

All 14 rats were injected with a combination of 170 μCi of [^{131}I]BMPDA and 10 μCi of [^{14}C]BMHDA through a lateral tail vein and killed 30 min later. Thirty-minute killing time was chosen because our previous studies showed that by this time the uptake of BMPDA in the heart reaches an equilibrium point. In 12 rats, the hearts and lungs were removed, frozen in liquid nitrogen, embedded in carboxy-methyl-cellulose, and sectioned with a cryomicrotome. The liver, kidney, and blood samples, as well as small parts of heart, muscle, and lung, were weighed and counted. The two remaining rats (one hypertensive and one normotensive) were processed for whole-body autoradiography. The tissue sections of 30-μm thickness and graded standards were placed on x-ray films† for exposure.

The first exposure for 16 hr revealed the ^{131}I distribution, the second exposure for ^{14}C was initiated 3 mo later following the decay of ^{131}I activity. Imaging of [^{14}C]BMHDA required 15 days for adequate image quality. Selected regions of ^{131}I and ^{14}C autoradiograms were digitized and quantitated using a videosensitometric system‡ coupled to a minicomputer§ (*14,15*). A



[1—C—14]—3 (R,S) - Methylheptadecanoic Acid (BMHDA)



15-(p-I-131-Iodophenyl)-3-R,S-Methylpentadecanoic Acid (BMPDA)

FIGURE 1
Structure of [^{14}C]BMHDA and [^{131}I]BMPDA.

2.0 × 2.0 cm field including the heart was digitized to 128 × 128 pixels, so that the pixel size was 0.15 × 0.15 mm. The digitized images were converted to quantitative images (nCi/g for ^{14}C, relative counts/pixel for ^{131}I) using a response curve relating the actual radioactivity and film density using graded standards exposed and digitized under the same condition as heart images.

RESULTS

The chemical structure of [^{14}C]BMHDA and [^{131}I] BMPDA is shown in Fig. 1. The whole-body autoradiograms of [^{14}C]BMHDA and [^{131}I]BMPDA of the same section of a hypertensive rat are depicted in Fig. 2. The tissue distribution data of [^{131}I]BMPDA and [^{14}C] BMHDA obtained in the current study for both hypertensive and normotensive rats are summarized in Table 1. In hypertensive rats, the myocardial uptake (% dose/g) was not significantly different between [^{14}C]BMHDA (2.42 ± 0.39) and [^{131}I]BMPDA (2.58 ± 0.26), but radioactivity in the liver (% dose/g) of [^{131}I]BMPDA (0.80 ± 0.19) was significantly lower ($p < 0.001$) than for [^{14}C]BMHDA (2.87 ± 0.66). Therefore, the radioactivity ratio of myocardium to liver of [^{131}I]BMPDA (3.29 ± 0.70) was much higher than that of [^{14}C] BMHDA (0.80 ± 0.11). In the blood and lung, [^{131}I] BMPDA retention was slightly higher than [^{14}C] BMHDA. Myocardium/lung ratios were 2.91 ± 0.72 for [^{131}I]BMPDA and 3.54 ± 0.65 for [^{14}C]BMHDA and myocardium/blood ratios were 3.29 ± 0.34 and 3.77 ± 0.37, respectively. In normotensive rats, the myocardial activity of [^{131}I]BMPDA and [^{14}C]BMHDA was significantly higher ($p < 0.001$) than the hypertensive rats.

The autoradiograms of [^{131}I]BMPDA and [^{14}C] BMHDA of the same myocardial section are shown in Fig. 3 (normotensive heart) and Fig. 4 (hypertensive heart). The myocardial distribution of these two tracers was almost homogenous in the normotensive rat; however, these tracers showed quite a heterogenous distribution in the hypertensive myocardium. The autoradiograms of both [^{14}C]BMHDA and [^{131}I]BMPDA showed decreased uptake in the endocardial region of hypertensive hearts.

Figure 5 depicts the quantitative images of [^{14}C] BMHDA and [^{131}I]BMPDA of the same section of hypertensive heart and lung. After the image registration processing, the activity in each pixel of this quantitated [^{131}I]BMPDA image was divided by the activity in the corresponding pixel of [^{14}C]BMHDA image. The divided result is shown on extreme right of this figure. Although the distribution of radioactivity in the hypertensive myocardium was very irregular in both quantitated [^{131}I]BMPDA and [^{14}C]BMHDA images, the myocardial region of the ratio image was displayed at almost the same gray level. This indicates that [^{131}I]BMPDA

