Annals of Nuclear Medicine Vol. 10, No. 3, 361–366, 1996

Evaluation of the brain uptake properties of [1-^{11}C]labeled hexanoate in anesthetized cats by means of positron emission tomography

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Positron emission tomography (PET) was performed on the cat brain to characterize [1-^{11}C]hexanoate and other [1-^{11}C]labeled short and medium-chain fatty acids as a tracer of fatty acid oxidative metabolism. After an intravenous injection the brain uptake of [1-^{11}C]hexanoate reached a peak followed by rapid washout until 2 min (first phase). Subsequently the total brain uptake was again increased and reached to a peak 7–10 min after tracer injection (second phase). The blood radioactivity of unmetabolized [1-^{11}C]hexanoate was rapidly decreased and almost eliminated within the first 2 min, whereas the blood radioactivity of [^{11}C]CO_2/HCO_3^- was gradually increased and reached a peak approximately 5 min after tracer injection. As the effect of circulating [^{11}C]CO_2/HCO_3^- was examined by a bolus intravenous injection of [^{11}C]CO_2/HCO_3^-, the brain uptake of [^{11}C]CO_2/HCO_3^- was rapidly increased right after the injection and changed parallel to the blood level of [^{11}C]CO_2/HCO_3^-.

These results suggest that, in contrast to the previous mouse data, the time-activity curve in the cat brain following intravenous injection of [1-^{11}C]hexanoate has a biphasic pattern, the second phase being determined by peripherally originating [^{11}C]CO_2/HCO_3^-, and therefore does not reflect the metabolism of ^{11}C-labeled fatty acid in the brain.

Key words: hexanoate, fatty acid, brain, cats, PET

INTRODUCTION

ALTHOUGH POSITRON LABELED fatty acids have been used to assess the myocardial metabolism,[1,2] few reports have applied them to the brain. The brain relies almost exclusively on glucose as a substrate for energy production in the normal condition.[3] In prolonged starvation the brain utilizes glucose and ketone bodies, but there is no evidence of a significant contribution of fatty acids to the energy metabolism,[4] but a previous study on the uptake of radiolabeled fatty acids by the mouse brain revealed that mitochondria in the brain have the ability to oxidize fatty acids.[5] Fatty acids such as butyrate and octanoate as well as glucose were converted to acetyl-CoA and entered the tricarboxylic acid (TCA) cycle, which was further metabolized to glutamine and glutamate.[6,7] On the basis of these observations, evaluation of the brain uptake of radiolabeled fatty acids *in vivo* was studied in mice and rats. Carbon-14 labeled octanoate, applied to mice *in vivo*, revealed the somatosensory activated area in autoradiography, and was regarded as a functional marker of the brain.[8] Our preliminary studies with mice have demonstrated that the initial brain uptake of [1-^{11}C]hexanoate occurred in the mouse brain, followed by a gradual clearance. We also confirmed that [1-^{11}C]hexanoate was rapidly incorporated into glutamine and glutamate.[9] Considering these findings, we speculated that if β-oxidation is rapid enough, the clearance rate of ^{11}C following injection of [1-^{11}C]hexanoate could reflect the rate of the oxidative process in the TCA cycle, or the retention of ^{11}C could reflect the size of the amino acid pool coupled to the TCA cycle. Although [1-^{11}C]labeled acetate is suitable because it is directly converted to ^{11}C-acetyl-CoA and enters the TCA cycle without a β-oxidative process, it may be inappropriate for brain imaging because of its low

Received October 18, 1995, revision accepted May 30, 1996.

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first pass extraction across the blood-brain barrier. It is known that medium-chain fatty acids such as hexanoate and octanoate penetrate the blood-brain barrier more easily than acetates.[10] Our preliminary study also indicate that the brain uptake of [1-^{11}C]hexanoate was twice as much as that of [1-^{11}C]acetate.[11] Since [1-^{11}C]hexanoate appeared promising for the evaluation of short and medium chain fatty acid metabolism in the mouse,[9,11] we planned to acquire time sequential images with a PET camera together with blood radioactivity analysis on larger animals. The clearance rate of radioactivity measured by PET possibly reflects the metabolism of hexanoate as described above. In this study the authors aim to evaluate the potential of [1-^{11}C]hexanoate as a radiopharmaceutical assessing oxidative metabolism of the cat brain by PET.

