Myocardial Accumulation of Iodinated Beta-Methyl-Branched Fatty Acid Analogue, Iodine-125-15-(p-iodophenyl)-3-(R,S)methylpentadecanoic Acid (BMIPP), in Relation to ATP Concentration

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To clarify the relationship between the myocardial accumulation of ^{125}I-15-(p-iodophenyl)-3-(R,S)-methylpentadecanoic acid (BMIPP) and intracellular adenosine-5′-triphosphate (ATP) content, the effect of 2,4-dinitrophenol (DNP, an electron transport uncoupler) on myocardial BMIPP accumulation was studied, in comparison with that of thallium-201-chloride (^{201}Tl-Cl). In the mouse myocardium, DNP decreased the intracellular ATP and ADP levels, without affecting either acyl-CoA synthetase activity or the level of CoA-SH. Following treatment with DNP, decreases in myocardial BMIPP accumulation correlated well with those of ATP, while ^{201}Tl-Cl showed slightly increased accumulation in the myocardium. Thus, in some diseases, BMIPP may be useful in evaluating myocardial ATP levels.

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A beta-methyl-branched fatty acid analogue, iodine-123-15-(p-iodophenyl)-3-(R,S)methylpentadecanoic acid (BMIPP) (Fig. 1) has been proposed as a probe for myocardial fatty acid utilization assessed by single-photon emission computed tomography (SPECT) because of its long retention in the myocardium (*1–3*). Although some studies have noted a dissociation between myocardial uptake of methyl-branched fatty acids and regional myocardial perfusion in animal disease models (*4–10*), the mechanism of decreased myocardial fatty acid uptake have not been elucidated. These methyl-branched fatty acids are not metabolized via beta-oxidation, but are mainly trapped in the triglyceride (TG) fraction (*11*) and myocardial BMIPP accumulation appears to be associated with TG synthesis (*12*).

We speculated that adenosine-5′-triphosphate (ATP) plays an important role in myocardial uptake and retention of BMIPP, because it is required in the first enzymatic conversion of fatty acids to acyl-CoA, a common pathway of fatty acid metabolism, such as TG synthesis and beta-oxidation. To examine this hypothesis, we studied BMIPP accumulation in 2,4-dinitrophenol- (DNP) induced ATP-deficient myocardium in vivo.

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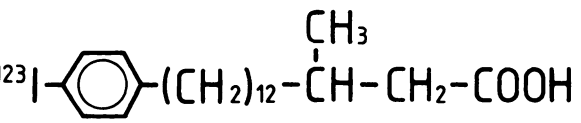
MATERIALS AND METHODS

DNP Treatment of Mice

After overnight fasting, ddY male mice (25 g body weight) were injected with 50 μl of DNP solution (7.5 mg/ml in 1.5% sodium bicarbonate) into the tail vein. Untreated mice served as controls.

Tissue Extraction

Tissue sample extraction was performed as previously reported (*13,14*). Mice were killed by cervical dislocation. The heart was quickly dissected, weighed, and frozen in liquid nitrogen. Heart tissue was ground in a pre-cooled mortar, extracted with 3 ml of 0.42 *M* perchloric acid, or with 0.42 *M* perchloric acid containing 20 m*M* 2-mercaptoethanol for coenzyme A (CoA-SH) analysis, and centrifuged at 3000 rpm for 10 min at 4°C. Supernatant (2 ml) was neutralized with 6 N potassium hydroxide (pH 5–8) and centrifuged at 3000 rpm for 10 min at 4°C to remove the precipitated potassium perchlorate. Next, supernatant (1 ml) was collected and stored at −20°C for HPLC analysis.



^{123}I-BMIPP

FIGURE 1
Structural formula of BMIPP.