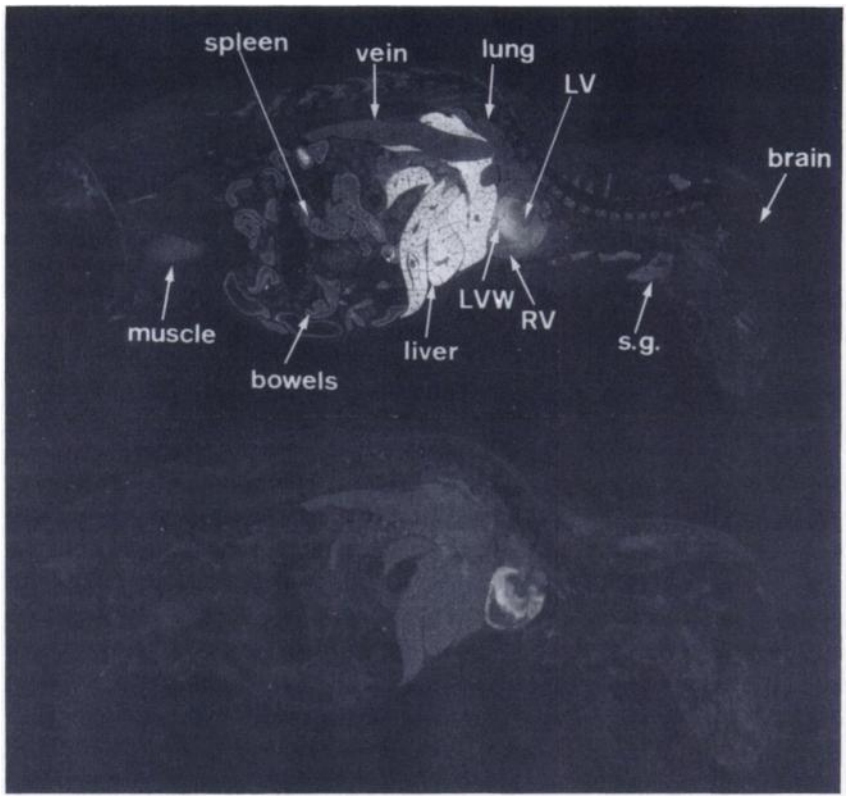


FIGURE 2
Whole-body autoradiograms of [^{14}C] BMHDA (upper) and [^{131}I]BMPDA (lower) of same section of hypertensive rat. LV: Left ventricle, LVW: left ventricular wall, RV: right ventricle, s.g.: salivary gland

distribution in the hypertensive myocardium was essentially equal to that of [^{14}C]BMHDA. While the lung region was scarcely visualized in controls in the quantitative images, it was clearly imaged in the ratio image because [^{131}I]BMPDA retention in the lung was relatively higher than [^{14}C]BMHDA. The relationship between the activities of [^{131}I]BMPDA and [^{14}C]BMHDA calculated from corresponding pixels in the myocardial region of the quantitated images showed an excellent linear correlation (Fig. 6). The correlation coefficients of nine hypertensive heart sections were from 0.88 to 0.96 (mean ± s.d. = 0.93 ± 0.03).

DISCUSSION

Iodine-123-labeled 17-iodoheptadecanoic acid (HDA) is routinely used at several institutions for the evaluation of ischemic heart disease by analysis of the time-activity curves of iodide washout resulting from catabolism (beta-oxidation) of this straight-chain fatty acid analog (*16–19*). Although high blood background requires correction of free radioiodide to differentiate regions of the myocardium from the blood pool, this technique is routinely used and altered clearance rates are associated with diseased segments. In an attempt to circumvent the rapid deiodination of the iodoalkyl-substituted HDA analog, investigators developed 15-(*p*-iodophenyl)-pentadecanoic acid (IPP) (*20,21*), where iodide is stabilized on a terminal phenyl ring to overcome facile deiodination. This agent shows less rapid myocardial washout (*22,23*), but imaging must be done rapidly to minimize the problem of label clearance. Because of the high blood levels and rapid myocardial washout, however, these agents would not be optimal for evaluation of regional fatty acid uptake by single photon emission computed tomography (SPECT). For this application the optimal properties of the radiolabeled fatty acid would include rapid extraction, high heart:blood ratios and very prolonged retention so that the initial distribution pattern is "frozen". In this manner SPECT can possibly be used to evaluate regional distribution of fatty acid analogs.

