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Application of a beta microprobe for quantification of regional cerebral blood flow with ^{15}O-water and PET in rhesus monkeys

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A beta microprobe was successfully applied to monitor arterial input function for quantification of regional cerebral blood flow (rCBF) in the monkey brain with ^{15}O-water and positron emission tomography (PET). The sensitivity of the probe was approximately 0.83 to 1.67 cps/kBq/m*l* depending on the studies. A preliminary study was performed to find a suitable use and to evaluate the performance of the system and data analysis procedure. The results showed that dispersion correction of measured input function was unnecessary if microprobes were connected directly to the arterial catheter. Then multiple CBF measurements were done in three monkeys under anesthesia. Identical regions of interest were placed with the aid of magnetic resonance imaging (MRI) of each monkey and rCBF values were estimated. Estimated rCBFs were reproducible for several measurements. The mean CBF value for a pentobarbital anesthetized monkey was 46.0 m*l*/min/100 g ($PaCO_2$ = 46.3 mmHg). This shows that the use of the beta microprobe for quantification of rCBF with PET was validated. The lack of a need for dispersion correction of observed input function is an advantage with the beta microprobe system because the probes are small enough to be placed near the arterial sampling site.

Key words: regional cerebral blood flow, positron emission tomography, arterial input function

INTRODUCTION

IT IS IMPORTANT to measure absolute regional cerebral blood flow (rCBF) in both basic and clinical studies not only for the evaluation of rCBF but for the quantification of regional oxygen extraction and consumption. Positron emission tomography (PET) is widely used for noninvasive measurement of rCBF. ^{15}O-water and ^{15}O-butanol are used as tracers for the rCBF measurements. Since these tracers are usually administrated as an intravenous bolus injection, one of the difficulties in the quantification of rCBF with PET is the accurate measurement of arterial input function. The arteries are too small and radioactivity in the arteries changes too rapidly for the PET system to detect it. Manual sampling of arterial blood from peripheral arteries and beta detector systems, have been applied to monitor the input function of the tracer into the brain. Generally measured input function is smeared out by a significant volume of blood in the detector and the passage (e.g. tubes) between the sampling site and the detector. Because direct use of the dispersed input function generally induces overestimation of rCBF, mathematical corrections are necessary with those methods.[1-3] We have developed a fiber-type beta-microprobe system for the direct detection of local beta particles.[4,5] In this study, we applied the beta-microprobe system for the measurement of arterial input function of ^{15}O-water during the PET scan. Because the microprobes are small, they were able to be positioned close to the arterial catheter to minimize possible dispersion of measured input function. First, we attempted to find a suitable method for the quantification of rCBF with PET and the beta microprobe in a monkey. Eight PET and microprobe measurements were performed and a microsphere method was applied to validate the method. Then a total of 17 measurements in three anesthetized monkeys were done

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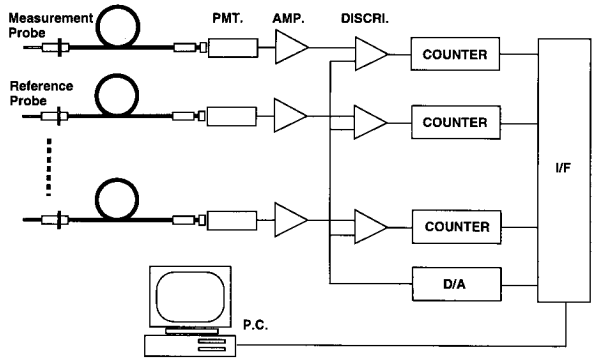

Fig. 1 Block diagram of beta microprobe system. The system consists of microprobes (PROBE), optical fibers, photomultiplier tubes (PMT), signal amplifier (AMP), discriminator (DISCRI), digital/analogue converter (D/A), counter circuit (COUNTER), interface (I/F) and personal computer (P.C.).

to evaluate the reproducibility of the rCBF values obtained with the PET and beta microprobe system.

