EFFECT OF HYPOTHERMIA AND ETHER ANESTHESIA ON THE DISPOSITIONS OF CREATININE AND UREA IN MICE*

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Hypothermal mice were used under the anesthetized condition with ether to clarify the effect of diminished blood circulation on the dispositions of creatinine and urea which are considered to pass through the water-filled pores of biological membranes easily.

The total body clearance of each chemical was considerably decreased in hypothermal mice compared with normal mice. This was considered to be caused by the decrease of glomerular filtration rate.

The whole-body autoradiograms obtained following intravenous administration of ^{14}C -urea and the rate of muscular blood flow showed that the transfer of urea from blood to muscle was apparently delayed by the decrease of muscular blood flow rate. However, the transfer of creatinine to muscle was not influenced by the change of muscular blood flow rate. This difference between creatinine and urea might be related to the higher permeability of muscular cell membrane to urea than to creatinine.

Keywords — creatinine; urea; hypothermal mouse; plasma level; whole-body autoradiography; disposition; total body clearance; muscular cell membrane permeability; muscular blood flow rate

INTRODUCTION

In a previous paper,¹⁾ the authors reported the dispositions of creatinine and urea, which are likely to pass through the water-filled pores of biological membranes easily, in 1-day-old, 1-week-old, 3-week-old and 8-week-old mice. It was clarified in the study that the transfer of creatinine from blood to muscle was more rapid in 1-day-old and 1-week-old mice, especially in 1-day-old mice, than in the more aged mice. The difference in muscular blood flow rate or muscular cell membrane permeability to creatinine was assumed to be responsible for this result.

In this paper, to clarify the significance of blood flow rate on the dispositions of creatinine and urea, the rectal temperature of mice (8 weeks old) was reduced by ether anesthesia and

ice-cooling, and these mice were used as model animals for diminished blood circulation. Whole blood concentration analysis and whole-body autoradiography following intravenous administration of each chemical were carried out, and muscular blood flow rate was measured. The results obtained were compared with those for normal mice (8 weeks old) reported previously.¹⁾

MATERIALS AND METHODS

Chemicals — [carbonyl- ^{14}C] Creatinine hydrochloride (specific activity, 12.0 mCi/mmol) and ^{14}C -urea (specific activity, 9.8 mCi/mmol) were purchased from Amersham International Ltd., Amersham, England, and New England Nuclear, Boston, Mass., U.S.A., respectively. All other chemicals were of analytical grade and

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were used without further purification.

Animals and Induction of Hypothermia

— Male mice of ddY strain were purchased from Shizuoka Agricultural Cooperative for Experimental Animals, Hamamatsu, Japan, and were used for experiments at 8 weeks old (30.4–38.1 g). After rectal temperature of mice (about 38 °C) was reduced to 25–27 °C by ether anesthesia and ice-cooling, each radiochemical was administered or muscular blood flow rate was determined. Therefore, hypothermal mice in this paper were kept under anesthesia with ether. Ether anesthesia and ice-cooling of mice were carried out when it is necessary to maintain the rectal temperature at 25–27 °C. To evaluate the effect of hypothermia, it may be necessary to use the anesthetized mice with normal body temperature as controls. However, it may be practically impossible to equalize the effect of ether anesthesia between hypothermal and control mice. In this paper, the data with hypothermal mice were compared with those for normal mice reported previously.¹⁾

Whole Blood Levels of ¹⁴C-Creatinine and ¹⁴C-Urea Following Intravenous Administration

— Mice were given 100 µCi/kg of ¹⁴C-creatinine (0.94 mg/kg as creatinine) and ¹⁴C-urea (0.61 mg/kg as urea) intravenously into the tail vein. Blood samples (5 µl) were taken periodically with Microcaps (Drummond Scientific Co., Broomal, Pa., U.S.A.) by wounding the other tail vein. These blood samples were dissolved in 200 µl of Soluene-350 (Packard Instrument Co., Downers Grove, Ill., U.S.A.) and then decolorized with 50 µl of hydrogen peroxide. In order to stanch a cut of the veins, an adhesive agent (Aron Alpha, Touagousei Co., Ltd., Tokyo, Japan) was applied.