Carbon-11-labeled BMHDA is a fatty acid that has been recommended as a potentially useful agent for evaluating myocardial fatty acid utilization with positron emission tomography (*8*). This agent enters the myocardium and remains in situ, permitting the re-

TABLE 1
Tissue Distribution Data at 30 min After Injection (Mean ± s.d. of Rats)

	Percent injected dose/g			
	Hypertensive rat		Normotensive rat	
	[^{131}I]BMPDA	[^{14}C]BMHDA	[^{131}I]BMPDA	[^{14}C]BMHDA
Blood	0.83 ± 0.14	0.63 ± 0.10	0.71 ± 0.06	0.25 ± 0.04
Myocardium	2.58 ± 0.26	2.42 ± 0.39	3.87 ± 0.63	5.08 ± 1.05
Lung	0.92 ± 0.31	0.68 ± 0.24	0.57 ± 0.13	0.67 ± 0.19
Liver	0.80 ± 0.19	2.87 ± 0.66	0.66 ± 0.10	2.72 ± 0.23
Kidneys	0.69 ± 0.09	0.71 ± 0.18	0.61 ± 0.03	0.89 ± 0.07
Muscle	0.24 ± 0.03	0.40 ± 0.07	0.21 ± 0.03	0.36 ± 0.03

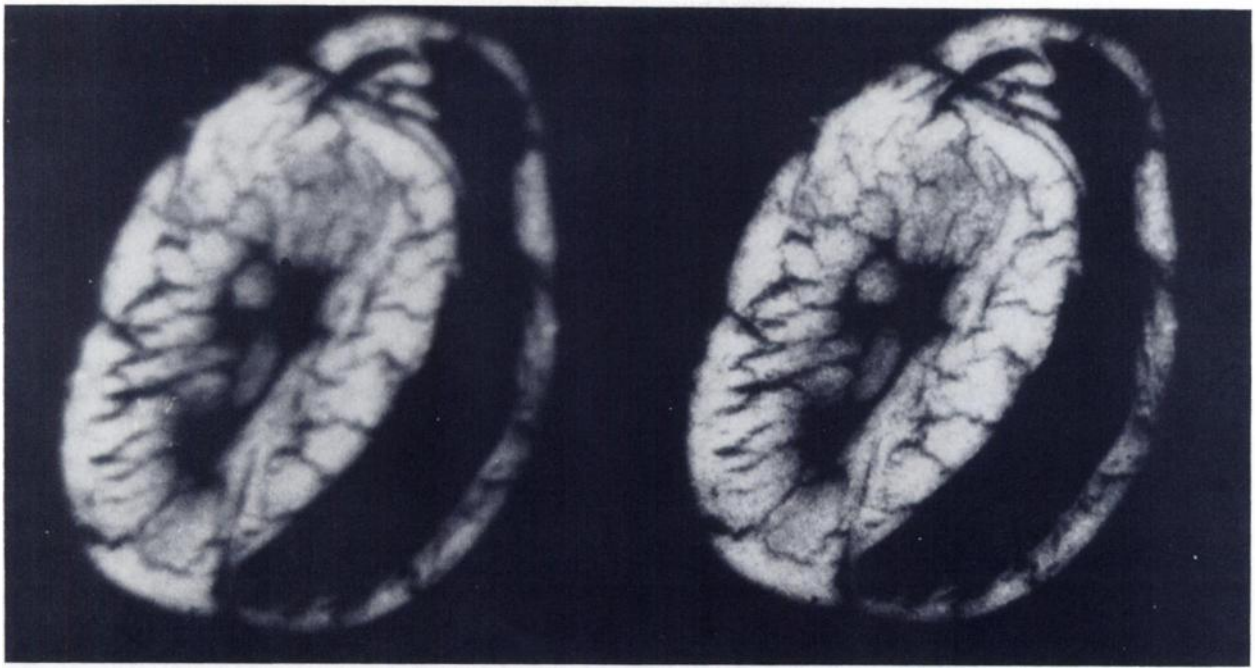


FIGURE 3
Autoradiograms of [^{14}C]BMHDA (left) and [^{131}I]BMPDA (right) of same section of normotensive heart

cording of high quality images. Our previous studies demonstrated that hypertensive rat myocardium had decreased uptake of [^{14}C]BMHDA in the endocardial region, although regional myocardial perfusion was unchanged as indicated by the homogeneous distribution of thallium-201 (*10*). Those data suggested that ^{11}C-labeled BMHDA might be useful in detecting alterations in myocardial substrate utilization in patients with prolonged, severe hypertension before any ischemic changes had occurred.

Previous studies with a 14-(*p*-iodophenyl)-3(R,S)-methyltetradecanoic acid in humans and rats showed rapid myocardial extraction and prolonged myocardial retention in both species (*24*). The addition of methyl group does not appear to alter myocardial uptake, but enhances the residence time in the heart. The autoradiographic comparison studies suggest that the behavior of BMPDA and BMHDA in the myocardium are similar. The long residence time in the heart should make [^{131}I]BMPDA useful for SPECT study with a rotating gamma camera.