MATERIALS AND METHODS

Beta Microprobe System

The beta microprobe system was developed especially for measurement of positron-emitting tracers in small animals because the fiber-type probes can be inserted directly into the target organs. Since the effective volume is up to the positron range of the isotopes, it is smaller than spatial resolution of PET. Furthermore, the combination of small probes and optical fiber facilitates the positioning of the probes.

As Figure 1 shows, the beta microprobe system consists of plastic scintillation fiber probes, optical fibers, photomultiplier tubes, a signal amplifier, discriminator and counter. As the scintillation light diminishes to a single photon as it passes through the optical fiber, the photomultiplier tube for photon counting (R2295, Hamamatsu Photonics, K.K., Japan) was chosen. Output signals are stored in a PC-9801 personal computer (NEC, Japan). The shortest sampling time with this system is one second, and the optimal sampling time can be fixed for the purpose. The computer acquires counts during the fixed sampling time. Up to 6000 data points can be stored. The time activity curves of the probes are displayed simultaneously during the measurement. The scintillation fibers are 0.5 mm in diameter and 3.0 mm in length. To measure β^+-rays, a pair of probes are needed as shown in Figure 2. The measurement (M)-probe detects both β^+-rays and background γ-rays while the other, the reference (R)-probe detects only γ-rays.

Calibration of the Beta Microprobe

The linearity of the beta microprobe was evaluated up to 10^5 cps (approximately 100 MBq/m*l*) prior to the following experiments (unpublished data). Radioactive counts generated purely by β^+-rays, Q_b [cps], were obtained by weighted subtraction of the counts of the R-probe, Q_R [cps], from those of the M-probe, Q_M [cps]:

$$Q_b = Q_M - r\,Q_R \quad (1)$$

Where r is the ratio of sensitivity for the γ-ray of the two probes. The r was obtained under the condition in which only γ-rays were measured. The activity concentration of β^+ particles, C_b [kBq/m*l*] is relative to Q_b:

$$C_b = Q_b/CCF \quad (2)$$

where CCF is the cross-calibration factor for the probes [cps/kBq/m*l*]. From equations (1) and (2), CCF was obtained with a known activity concentration C_b [kBq/m*l*]:

$$CCF = (Q_M - r\,Q_R)/C_b \quad (3)$$

In vitro measurement was performed to obtain CCF. The ratio of sensitivity for γ-rays of M- and R-probes was determined from count rate of the attached natrium line source. Then microprobes were immersed in ^{15}O-water and data were acquired for 10 seconds. At the beginning of the measurement, 1 m*l* of ^{15}O-water was sampled and counted in an autowell gamma counter (AUTO WELL GAMMA SYSTEM ARC-2000, Aloca, Japan) which had already been cross-calibrated with a dose calibrator (CURIEMETER 3, Aloca, Japan). All data were corrected for the physical decay of ^{15}O to the starting time. CCF was then calculated with equation (3) by using the averaged count rate.

CCF from the *in vitro* measurement above was applied for the preliminary study to estimate rCBF. After the preliminary study, numerous measurements in different animals were performed and the necessity of individual CCFs for individual animals was found. Therefore in the rest of the measurements, individual CCFs were calculated, and for each PET scan an arterial blood sample was withdrawn during PET and microprobe measurement. The sample was weighted and counted in an autowell gamma counter. The CCF value was obtained from the activity concentration of the sample and beta microprobe outputs at the time when the sample was withdrawn. The calibration was performed in every PET study and the CCF value was determined by averaging the values obtained in repeated measurements in individual monkeys.

I. Preliminary Study

Preliminary experiments were performed in a monkey. A total of eight PET and microprobe measurements were done under several conditions of microprobe measurement, such as different rates of withdrawal of arterial blood and different distances from the arterial catheter to the probe, to investigate observed input functions. To evaluate estimated rCBF, we attempted to compare rCBF values estimated with the combination of PET and the beta microprobe and those with the microsphere method.