Whole-Body Autoradiography Following Intravenous Administration — Mice were given 100 µCi/kg of ¹⁴C-creatinine (0.94 mg/kg as creatinine) and ¹⁴C-urea (0.61 mg/kg as urea) intravenously into the tail vein and sacrificed at 30 s, 2 and 5 min following the administration by soaking them in dry ice-acetone (–78 °C). Sections (40 µm) were obtained with a microtome

(Yamato 1111, Tokyo, Japan) at about –25 °C, and attached to Salotape (Hisamitsu Pharmaceutical Co., Ltd., Tosu, Japan). After being dried in a freeze-dryer for a few days, the sections were placed in contact with X-ray films (No. 150, Fuji Photo Film Co., Ltd., Tokyo, Japan) for 20 d at 4 °C. A densitometer (PDA-11, Konishiroku, Tokyo, Japan) was used for determining the optical density of autoradiograms.

Muscular Blood Flow Rate — The skin at the back of hypothermal and normal (unanesthetized condition) mice was incised and the muscle was exposed. The elimination half-life of hydrogen gas in muscle (*M. latissimus dorsi*) was measured using a blood flowmeter (PHG-300, MT Giken, Co., Ltd., Tokyo, Japan) following inspirational administration of proper amount of hydrogen gas to the mice. Muscular blood flow rate was calculated by the equation²⁾ shown below.

$$F = \frac{0.693}{t_{1/2}}$$

F: muscular blood flow rate (ml/min/g muscle)

*t*_{1/2}: biological half-life of hydrogen gas in muscle (min)

Radioactivity Measurement — The radioactivity was determined in a Mark II liquid scintillation spectrometer (Nuclear-Chicago Corporation, Des Plaines, Ill., U.S.A.). All samples were determined with 10 ml of toluene-Triton X-100 liquid scintillator (2,5-diphenyloxazole (PPO) 5 g, 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP) 0.3 g, toluene 700 ml, Triton X-100 300 ml). The counting efficiencies were automatically determined by the ¹³³Ba external standard ratio method and cpm was converted to dpm.

RESULTS

1. Whole Blood Levels of ¹⁴C-Creatinine Following Intravenous Administration

As ¹⁴C-creatinine was quantitatively excreted as unchanged form into the urine following intravenous administration to adult mice,³⁾ all of the radioactivity in whole blood were regarded as originating from ¹⁴C-creatinine.

Whole blood levels of ^{14}C -creatinine following intravenous administration to hypothermal mice and normal mice,¹⁾ which were analyzed on the basis of a one-compartment open model, are shown in Fig. 1, and the estimated pharmaco-

FIG. 1. Whole Blood Levels of ^{14}C -Creatinine Following Intravenous Administration to Hypothermal (●) and Normal (○) Mice

Each point represents the mean $\pm$ S.D. for four to six mice. The points without a vertical bar have S.D. values smaller than the circles. The plots are computer-fitted lines (weight (i)=1). Rectal temperature of hypothermal mice was 25–27°C. The data for normal mice are reported previously.¹⁾

kinetic parameters are summarized in Table I. The distribution volume of creatinine was significantly ($p<0.05$) but barely different between hypothermal (440 ± 11 ml/kg) and normal (498 ± 52 ml/kg) mice. However, the elimination of creatinine from whole blood in hypothermal mice ($t_{1/2}$; 76.8 ± 4.6 min) was considerably delayed than that in normal mice ($t_{1/2}$; 30.8 ± 8.0 min) ($t<0.01$). Accordingly, the total body clearance ($k_{el} \cdot V_d$) was much different between hypothermal (4.0 ± 0.3 ml/min/kg) and normal (11.2 ± 3.1 ml/min/kg) mice ($p<0.01$).

2. Whole Blood Levels of ^{14}C -Urea Following Intravenous Administration

^{14}C -Urea administered intravenously to adult mice was excreted into the urine as unchanged form (82.2%) or metabolized and excreted into the expired air as $^{14}\text{CO}_2$ (11.1%) in 24 h.³⁾ However, in this paper, all of the radioactivity in whole blood was provisionally regarded as originating from ^{14}C -urea.