METHODS

Four male cats aged 7–8 years weighing 3.6–4.0 kg were used for the present experiment. The cats were anesthetized with halothane. During the experiment the halothane concentration was kept at 1.0%. The animals were immobilized with galamine triethiodide and mechanically ventilated to maintain the end-tidal carbon dioxide at 3.0%. The body temperature was maintained at 37.5°C with a heating pad and lamp with thermocontroller, and the systemic arterial blood pressure was maintained at 90–120 mmHg. These physiological conditions were continuously monitored on a polygraph recorder. The anesthetized animal was positioned in a holder made of polyacrylate, a modification of a stereotaxic holder for physiological experiments (Narishige, Tokyo, Japan).

The synthesis method for the ^{11}C-labeled compounds is described elsewhere.[11,12] [^{11}C]CO_2/HCO_3^- was produced by dissolving $^{11}CO_2$ in a 7% sodium bicarbonate solution. Carrier-loaded [1-^{11}C]hexanoate was obtained by adding 1 mmol/kg of hexanoate to the [1-^{11}C]hexanoate solution. The cat underwent a consecutive PET measurement with [^{15}O]labeled water and one or two of the ^{11}C-labeled compounds: [1-^{11}C]hexanoate, [1-^{11}C]octanoate, [1-^{11}C]butanoate, [1-^{11}C]acetate, [^{11}C]CO_2/HCO_3^- and carrier-loaded [1-^{11}C]hexanoate. Each measurement was performed after the radioactivity ceased. Measurement with [1-^{11}C]hexanoate was done on three cats, and other tracer experiments were each performed once. The SHR-2000 PET camera (Hamamatsu Photonics, Hamamatsu, Shizuoka, Japan) was used and provided a set of 7-slice images at 6.5-mm intervals with an image spatial resolution of 4.0-mm full width at half maximum (FWHM).[13] Following an injection of 1 GBq of [^{15}O]labeled water, scanning was performed for 3 min to confirm the positioning. After the radioactivity ceased, transmission data were obtained with a rotating ^{68}Ge/^{68}Ga rod source. Subsequently 600 MBq of ^{11}C-labeled compound was injected and scanned for 60 min in a dynamic mode. After the measurement, tomographic images were reconstructed with a Butterworth filter with a cutoff frequency of 144 cycles/cm. To measure the radioactivity in the whole brain, the region of interest (ROI) was determined on each slice of the image of [^{15}O]labeled water by cutting off at 30% of the maximal pixel value. All analysis procedures were taken on a workstation with an image analysis software Dr. View version 4.0 (Asahi Kasei Joho System, Tokyo, Japan).

The time course of radioactivity in the arterial blood was measured in blood samples manually collected from the femoral artery catheter. Samples were collected at 20 sec, 40 sec, and 1, 1.5, 2, 3, 5, 10, 15, 30 min after tracer injection. Analysis of metabolites in the blood was performed by the modified method of Shields et al.[14] An aliquot (30 μl) of each blood sample was immediately mixed with 1 M NaOH solution to measure the total ^{11}C concentration in the blood. Another 30 μl aliquot of each sample was mixed with 1 M HCl solution to expel CO_2/HCO_3^-, and then applied to an SPE column ODS-4 (Whatman Co.) to separate unmetabolized [1-^{11}C]hexanoate. The concentration of [^{11}C]CO_2/HCO_3^- was estimated by subtracting the radioactivity in the HCl-treated aliquot from that in the NaOH-treated one. The radioactivity was measured in a well counter.

The tissue or blood uptake of tracer was evaluated by the differential uptake ratio (DUR) or area under the curve (AUC) of DUR calculated by the following equation.

$$\mathrm{DUR} = \frac{\text{tissue (or blood) radioactivity (MBq/g)}}{\text{injected dose (MBq) / body weight (g)}}$$

$$\mathrm{AUC}_{\mathrm{hexanoate}} = \Sigma\, \mathrm{DUR(hexanoate)}\, \Delta t$$

RESULTS

Figure 1 summarizes the images of three brain slices (rostral, middle, caudal) following i.v. injection of [^{15}O]labeled water and [1-^{11}C]hexanoate. Both early phase images (2–5 min) and later phase images (5–10 min) of [1-^{11}C]hexanoate showed that the distribution was not much different from the images for [^{15}O]labeled water. Based on the images for [^{15}O]labeled water, the ROIs of the whole brain were determined.