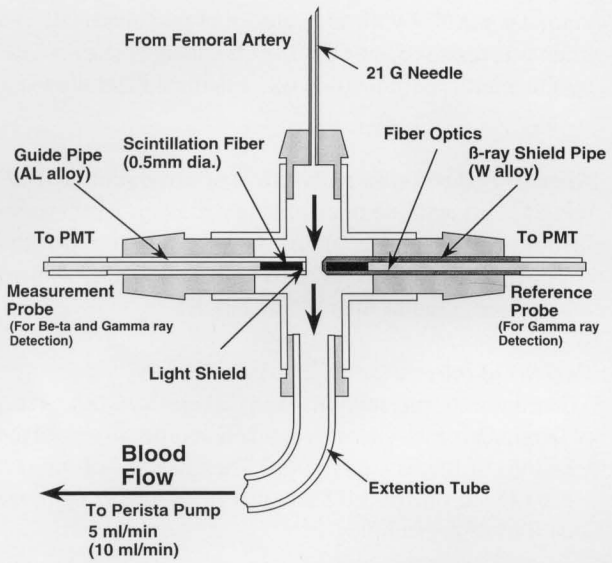

Fig. 2 The structure of beta microprobes for the monitoring of arterial input function. Arterial blood is withdrawn from the catheter to the extension tube, connected to peristatic pump which controls withdrawal rate. Microprobes are composed of scintillation fiber to detect β^+- and γ-rays, optical fibers to transmit scintillation lights to PMT. The scintillation and optical fibers of measurement probe are covered with an aluminum tube and light shield while those of reference probe are covered with tungsten tube to detect only γ-rays.

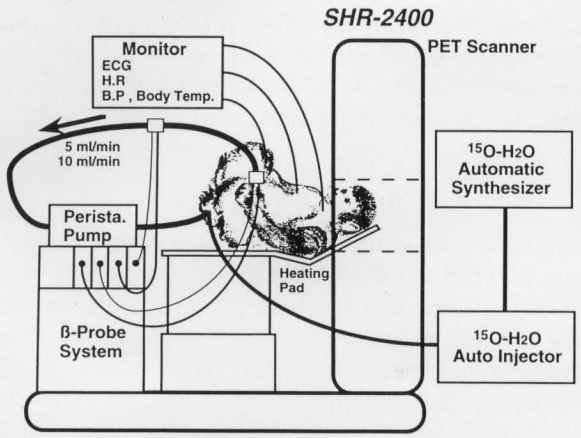

Fig. 3 Experimental set-up of the monkey, PET and beta microprobe system. Anesthetized monkey is fixed in PET scanner. ^{15}O-water is injected by auto-injector to the left saphena and arterial blood was withdrawn from right femoral artery. A pair of microprobes is connected directly to the arterial catheter and another is connected via extension tube.

The physiological conditions of the monkey were maintained throughout the experiments.

Animal preparations

Monkeys were maintained and handled in accordance with recommendations by the US National Institutes of Health and also the guidelines of the Central Research Laboratory, Hamamatsu Photonics K.K.

One male rhesus monkey (5.5 kg) was used in the preliminary study. The monkey was anesthetized with intravenous pentobarbital (25 mg/kg). Maintenance doses of anesthesia were administered as needed. A catheter was placed in a femoral artery for measurement of input function with beta microprobes and another was placed in a saphena for administration of ^{15}O-water and return of the arterial blood. Arterial blood pressure, body temperature and heart rate were monitored throughout the study. Arterial partial pressure of O_2 (PaO_2), CO_2 ($PaCO_2$) and pH were measured with a blood gas tension analyzer (NOVA Stat Profile 3 Analyzer, NOVA Biochemical, USA). Two pairs of beta microprobes were connected to the arterial line. The microprobes were set as described in Figure 3. Each pair of microprobes were encased in lead to minimize background γ-rays. Arterial blood was withdrawn with a peristatic pump (PERISTA BIO-MINIPUMP, AC-2120, ATTO, Japan) at a constant rate (5 m*l*/min or 10 m*l*/min) and was returned to the saphena to avoid excessive loss of blood. When manual samplings were applied, arterial blood samples were withdrawn from a three-way cock which was connected between the probes and the extension tube. The probes and the tube were flushed with heparinized saline after every PET measurement.