Figure 2 shows the whole blood levels of ^{14}C -urea following intravenous administration to hypothermal mice and normal mice.¹⁾ In contrast with the data for normal mice, apparent distribution phase was observed in the whole blood concentration in hypothermal mice. The estimated pharmacokinetic parameters for hypothermal mice on the basis of a two-compartment open model are summarized in Table II, accompanied with those for normal

TABLE I. Pharmacokinetic Parameters for ^{14}C -Creatinine Following Intravenous Administration to Hypothermal and Normal Mice (Value for Parameter $\pm$ Standard Error^{a)})

Parameter	Hypothermal ($n=7$) ^{b)}	Normal ^{c)} ($n=8$) ^{b)}
V_d (ml/kg)	4.40×10^2 ^{d)} $\pm 1.10 \times 10^1$	4.98×10^2 $\pm 5.23 \times 10^1$
k_{el} (min^{-1})	9.02×10^{-3} ^{e)} $\pm 5.35 \times 10^{-4}$	2.25×10^{-2} $\pm 5.83 \times 10^{-3}$
$t_{1/2}$ (min)	7.68×10^1 ^{e)} $\pm 4.55 \times 10^0$	3.08×10^1 $\pm 7.98 \times 10^0$
$k_{el} \cdot V_d$ (ml/min/kg)	3.97×10^0 ^{e)} $\pm 2.55 \times 10^{-1}$	1.12×10^1 $\pm 3.13 \times 10^0$

a) W. E. Deming, "Statistical Adjustment of Data," John Wiley and Sons, Inc., New York, 1946.

b) The number of input data, each of which is the mean for four to six mice.

c) Data in previous report.¹⁾

d) Significantly different from the value for normal mice at $p<0.05$.

e) Significantly different from the value for normal mice at $p<0.01$.

FIG. 2. Whole Blood Levels of ^{14}C -Urea Following Intravenous Administration to Hypothermal (●) and Normal (○) Mice

Each point represents the mean $\pm$ S.D. for four mice. The plots are computer-fitted lines (weight $(i) = 1/C_i^2$ in hypothermal mice, where C_i is the whole blood levels and weight $(i) = 1$ in normal mice). Rectal temperature of hypothermal mice was $25-27^\circ\text{C}$. The data for normal mice are reported previously.¹⁾

mice whose data were fitted to a one-compartment open model. In line with the case of creatinine, the elimination half-life of urea in hypothermal mice (18.2 ± 6.4 h) was markedly longer than that in normal mice (1.1 ± 0.1 h), and the total body clearance of urea in hypothermal mice ($k_{10} \cdot V_1'$; 0.58 ± 0.22 ml/min/kg) was about one tenth of the normal value ($k_{el} \cdot V_d'$; 5.79 ± 0.47 ml/min/kg). The extent of decrease in total body clearance of urea due to hypothermia was much greater than that of creatinine.

3. Whole-Body Autoradiography Following Intravenous Administration of ^{14}C -Creatinine

Whole-body autoradiogram at 5 min following intravenous administration of ^{14}C -creatinine to hypothermal mice is shown in Fig. 3, with that for normal mice.¹⁾ Any difference was not observed visually between the two autoradiograms. Muscle is the very important tissue for an observer to decide visually whether radioactivity is distributed homogeneously or heterogeneously in the body. The relative optical densities of muscle to heart blood in the autoradiogram at 30 s, 2 and 5 min following the admin-

TABLE II. Pharmacokinetic Parameters for ^{14}C -Urea Following Intravenous Administration to Hypothermal and Normal Mice (Value for Parameter $\pm$ Standard Error^{a)})