ATP, Adenosine-5′-diphosphate (ADP), Adenosine-5′-monophosphate (AMP), and CoA-SH Determination

ATP, ADP, and AMP were determined by high-performance liquid chromatography (HPLC) as previously reported with modification (*13*). A Cosmosil C18 column (4.6 × 50 + 4.6 × 150 mm, Nacarai Tesque Co., Ltd., Japan) was attached to a dual-pump gradient chromatographic system (LC-6A, Shimadzu Co. Ltd., Japan). The mobile phase contained 215 m*M* potassium dihydrogen phosphate, 2.3 m*M* tetrabutylammonium hydrogen sulfate, and 10% acetonitrile. The solvent pH was adjusted to 6.25 with 1 N sodium hydroxide. The flow rate was maintained at 1 ml/min. Samples (10 μl) were injected and absorbance was detected at 206 nm. Signals from the spectrometer were input to a reporting integrator (CHROMATOPAC, Shimadzu Co., Ltd., Japan).

CoA-SH was determined by the slightly modified method previously reported (*14*). A ZORBAX C8 column (6.0 × 250 mm, 5 μm, Du Pont, N. Billerica, MA) was attached to the same HPLC system used in the ATP, ADP, and AMP determinations. The mobile-phase solution was as follows: (a) 220 m*M* KH_2PO_4, 0.05% beta-thiodiglycol, adjusted to pH 3.9 with phosphoric acid; (b) methanol (40%), chloroform (0.9%). Beta-thiodiglycol (0.05%), 120 m*M* KH_2PO_4 was adjusted to pH 3.9 with phosphoric acid. The chromatographic separation was performed at a flow rate of 0.5 ml/min. The mobile-phase composition was divided into a linear gradient system: 88% solvent A (12% solvent B) at time 0, 85% solvent A (15% solvent B) at 8 min, 75% solvent A (25% solvent B) at 15 min, 55% solvent A (45% solvent B) at 21 min, 26% solvent A (74% solvent B) at 26 min, and 100% solvent A at 27 min, and this composition was maintained from 27 to 30 min. The original conditions were reestablished by a reverse gradient to 88% solvent A (12% solvent B) from 30 to 31 min.

Acyl-CoA Synthetase Activity Measurement

Long-chain acyl-CoA synthetase activity was determined as described previously (*15*). [1-^{14}C]-palmitic acid (ECC-075, NEN, N. Billerica, MA) (3.7 KBq in 10 μl ethanol) was mixed with 20 μl of 1% WR-1339 and 0.2 ml of incubation buffer containing 70 μmole of Tris-HCl buffer (pH 7.5), 1 μmole of dithothreitol, 2 μmole of ATP, 0.25 μmole of CoA-SH, and 0.8 μmole of $MgSO_4$. DNP solution (10 μl) was added to the mixture to bring the final DNP concentration of 0–2 μ*M*. An acyl-CoA synthetase (microsome) fraction obtained from mouse heart (*16*) (25 μg protein in 20 μl) was added to the solution and incubated for 1.5 min at 37°C. The enzyme reaction was stopped by the addition of 0.6 ml of 1 N H_2SO_4 and unreacted ^{14}C-palmitic acid was extracted twice with 5 ml of ethyl ether. The organic layer was collected, evaporated, and the radioactivity was measured by liquid scintillation counter (Aloka LSC-900, Japan). Reaction buffer without CoA-SH was used as the reference.

Mouse Biodistribution Studies

Figure 2 shows the protocol of the biodistribution studies in ddY mice (*17*). BMIPP (^{125}I-labeled, 18.9 GBq (0.51 Ci)/mmol) dissolved in 6% HSA solution (7.4 KBq in 0.1 ml) or thallium-201-chloride (^{201}Tl-Cl) (carrier-free, 9.3 KBq in 0.1 ml) was injected into the tail vein at 5–120 min after DNP injection. Mice were anesthetized with ether and killed. Injection-sacrifice intervals of 30 min were used to evaluate the metabolic retention of BMIPP, and of 5 min in the case of ^{201}Tl to evaluate it as a blood flow indicator. Tissues of interest were weighed and radioactivity was measured by a well-type scintillation counter (Aloka ARC-300, Japan).

Statistical Analysis

Statistical significance of the data was evaluated by analysis of variance and Fisher's paired t-test.

RESULTS

The effects of DNP on ATP, ADP, and AMP levels in the mouse heart are shown in Figure 3. The ATP level was quickly dropped upon DNP injection to one-third that of controls, but returned to the control level after 2 hr. The ADP level showed a pattern similar to that of ATP. In contrast, the AMP level increased after DNP treatment, before returning to normal levels.