Conditions for beta microprobe measurement

(1) Distance from arterial sampling site

Two pairs of microprobes were used in this study to investigate the influence of the path length and dead volume of the tube between the probes and the catheterized femoral artery. A pair of microprobes was placed directly into the catheter in the artery and another pair was connected to it by way of a manometer tube of 50 cm long and 0.5 mm in inner diameter. The volume of the tube was 0.4 m*l*. Output data for the probes were acquired simultaneously and compared so that two input functions were measured simultaneously in one PET measurement.

(2) Rate of withdrawal of arterial blood

The rate of withdrawal of arterial blood for monitoring input function was considered to affect observed input functions. Arterial blood was withdrawn at the constant rate of 5 m*l*/min or 10 m*l*/min with a peristatic pump.

Manual samplings

Manual arterial blood samplings were applied during PET scan and beta microprobe measurement in a study to compare measured input function detected with the beta microprobe and that with manual sampling. The arterial samples were taken approximately every five seconds and the sampling times were recorded. The samples were weighed and the radioactivity in each sample was counted in an autowell gamma counter. Then the input function was constructed with the sampling times and the radioactivity concentrations of the samples.

Beta microprobe measurement

Beta microprobe data acquisition lasted for 150 seconds after the tracer injection. At 120 seconds after the beginning of the measurement, approximately 1 m*l* of arterial blood was withdrawn. The sample was weighed and the radioactivity was counted in an autowell gamma counter, which had already been calibrated with a dose calibrator. The ratio of gamma sensitivity of two microprobes was evaluated by microprobe measurement after the injection of ^{15}O-water without withdrawal of arterial blood from each monkey.

PET scan protocol

The monkey was placed in a PET scanner (SHR2400, Hamamatsu Photonics K.K., Japan) in the supine position. Details of the PET system are given elsewhere.[5] The scanner consists of five detector rings covering a field of view (FOV) 291 mm in diameter by 74 mm in the axial direction. The scanner simultaneously acquires 9 planes with an interplane spacing of 6.75 mm. The transaxial resolution of the scanner was 2.8 mm full width at half maximum (FWHM) measured with an ^{18}F line source (inner diameter of 1.0 mm) in the center of the FOV. That of ^{15}O-water images shown in Figure 4 was 4.5 mm FWHM. The axial resolution was 5.7 mm FWHM in the direct plane and 5.3 mm FWHM in the cross plane.

The head of the monkey was fixed in a head holder developed by Hamamatsu Photonics and positioned in the scanner parallel to orbitomeatal (OM) line in the second slice of the PET image. Just after a bolus injection of 1.2 GBq of ^{15}O-water in 1.5 m*l* saline, PET scan and microprobe measurement were started simultaneously. Twelve frames of 10 second PET data were acquired dynamically.

Altogether eight PET measurements with different peristatic pump flow rates and with or without manual samplings: three withdrawal rates of 5 m*l*/min without manual sampling, three 10 m*l*/min without manual samplings and two 5 m*l*/min with manual samplings, were performed in the monkey with interval times of 10 minutes to allow the radioactivity of O-15 to decay. Figure 3 shows the outline of the settings.