Figure 2 shows time-activity curves of the total ^{11}C concentration in the whole brain following i.v. injection of [1-^{11}C]hexanoate into three cats. Up to 2 min after an injection the brain uptake of [1-^{11}C]hexanoate was rapidly increased and then rapidly decreased (early phase). Subsequently the brain uptake of the total ^{11}C increased again by approximately 30%, and reached another peak 7–10 min after tracer injection (second phase). Then up to the end of the experiment the level of the total ^{11}C was gradually decreased.

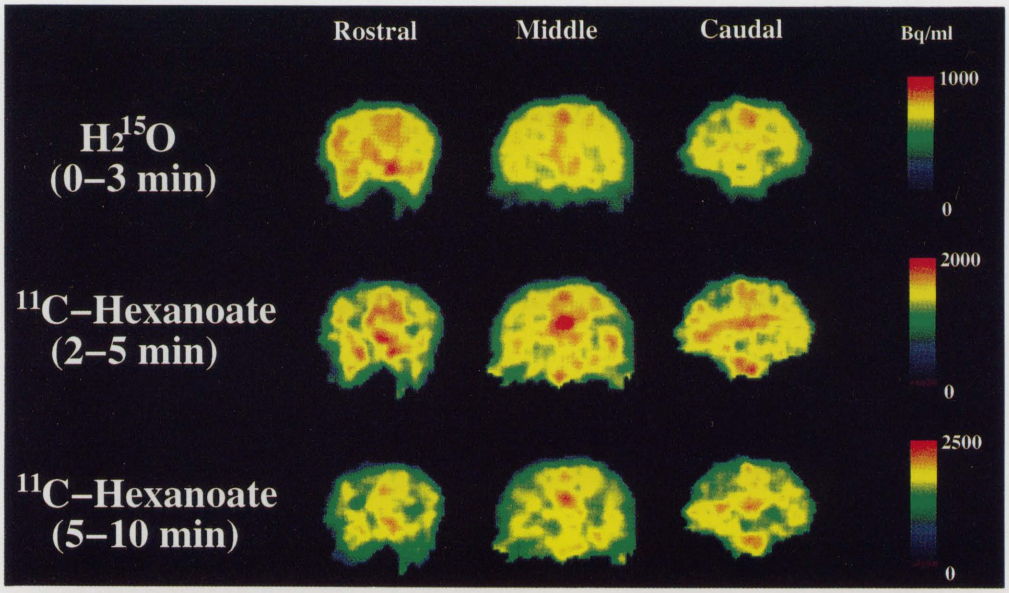

Fig. 1 PET images of cat brain acquired with [^{15}O]labeled water, [1-^{11}C]hexanoate (average of 2–5 min) and [1-^{11}C]hexanoate (average of 5–10 min). Three out of 7 slices were selected (rostral, middle, caudal), anatomically corresponding to the coronal planes crossing 0 mm, 6.5 mm and 13.0 mm anterior to the interaural line. Values were expressed by Bq/m*l*.

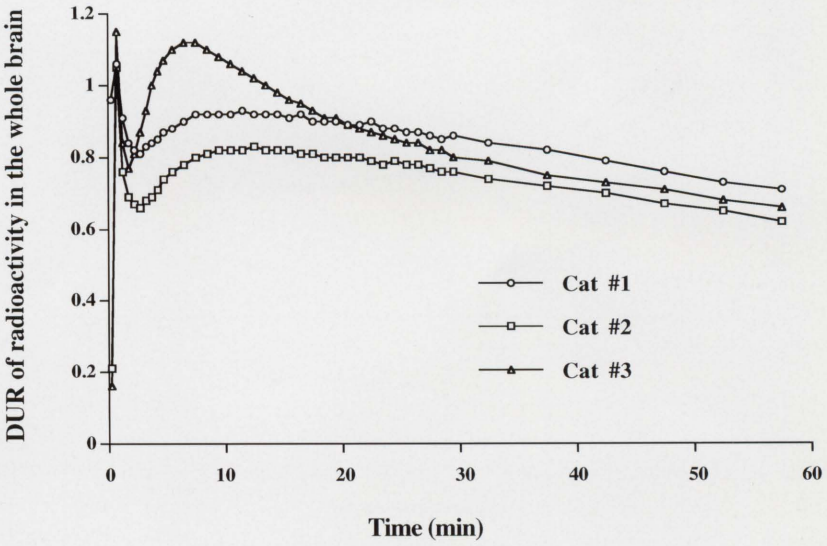

Fig. 2 Time course of total ^{11}C concentrations in the whole brain following i.v. injection of [1-^{11}C]hexanoate into three cats. Values represent differential uptake ratio (DUR) as defined in the text.