Iodine-123 BMPDA can be prepared rapidly and in high yield and high specific activity (8–10 Ci/mmol) by ^{123}I displacement of the *p*(bis-(trifluoroacetoxy)-thallium) phenyl-substrate. The aryl thallium intermediate rapidly collapses to 15-(*p*-iodophenyl)-3(R,S)-methyl-

FIGURE 4
Autoradiograms of [^{14}C]BMDHA (left) and [^{131}I]BMPDA (right) of same section of hypertensive rat heart. Note heterogenous distribution of radioactivity

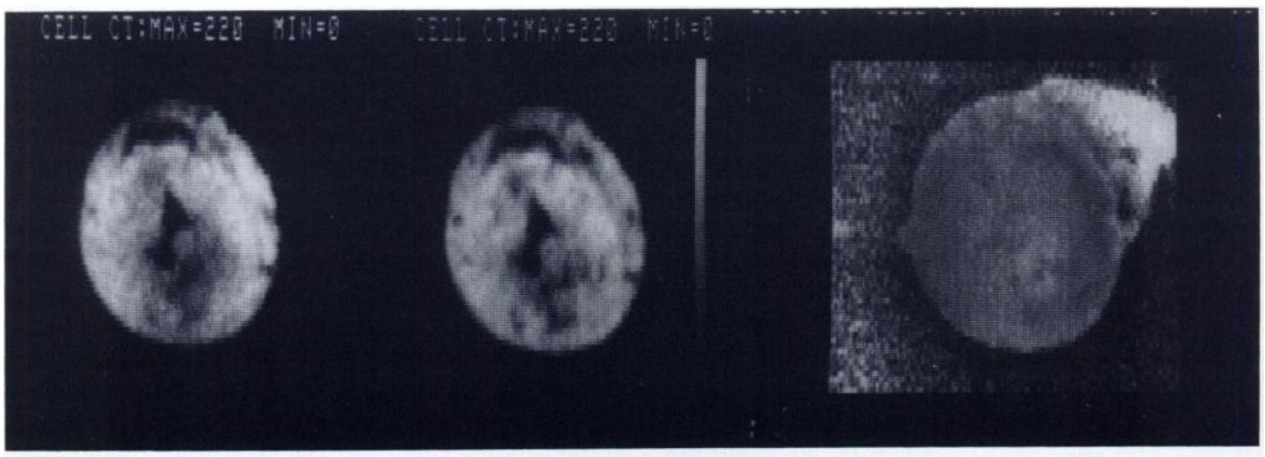


FIGURE 5
Quantitative images of [^{14}C]BMDHA (left) and [^{131}I]BMPDA (center) of same section of hypertensive heart and lung. [^{131}I]BMPDA image was divided by [^{14}C]BMHDA image and ratio image is shown on extreme right of this figure

pentadecanoic acid at 100°C in the presence of one equivalent of ^{123}I (*13*).

Our data showed that [^{131}I]BMPDA radioactivity in myocardium (% dose/g) was almost the same as [^{14}C] BMHDA at 30 min after injection, and both tracers depicted essentially the same heterogenous distribution in the hypertensive heart.

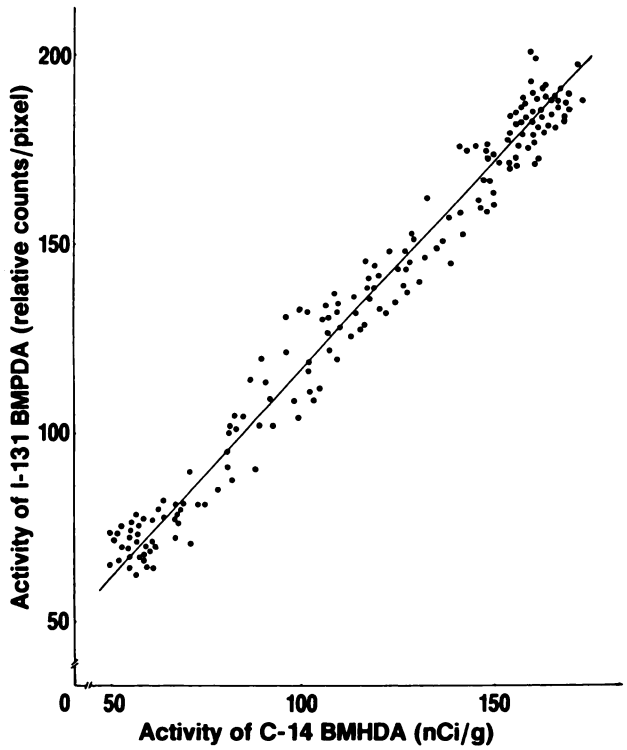


FIGURE 6
Correlation of regional myocardial distribution of [^{14}C] BMHDA and [^{131}I]BMPDA ($r = 0.93 \pm 0.03$)

FOOTNOTES

† DuPont NEN Medical Products, No. Billerica, MA (Lo-Dose Mammography Film).
‡ Hammamatsu TV.
§ DEC, PDP 11/34.

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