Hypothermal ($n=7$) ^{b)}		Normal ^{c)} ($n=5$) ^{b)}
A (dpm/ml)	$3.21 \times 10^5 \pm 4.11 \times 10^4$	V_d' (ml/kg)
B (dpm/ml)	$2.42 \times 10^5 \pm 6.29 \times 10^3$	$5.57 \times 10^2 \pm 2.53 \times 10^1$
α (min^{-1})	$1.47 \times 10^{-1} \pm 2.33 \times 10^{-2}$	k_{el} (min^{-1})
β (min^{-1})	$6.38 \times 10^{-4} \pm 2.27 \times 10^{-4}$	$1.04 \times 10^{-2} \pm 6.89 \times 10^{-4}$
k_{10} (min^{-1})	$1.48 \times 10^{-3} \pm 5.39 \times 10^{-4}$	$t_{1/2}$ (min)
k_{12} (min^{-1})	$8.24 \times 10^{-2} \pm 1.65 \times 10^{-2}$	$6.66 \times 10^1 \pm 4.41 \times 10^0$
k_{21} (min^{-1})	$6.34 \times 10^{-2} \pm 7.46 \times 10^{-3}$	$k_{el} \cdot V_d'$ (ml/min/kg)
V_1' (ml/kg)	$3.94 \times 10^2 \pm 3.03 \times 10^1$	$5.79 \times 10^0 \pm 4.65 \times 10^{-1}$
V_2' (ml/kg)	$5.13 \times 10^2 \pm 1.25 \times 10^2$	
AUC (dpm $\cdot$ min/kg)	$3.81 \times 10^8 \pm 1.35 \times 10^8$	
$(V_d')_{\text{extrap}}$ (ml/kg)	$9.17 \times 10^2 \pm 2.35 \times 10^1$	
$(V_d')_{\beta}$ (ml/kg)	$9.14 \times 10^2 \pm 4.73 \times 10^2$	
$t_{1/2\beta}$ (min)	$1.09 \times 10^3 \pm 3.86 \times 10^2$	
$k_{10} \cdot V_1'$ (ml/min/kg)	$5.83 \times 10^{-1} \pm 2.17 \times 10^{-1}$	

a) W. E. Deming, "Statistical Adjustment of Data," John Wiley and Sons, Inc., New York, 1946.

b) The number of input data, each of which is the mean for four mice.

c) Data in previous report.¹⁾

FIG. 3. Whole-Body Autoradiograms Showing the Distribution of Radioactivity (Dark Areas) at 5 min Following Intravenous Administration of ^{14}C -Creatinine to Hypothermal (A) and Normal (B) Mice. The data for normal mice are reported previously.¹⁾

FIG. 4. Relative Optical Densities of Muscle to Heart Blood in Autoradiograms Following Intravenous Administration of ^{14}C -Creatinine (A) or ^{14}C -Urea (B) to Hypothermic (●) and Normal (○) Mice. The data for normal mice are reported previously.¹⁾

istration are shown in Fig. 4 (A). As is expected by visual observation of autoradiograms, the relative optical densities were scarcely different between hypothermal and normal mice.

4. Whole-Body Autoradiography Following Intravenous Administration of ^{14}C -Urea

The autoradiogram at 5 min following the administration of ^{14}C -urea to hypothermal mice is shown in Fig. 5, with that for normal mice.¹⁾ As discussed in a previous paper,¹⁾ the distribution pattern of radioactivity in normal mice was almost homogeneous throughout the body at 5

min. In hypothermal mice, however, radioactivity contained in the muscle seemed to be fairly less than that in the heart blood and radioactivity fairly existed in the liver. The relative optical densities of the muscle to the heart blood in the autoradiograms (Fig. 4 (B)) supported this visual observation, *i.e.*, the relative optical densities for hypothermal mice were lower than those for normal mice at any time.

5. Muscular Blood Flow Rate

Table III shows the determined rates of muscular blood flow in hypothermal and normal

FIG. 5. Whole-Body Autoradiograms Showing the Distribution of Radioactivity (Dark Areas) at 5 min Following Intravenous Administration of ^{14}C -Urea to Hypothermal (A) and Normal (B) Mice. The data for normal mice are reported previously.¹⁾

mice. The value for hypothermal mice (0.11 ± 0.05 ml/min/g muscle) was remarkably lower than that for normal mice (0.94 ± 0.45 ml/min/g muscle) with statistical significance ($p < 0.01$).