Figure 3 shows time-activity curves for total ^{11}C, [1-^{11}C]hexanoate and [^{11}C]CO_2/HCO_3^- fractions in the blood following intravenous injection of [1-^{11}C]hexanoate. Up to 5 min after the tracer injection the radioactivity of [1-^{11}C]hexanoate in the blood rapidly decreased and was almost completely eliminated. On the other hand, the radioactivity of [^{11}C]CO_2/HCO_3^- in the blood gradually increased and reached a peak value 5 min after injection, then gradually decreased until the end of the experiment.

Figure 4 shows the time course of [^{11}C]CO_2/HCO_3^- concentrations in the whole brain and blood following i.v. injection of [^{11}C]CO_2/HCO_3^- into a cat. Following the initial peak the brain uptake of [^{11}C]CO_2/HCO_3^- gradually decreased until the end of the experiment. The time courses for the brain and blood level of [^{11}C]CO_2/HCO_3^- almost exactly paralleled each other. Up to 10 min after tracer injection, the AUC of the brain level was about 50% of that of the blood level.

Figure 5 shows the time courses of radioactivity in the whole brain and blood following intravenous injection of [1-^{11}C]hexanoate (carrier-free) and [1-^{11}C]hexanoate with 1 mmol/kg of hexanoate (carrier-added) into a cat. During the first 5 min both the brain and blood radioactivities were higher in the carrier-added condition than in the

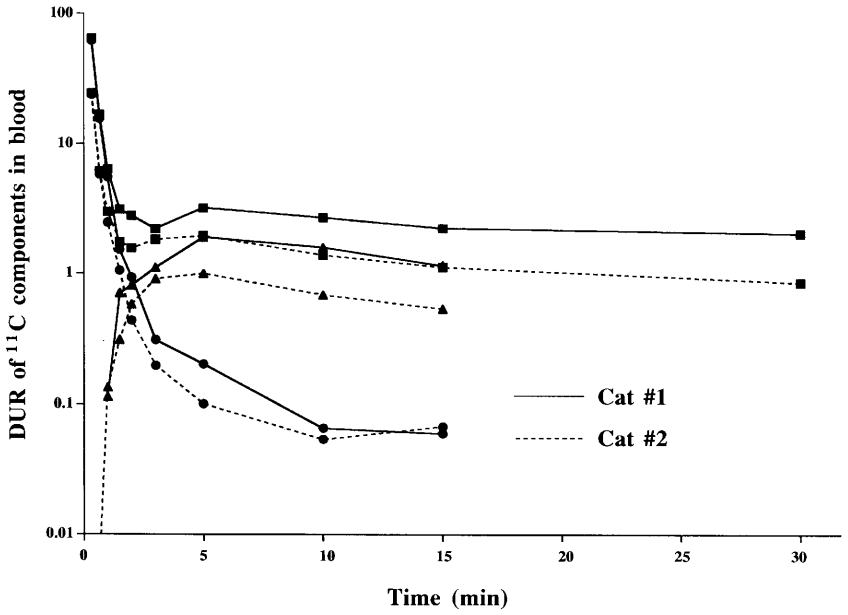

Fig. 3 Time course of radioactivity of total ^{11}C (closed square), unmetabolized [1-^{11}C]hexanoate (closed circle) and [^{11}C]-CO_2/HCO_3^- (closed triangle) in the blood following i.v. injection of [1-^{11}C]hexanoate into two cats. Values represent differential uptake ratio (DUR), index of tissue or blood radioactivity as defined in the text.