The CoA-SH levels slightly increased after DNP treatment and then returned to the control level (Fig. 4).

The effect of DNP on acyl-CoA synthetase activity also was studied in vitro. No alteration in acyl-CoA synthetase activity was found in the presence of 2 × 10^{-2}, 2 × 10^{-1}, or 2 μ*M* DNP (96.7, 83.4, and 106.3% of control, respectively).

Table 1 shows the effect of DNP on BMIPP biodistribution in mice. DNP reversibly decreased radioactivity accumulation in the heart. On the other hand, the blood levels remained unchanged during the experiment.

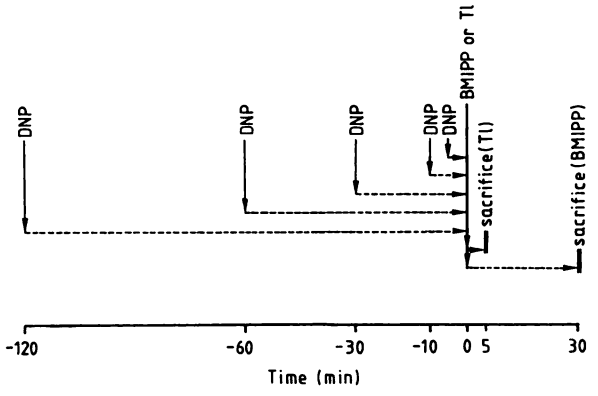


FIGURE 2
Protocol of the biodistribution studies in mice.

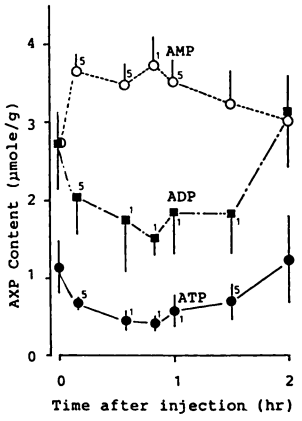


FIGURE 3
Time course of adenosine phosphate (ATP, ADP, AMP) levels in mouse heart after DNP treatment (15 mg/kg. i.v.). Each value represents an average of five animals (1 s.d.). Data at time zero were those of non-treated controls. Statistical significance (^{1}:$p < 0.01$, ^{5}:$p < 0.05$) compared with the control.

Myocardial ^{201}Tl-Cl accumulation increased with DNP treatment without any change in the blood level. These results suggest increased fractional myocardial blood flow (*18*).

Gathering the results of Figure 3 and Table 1, positive correlation ($r = 0.91$) between ATP content and relative myocardial BMIPP accumulation (heart/blood accumulation ratio) was visualized (Fig. 5).

TABLE 1
The Effect of DNP on ^{125}I-BMIPP and ^{201}Tl-Cl Biodistribution in ddY Male Mice*

^{125}I-BMIPP					
Time†	Control	10 min	30 min	1 hr	2 hr
Heart	18.81 (3.13)	14.75‡ (2.31)	14.72‡ (2.55)	13.59‡ (2.18)	15.53 (3.23)
Blood	11.37 (0.96)	11.94 (1.32)	12.77 (0.75)	12.93 (0.71)	10.91 (0.62)
Lung	6.47 (0.35)	7.43 (0.38)	7.78 (0.88)	7.85 (0.74)	7.99 (0.22)
Liver	6.93 (0.53)	8.52 (0.94)	7.30 (0.94)	9.72 (1.15)	14.71 (0.77)
Kidney	7.37 (0.40)	6.70 (0.21)	6.98 (0.50)	7.74 (0.59)	8.52 (0.15)
Stomach	1.58 (0.14)	1.69 (0.16)	1.59 (0.30)	1.60 (0.29)	1.48 (0.16)
Intestine	2.11 (0.14)	2.28 (0.21)	2.38 (0.26)	2.65 (0.15)	2.30 (0.12)
^{201}Tl-Cl					
Time†	Control	5 min	10 min	30 min	1 hr
Heart	20.50 (5.17)	22.72 (2.85)	25.02 (3.01)	25.21 (5.24)	25.63‡ (3.03)
Blood	0.77 (0.08)	0.83 (0.04)	1.04 (0.08)	1.08 (0.09)	1.04 (0.09)
Lung	10.64 (1.53)	10.67 (1.25)	11.89 (0.83)	12.53 (1.22)	11.75 (0.92)
Liver	3.21 (0.35)	3.20 (0.25)	4.19 (0.71)	3.61 (0.37)	3.45 (0.30)
Kidney	27.05 (2.65)	25.63 (3.16)	27.76 (6.19)	28.40 (4.76)	32.28 (6.14)
Stomach	4.21 (0.64)	3.53 (0.57)	3.98 (0.92)	4.50 (0.83)	4.26 (2.37)
Intestine	5.02 (0.60)	5.64 (0.60)	5.96 (0.98)	6.62 (0.72)	6.64 (1.13)