DISCUSSION

As both creatinine⁴⁾ and urea⁵⁾ are known to bind negligibly with plasma protein, the effect of hypothermia on the protein binding of creatinine and urea was not considered in this paper.

During progressive hypothermia, the glomerular filtration rate and effective renal plasma flow were reported to decrease in a linear manner with decreasing rectal temperature.⁶⁾ This may be the reason for the decreased total body clearance of creatinine and urea in hypothermal mice, because the eliminations of these two chemicals from the body are mainly dependent on renal excretion in mice.³⁾

However, the extent of decrease in total body clearance of urea was much greater than that of creatinine. Urea filtrated through the glomeruli is partly reabsorbed and the extent is dependent on the urinary flow rate in the tubule.⁷⁾ Accordingly, the greater decrease in urea total body clearance observed by decreasing rectal temperature is supposed to be based on the high rate reabsorption of urea in hypothermal mice whose urinary flow rate may be slower than that in normal mice.

Higashi *et al.*⁸⁾ reported the dispositions of gentamicin and tobramycin, which are excreted into urine by the kidney without metabolism in the body, in rats whose rectal temperature was reduced by pentobarbital or ether anesthesia to

about 33–36 °C. The elimination of each antibiotics in the rats was slower than that in unanesthetized rats, without any change in distribution volume. In our study, the distribution volume of creatinine in hypothermal mice was almost same as that in normal mice. These results may propose the possibility that the distribution volumes of chemicals which have little binding capacity to serum or plasma protein, such as creatinine⁴⁾ and above aminoglycoside antibiotics,⁹⁾ are not changed remarkably by hypothermia.

The comparison of urea distribution volume could not be made directly between hypothermal and normal mice, because apparent distribution phase appeared in the whole blood concentration-time data for ¹⁴C-urea by decreasing rectal temperature of mice (Fig. 2). As will be discussed later with whole-body autoradiograms, this is likely to be caused by slower transfer of urea, which is more diffusible than creatinine in the body,¹⁰⁾ from blood to various tissues by the decreased blood circulation rate due to hypothermia.

It was suggested from whole-body autoradiograms and the determined rates of muscular blood flow that the transfer of urea from blood to muscle was apparently delayed by the decrease of muscular blood flow rate observed in this study. Accordingly, the distribution kinetics of urea to various tissues is considered to be more sensitive to the supply (blood perfusion) rate to the tissue than that of creatinine. This may be brought about by the higher permeability of cell membrane to urea (M.W. 60.1) than to creatinine (M.W. 113.1). In a previous study,¹⁾ it

TABLE III. Muscular Blood Flow Rates in Hypothermal and Normal Mice

Groupe of mice	Blood flow rate (ml/min/g muscle)
Hypothermal ($n=9$) ^{a)}	0.11 ± 0.05 ^{b)}
Normal ($n=6$)	0.94 ± 0.45

a) The number of data.

b) Standard deviation.

was observed that the transfer of creatinine from blood to muscle was more rapid in 1-day-old and 1-week-old mice than in the more aged mice. The difference in muscular blood flow rate or muscular cell membrane permeability to creatinine was assumed to be responsible for the result. The data obtained in this study suggest that the result is likely to be caused by the difference in muscular cell membrane permeability to creatinine.

Hypothermal mice in this study were kept under anesthesia with ether. However, non-treated mice were used as the controls, because it may be practically impossible to equalize the effect of ether anesthesia between hypothermal and control mice. Therefore, the difference of the data between hypothermal and normal mice observed in this study may be partly brought about by ether anesthesia of hypothermal mice. The exclusion of the effect of ether anesthesia remains to be carried out. Furthermore, in this paper, hypothermal mice with other body temperature were not used. To examine the appropriateness of discussion mentioned above, the study using the hypothermal mice with various body temperature may be necessary.

We are studying the effect of hypothermia on the disposition of thiourea which is considered to be covalently bound to protein or peptide in the body.¹¹⁾

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