Microsphere method

The standard microsphere method was applied to evaluate rCBF with PET and the beta microprobe system. ^{99m}Tc labeled macroaggregated human albumin particles (^{99m}Tc-MAA, Dai-ichi Radiopharmaceutical Laboratory Inc., Japan) were used for this purpose. Ninety percent of the particles are 10 to 60 μm in diameter and none is larger than 100 μm so that all of them should be captured in arterioles or capillaries. After a series of PET measurements, a left thoracotomy was performed and 0.48 GBq of ^{99m}Tc-MAA in 1 m*l* saline was injected directly into the left ventricle. Arterial blood was collected with a Harvard pump at the rate of 5 m*l*/min for 5 minutes from the left femoral artery. Immediately after the procedure. The animal was killed with an overdose of pentobarbital. The brain was removed and sliced as PET image planes. rCBF by the microsphere method was calculated as follows:

$$\text{Microsphere CBF} = F\, C_m/Q_{mb},$$

where F is the rate of withdrawal of arterial blood [m*l*/min], C_m is the tissue microsphere activity concentration [kBq/g] and Q_{mb} is the microsphere radioactivity of the arterial blood [kBq]. rCBF measured by the microsphere method was used as the reference rCBF.

Regions of Interest

When brain tissue samples were taken from the slices, photographs of the slices were taken in order to record the locations of the tissue samples. Then regions of interest were placed on the PET images in identical to those locations of the samples.

Estimation of rCBF

PET and microprobe data were decay corrected to the time when each measurement was started. PET data and beta microprobe data were calibrated in units of kBq/m*l*. The density of the brain tissue was assumed to be 1.04 g/m*l*. Kety's model[7] was applied for the tracer kinetic model of water in the brain. Integration of the differential equation and non-linear least squares minimization were applied to estimate rCBF and other parameters.[8,9] Estimated parameters were uptake rate K_1 (= FE) [m*l*/min/g], washout rate k_2 (= FE/p) [min^{-1}], and arrival time shift of the tracer between brain ROI and beta microprobes Δt [min]. Dispersion of observed input function with beta microprobes was also estimated in the preliminary study.[3]

$$dCi(t)/dt = K_1\, Ca(t-\Delta t) - k_2\, Ci(t)$$
$$Ca(t) = (1/\tau)\int I(t-t') \exp(-t'/\tau)dt',$$

where Ci(t) is the brain tissue activity concentration of i-th ROI [kBq/g], F is rCBF, E is the fraction of water extracted from blood to tissue, Δt is the tracer arrival time lag between the ROI and beta microprobe, p is the blood brain partition coefficient of water [g/m*l*], Ca(t) is the true input function [kBq/m*l*], I(t) is the measured input function [kBq/m*l*] and τ is the dispersion constant [min]. In this study, it was assumed that the brain tissue region observed with PET was homogeneous and that E was 1. In addition, the partial volume effect of PET was ignored.

Evaluation of estimated rCBF

There were two measured input functions in each PET study. One was measured with microprobes connected directly to the arterial catheter and the other was connected via a tube. Two CBF estimation methods, with and without dispersion correction, were applied. To evaluate the accuracy of the estimated rCBF, we calculate R as the ratio of estimated rCBF to reference rCBF:

$$R = \text{estimated rCBF/reference rCBF}$$

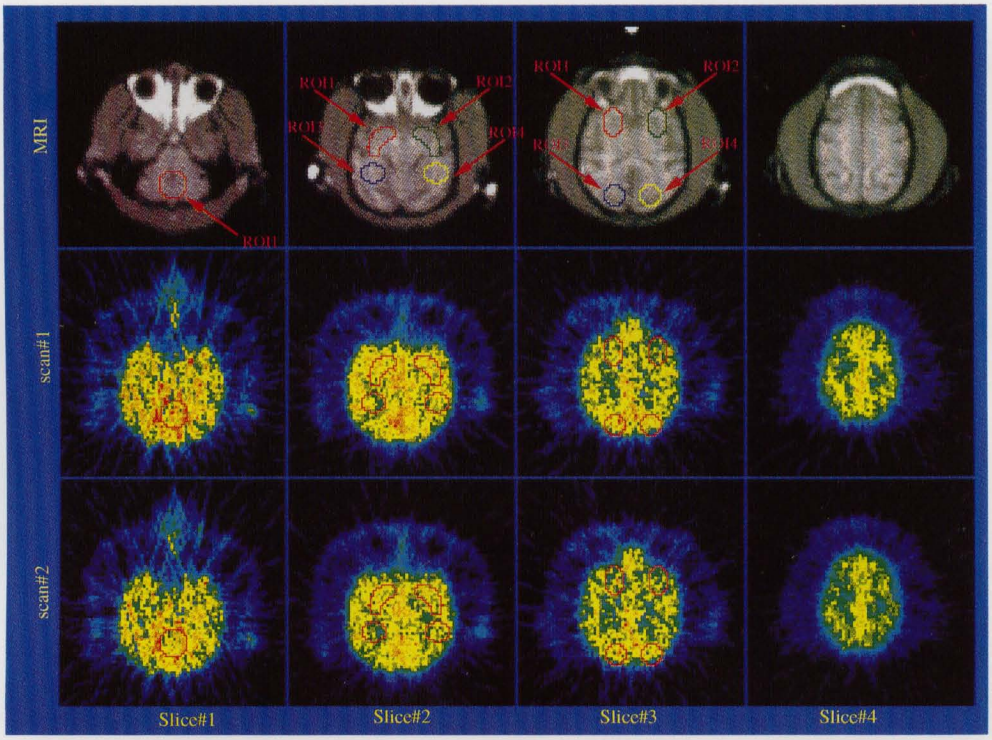

Fig. 4 Regions of interest of the main studies. Common regions of interest were drawn on MRI images of each monkeys. Slices of MRI images and those of PET images are identical.

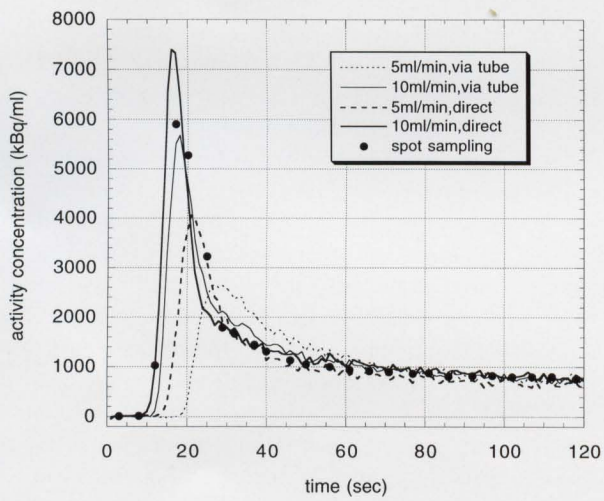

Fig. 5 Comparison of measured input functions. Solid and dotted lines are from beta microprobe system and scattered points represents data taken with manual samplings while withdrawal rate of 10 ml/min. Extension tube between arterial catheter and probes smears input function significantly and manual sampling method missed the peak.

If R is 1, it means that the estimated rCBF agrees with the reference rCBF. Then the mean ratios of the same conditions such as the pump flow rate, position of the probes and with/without dispersion correction were calculated.

Table 1 Ratio of estimated CBF by various conditions to reference (microsphere) CBF

withdrawal rate [ml/min]	probe position	dispersion correction	mean	sd
5	direct	no	1.038	0.093
10	direct	no	1.047	0.084
5	via tube	no	1.282	0.327
10	via tube	no	1.155	0.028
5	direct	yes	1.066	0.088
10	direct	yes	1.024	0.053
5	via tube	yes	1.062	0.131
10	via tube	yes	1.093	0.040

II. Main Studies

Three male rhesus monkeys (5.35 ± 0.33 kg) were used. Protocols of animal preparations and PET scan were the same as for the preliminary study. The microsphere method was not used. Beta microprobes were connected directly to the arterial catheter. Five or six of PET and beta microprobe measurements were done in each monkey. The total number of measurements was 17. Beta microprobes were connected directly to the arterial catheter. The rate of withdrawal of arterial blood was 5 ml/min or 10 ml/min with a peristatic pump.