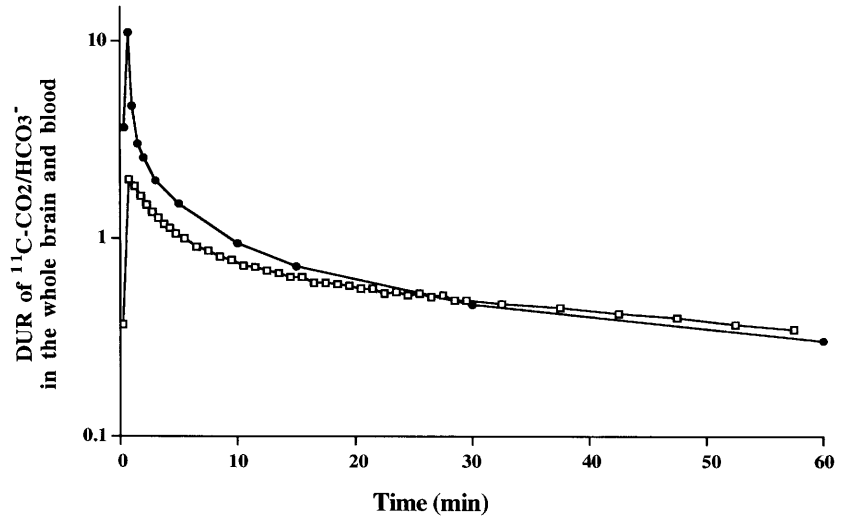

Fig. 4 Time course of radioactivity in the whole brain (open square) and blood (closed circle) following i.v. injection of [^{11}C]CO_2/HCO_3^- into a cat. Values represent differential uptake ratio (DUR) as defined in the text.

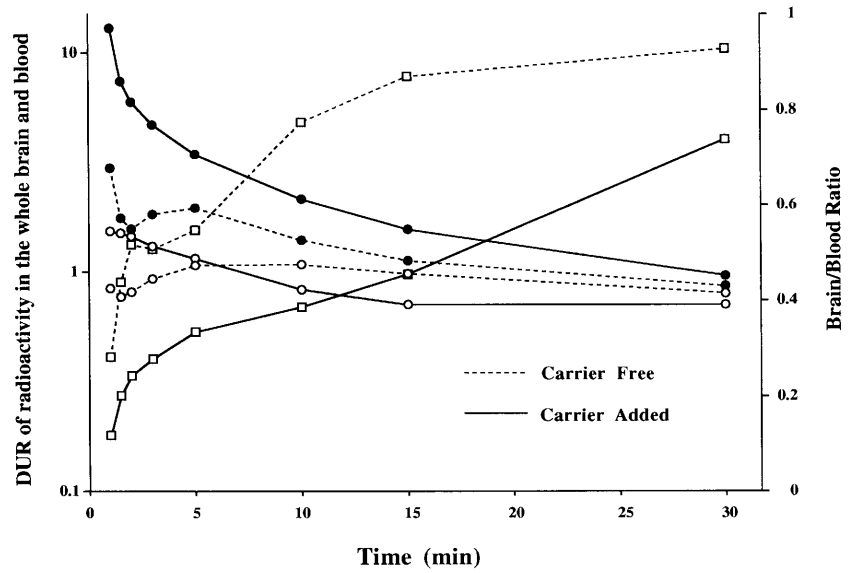

Fig. 5 Time course of radioactivity in the whole brain (open circle) and blood (closed circle) and their ratio (open square) following i.v. injection of [1-^{11}C]hexanoate (carrier-free, dotted line) and [1-^{11}C]hexanoate with 1 mmol/kg of hexanoate (carrier-added, solid line) into a cat. Values represent differential uptake ratio (DUR) as defined in the text.

carrier-free, but as the brain activity was compared with the blood activity, which is considered input function, the brain/blood ratio was noticeably diminished when carrier was added.

The difference in the brain uptake of various [1-^{11}C]labeled fatty acids was evaluated by the ratio of AUC to $AUC_{hexanoate}$ in the same animal estimated during the initial 5 min, and the results were as follows: $AUC_{acetate}$/$AUC_{hexanoate}$, $AUC_{octanoate}$/$AUC_{hexanoate}$ and $AUC_{butanoate}$/$AUC_{hexanoate}$ were 0.65, 0.93, 1.13, respectively. The brain uptake of [1-^{11}C]acetate was lower than [1-^{11}C]hexanoate, whereas the brain uptake of [1-^{11}C]butanoate, [1-^{11}C]octanoate and [1-11C]hexanoate were almost the same.