* %dose/g tissue, average of 3–8 animals (1 s.d.).
† Time after DNP treatment (15 mg/kg).
‡ $p < 0.05$ compared to the control, by analysis of variance and Fisher's paired t-test.

DISCUSSION

Under aerobic conditions, the energy requirements of the normal myocardium are usually met by the catabolism of free fatty acids. Goodman et al. suggested the usefulness of radioiodinated methyl-branched fatty acids as potential myocardial imaging agents because of their prolonged retention in the myocardium (*1*). We also obtained better quality myocardial images with BMIPP due to its rapid blood clearance and minimal washout from the heart (unpublished data). However, the significance of myocardial retention of these modified fatty acids should be clarified in order to determine whether they can be used for assessing myocardial metabolism.

Fatty acids in plasma are transported into myocytes through the sarcolemmal membrane and are metabolized via beta-oxidation or stored in lipid fractions. The initial step is the enzymatic conversion of fatty acid to acyl-CoA (*19*). This reaction is irreversible (*20*) and the acyl-CoA produced is hydrophilic (*21*), thus facilitating its cellular retention.

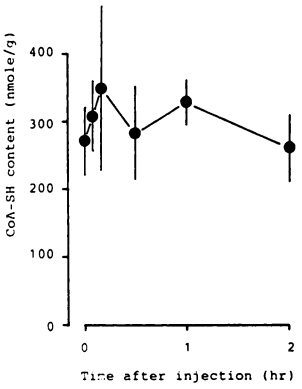


FIGURE 4
Time course of coenzyme-A (CoA-SH) content in mouse heart after DNP treatment (15 mg/kg, i.v.). Each value represents an average of five animals (1 s.d.). Data at time zero were those of non-treated controls. All data were statistically insignificant compared with the control.

Knapp et al. reported that most of the radioactivity was in the free fatty acid fraction in the initial phase of accumulation, but in the latter period it entered the triglyceride fraction (*11*), indicating that 3-methyl branching did not interfere with incorporation into the lipid pool via the enzymatic reaction producing BMIPP-CoA. Therefore, we hypothesize that this enzymatic reaction plays a role in the myocardial retention of BMIPP. This enzymatic reaction may be affected by the contents of ATP, CoA-SH, and acyl-CoA synthetase (Fig. 6). Of those, we focused attention to the ATP content, because of its importance in myocardial function. Therefore, we examined the effect of DNP treatment on myocardial accumulation of BMIPP in rela-

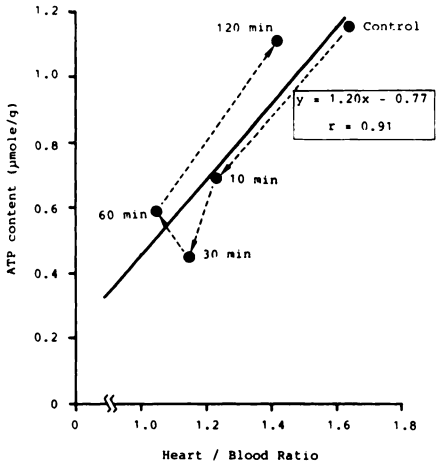


FIGURE 5
Relationship between myocardial ATP content and relative myocardial BMIPP accumulation (heart/blood ratio) in mice. Biochemical analysis and radioactivity distribution studies were performed with different sets of animals.