In order to choose identical regions of interest in the monkeys, brain MRI images of the monkeys were obtained prior to the PET studies. 10 regions of interest

Table 2 Estimated rCBF values [ml/min/g] of main studies

withdrawal rate [ml/min]	5		10		whole study	
ROI#	mean	sd	mean	sd	mean	sd
1	0.4121	0.0350	0.4182	0.0487	0.4149	0.0407
2	0.5155	0.0625	0.5140	0.0572	0.5148	0.0582
3	0.4139	0.0287	0.4353	0.0534	0.4240	0.0422
4	0.4056	0.0192	0.4060	0.0215	0.4058	0.0196
5	0.4502	0.0381	0.4557	0.0163	0.4528	0.0292
6	0.4431	0.0330	0.4483	0.0323	0.4455	0.0318
7	0.3719	0.0354	0.3719	0.0266	0.3719	0.0306
8	0.3679	0.0588	0.3693	0.0631	0.3685	0.0589
9	0.6401	0.1996	0.6019	0.1796	0.6221	0.1855
10	0.5793	0.0605	0.5808	0.0477	0.5800	0.0531

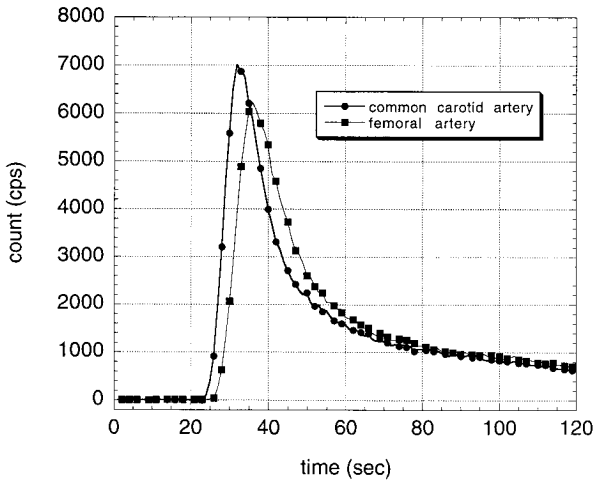

Fig. 6 Comparison of arterial time activity curves. The tracer was injected into saphena and arterial time activity curves were measured at femoral artery and at common carotid artery with beta microprobes. Later tracer arrival and slight dispersion were observed at femoral artery compared to internal carotid artery.

(ROIs) were placed on MRI image slices of each monkey, and matched with PET image planes. The regions of interest are shown in Figure 4.

Since it had been found that dispersion of input function can be ignored if it is monitored close to the arterial catheter, rCBF, partition coefficient and delay were estimated by a non-linear least squares method from arterial input function measured with the beta microprobe and brain time activity curves measured with PET.

RESULTS

Physiological Conditions

Mean $PaCO_2$ was 46.3 ± 1.39 mmHg and mean arterial systolic and diastolic blood pressures were 139.3 ± 11.5 and 78.3 ± 15.8 mmHg. Mean heart rate was 130 ± 13. Body temperature was maintained at 37°C with a heating pad.

Cross Calibration Factor for Beta Microprobe

The ratio of sensitivity for γ-ray of M- and R-probes was 0.98. The radioactivity concentration of the water was 6.18 MBq/m*l* and the calculated CCF was 0.781 cps/kBq/m*l*. When CCFs were obtained in the monkeys during the PET studies, they differed from monkey to monkey and varied from 0.853 to 1.536 cps/kBq/m*l*.

I. Preliminary Study

(1) Observed input functions

Arterial input functions obtained under various conditions are shown in Figure 5. Significant dispersion of measured input function was observed with beta-microprobes connected through a tube. Input function obtained with manual samplings was not frequent enough to trace the rapid changes in the arterial tracer concentration.