DISCUSSION

The present results have demonstrated a biphasic pattern in the time-activity curve of the brain following intravenous injection of [1-^{11}C]hexanoate as shown in Figure 2. The first phase (initial 2 min) may represent the blood pool passage together with rapid uptake and washout of [1-^{11}C]hexanoate in the brain tissue. As shown in Figure 1, the images of [^{15}O]labeled water and the images of [1-^{11}C]hexanoate at 2–5 min showed similar patterns of distribution reflecting perfusion, indicating that [1-^{11}C]hexanoate penetrated into the brain according to the regional blood flow. Although the images of [1-^{11}C]hexanoate at 5–10 min showed similar distribution as well, this second phase must be interpreted in a different way. As shown in Figure 3, [^{11}C]CO_2/HCO_3^- was produced in the blood and reached a peak at 5 min, whereas [1-^{11}C]hexanoate in the blood was rapidly eliminated and almost negligible at 5 min. Furthermore, as shown in Figure 4, [^{11}C]CO_2/HCO_3^- easily penetrated into the brain and remained equilibrated at 50% of the blood level. These results suggest that the second phase (5–10 min after tracer injection) of the [1-^{11}C]hexanoate time course in the brain reflects redistribution of the peripherally originating [^{11}C]CO_2/HCO_3^-. Our preliminary results have demonstrated that [^{11}C]CO_2/HCO_3^- in the mouse brain following injection of [1-^{11}C]hexanoate was approximately 9% of the total ^{11}C 3 min after tracer injection, which was completely eliminated 15 min after tracer injection. The results were not consistent with the data for cats, indicating that the clearance rate of [^{11}C]CO_2/HCO_3^- in the mouse brain was faster than that in the brain of larger animals. In addition, amino acid (glutamine and glutamate) in the mouse brain was about 70% of total radioactivity 3 min after the injection of [1-^{11}C]hexanoate, demonstrating that the major metabolites were glutamine and glutamate,[9] and this is also true of labeled octanoate.[15]

On the other hand, the brain uptake in the 2–5 min after the injection may reflect the regional cerebral blood flow, if the rate of β-oxidation is low enough.

The results of the carrier-loading study demonstrated that, as shown in Figure 5, the brain/blood ratio was lower in the carrier-added than in the carrier-free condition. These results were compatible with the preliminary results for mice,[9] indicating the existence of a carrier-mediated transport mechanism.

The chain-length dependency was examined by using [1-^{11}C]acetate(C2), [1-^{11}C]butanoate(C4), [1-^{11}C]hexanoate(C6) and [1-^{11}C]octanoate(C8). The brain uptake of C2 was lower than C6, whereas the brain uptake C4 and C8 were almost the same as C6. These results suggest that [1-^{11}C]hexanoate was suitable for use in this study because the brain uptake of fatty acid is almost the same if the carbon number of the chain is within the 4 to 8 range, although a different brain uptake index was reported by Ordendorf.[10] The lower brain uptake of [1-^{11}C]acetate may be due to the low lipophilicity of acetate, which makes it difficult to penetrate into the blood-brain barrier. The results were consistent with the results of a previous mouse study.[11]

As previously mentioned, we hypothesized, from the mouse data, that the radiolabeled fatty acid which enters the brain and is metabolized in the TCA cycle can be used in assessing the oxidative pathway or the pool of glutamate and glutamine in the brain by PET, but the present results revealed that it would be difficult to evaluate the oxidative process or the size of the amino acid pool by the total ^{11}C uptake or clearance in the cat brain with [1-^{11}C]hexanoate, because the redistribution of peripherally originating [^{11}C]-CO_2/HCO_3^- into the brain makes it difficult to interpret the clearance curve. In other words, the initial uptake of [1-^{11}C]hexanoate was not sufficient to overcome the confounding effect of peripheral metabolite. To properly evaluate the behavior of [1-^{11}C]hexanoate in the brain, correction for CO_2/HCO_3^- in the blood, and its contribution to brain activity would be necessary.

ACKNOWLEDGMENT

This work was supported by Grant-in-Aid for Scientific Research (C) No. 06807076 from the Ministry of Education, Science and Culture, Japan, and the Life Science Foundation of Japan. The authors thank Dr. T. Nozaki and Dr. K. Ogawa of the Faculty of Hygienic Sciences, Kitazato University, for their valuable advice. The authors also thank Atsuko Suzuki, of the Department of Autonomic Nervous System in the Tokyo Metropolitan Institute of Gerontology, for her technical assistance.

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