tion to this enzymatic reaction. DNP is a well known "uncoupler," which dissociates electron-transfers from phosphorylation in mitochondria. This uncoupling is achieved by causing a discharge of the high-energy state that is used to generate ATP (*22*). As a result, DNP stimulates substrate catabolism (fatty acid, glucose), oxygen consumption, and blood flow (*18*) without ATP production (*23*). These phenomena were also noted in this study. Namely, DNP treatment increased ^{201}Tl uptake in the myocardium, indicating an increase in myocardial blood flow. On the other hand, ATP and ADP levels quickly decreased and consequently AMP increased. In addition, the DNP-induced decrease in BMIPP accumulation in the myocardium was similar to that of ATP. These results suggest that the myocardial accumulation of BMIPP is not related to blood flow or fatty acid metabolism, but to ATP content. Moreover, CoA-SH was not considered to affect myocardial BMIPP accumulation. The small increase in CoA-SH was considered to be due to decrease in acyl-CoA synthesis.

Another possibility concerning the effect of DNP on myocardial BMIPP accumulation is the direct interaction of DNP with acyl-CoA synthetase. However, DNP had no marked effect on the enzyme activity in the in-vitro DNP inhibition study using mouse heart microsomal fractions as the enzyme source.

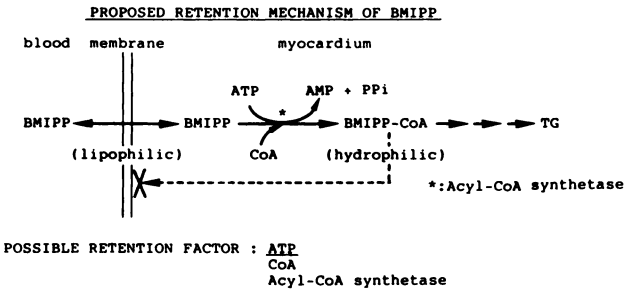


FIGURE 6
Proposed retention mechanism of BMIPP.

Our study suggests that BMIPP accumulation in the myocardium is closely correlated with the intracellular concentrations of high-energy phosphates, especially that of ATP (Fig. 5). The myocardial ATP content and the heart/blood ratio of BMIPP biodistribution showed a positive correlation. Moreover, the sensitivity of BMIPP to changes in ATP levels was higher than that of ^{201}Tl, a potassium analogue transported by Na,K-ATPase. Thus, BMIPP may be an indicator of ATP levels in the myocardium. However, it is possible that the ability of the myocardium to transport and metabolize fatty acids lags behind the return of ATP to pre-DNP levels, as suggested by the difference in time course of the myocardial ATP concentration and myocardial BMIPP. To clarify this point, further investigation is necessary.

The maximum reduction of BMIPP uptake in the myocardium was 28% at 60 min after DNP treatment. At this point, ^{201}Tl-Cl showed a 25% increase in myocardial uptake. From these data, it is estimated that the net extraction of BMIPP decreased more than 38% compared with the control state. The most likely explanation for this decreased net extraction of BMIPP is the effect of back-diffusion of BMIPP from the myocardium, probably as a result of the lack of metabolic retention. It has been demonstrated by positron emission tomography that ^{11}C-palmitate, a radiolabeled natural fatty acid, showed significant back-diffusion under disease conditions (*24*). Further investigation is required to clarify the myocardial kinetics of BMIPP in order to understand the retention mechanism in the myocardium.

In previous studies, decreased and nonhomogenous myocardial uptake of methyl-branched fatty acids were observed in various animal disease models, such as myocardial hypertrophy induced by high blood pressure (*4,5,9*), in cardiomyopathic hamsters (*6,7*), and in adriamycin treated rats (*10*). The present results were obtained via acute studies using a so-called "uncoupler" (DNP); therefore, this conclusion may not be directly applied to other interventional studies or chronic abnormalities. However, this study may contribute to understanding the behavior of beta-methyl-branched fatty acids in the myocardium.

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