(2) Evaluation of estimated rCBF

Table 1 shows the ratio of estimated rCBF and reference rCBF. The use of input function measured with probes connected via the tube without dispersion correction resulted in significant overestimation of rCBF, and a slower arterial blood flow rate caused overestimation of rCBF when input function was measured with probes via the tubes. On the other hand, the use of input function measured with probes connected directly to the arterial catheter resulted in close agreement between estimated rCBF values and reference rCBF values even without dispersion correction. And the rate of withdrawal of arterial blood did not affect the ratio. In addition, when dispersion correction was performed, the estimated τ was almost 0 s for input function measured with the beta microprobe connected directly to the arterial catheter and about 5 s via the tube when dt (delay) was fixed. Dispersion correction was therefore necessary for input functions measured with microprobes connected via a tube but it was unnecessary when they were measured with probes connected directly to the arterial catheter. Although each estimated rCBF value was different from those obtained by the microsphere method, the mean estimated CBF value and mean microsphere CBF value were equal. Especially the mean CBF value estimated without disper-

sion correction on input function and measured with probes connected directly to the catheter agreed closely with the mean microsphere CBF. It is evident that dispersion correction is unnecessary if microprobes are set close enough to the catheter.

II. Main Study

Table 2 shows rCBF obtained from 17 measurements in three monkeys. There was no significant difference between estimated rCBF values for two different rates of withdrawal of arterial blood (5 and 10 ml/min). This agrees with the result of the preliminary study. The mean CBF value for the pentobarbital anesthetized monkey was 46.00 ml/min/100 g when $PaCO_2$ was 46.3 mmHg.

DISCUSSION

Dispersion correction

Table 1 indicates that dispersion of the measured input function can be negligible if microprobes are connected closely enough to the arterial catheter. It is considered that the small size of the monkey lowered relative dispersion between arteries in the brain and microprobes connected to the catheter in the femoral artery. In fact there were slight differences between measured input functions in the femoral artery and the common carotid artery in a monkey (Fig. 6). This suggests that the longer passage from the heart to the femoral artery smeared the input function more than that of the common carotid artery. But the "true" input function for brain tissue was considered to be distorted further by additional pathways to the brain tissue, and became almost equivalent to the input function observed in the femoral artery. Furthermore, that there was no significant difference between estimated rCBF values for the two withdrawal rates (5 and 10 ml/min) indicates negligible dispersion of the measured input function with the beta microprobe. A faster rate of withdrawal of arterial blood is desirable to minimize dispersion of the measured input function if there is a significant dead volume between the arterial catheter and the probes. On the other hand, an excessive withdrawal rate can alternate the blood circulation of the subject, which may result in a change in rCBF. But the use of the microprobe system minimizes the dead volume so that there is no need for a faster withdrawal rate, and it does not require mathematical correction for dispersion. It can facilitate the estimation of rCBF without sacrificing the accuracy of the measurement of input function.

Possible improvements and applications

In order to monitor the input function more accurately, microprobes can be inserted through major arteries to the target organ to minimize dispersion and loss of blood. The reference probe (R-probe) may not be necessary because background subtraction without withdrawing blood is enough to correct background γ-rays from surrounding objects such as the auto-injector and cardiac blood pool. Appropriate settings of the probe, which minimize background counts, improve the accuracy of the calibrated radioactivity concentration. The probes and connected optical fibers should be placed away from highly radioactive objects such as the injector and the subject's body, and the use of a lead shield is also advisable.

In this study, the microprobe system was applied for the measurement of the arterial input function of ^{15}O-water. This method can be applied for other tracers such as ^{18}F-fluoro-deoxyglucose (FDG) and ^{11}C labeled tracers. Although they require a plasma input function, several blood samplings during the PET scan should be enough for the purpose. The microprobe is advantageous for the early phase of the scan, which requires very frequent blood samplings.

The system can be used for directly monitoring positron-emitting tracers in target organs. For example, inserting the beta microprobe into a cardiac chamber through a femoral artery is possible not only for monitoring input function but also for evaluating of cardiac function. This strategy can also be applied during surgery on a tumor labeled with positron-emitting tracers which bind specifically to tumor cells. Not only faster temporal resolution than PET but also its practicality can be an advantage in animal studies. The system can be easily applied to small laboratory animals, and it should accelerate the development of useful positron-emitting tracers for clinical use.

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