A femoral arteriovenous shunt facilitates arterial whole blood sampling in animals

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Abstract. In this study we evaluated on-line continuous blood sampling in a femoral arteriovenous (a-v) shunt for use in quantitative tracer studies using gamma-emitting radionuclides in animals. The shunt consisted of 40 cm polyethylene tubing (PE-50) guided through a coincidence probe. Two three-way valves allowed blood pressure measurements and tracer injection. Blood flow in the shunt and the impulse response function (IRF) were assessed using heparinized human blood mixed with fluorine-18 fluorodeoxyglucose (FDG). In vivo experiments were performed in eight male rats (300–350 g) anaesthetized with halothane. In three rats, manual blood sampling was performed in parallel with on-line sampling. In another five animals, the arterial whole blood activity was recorded on-line for 40 min. For the experiments 150–180 MBq FDG was injected over 35 s. Blood flow in the shunt was 23.6, 29.2 and 42.8 ml/h at 100, 120 and 160 mmHg, respectively. The IRF was characterized by minimal dispersion (1–2 s FWHM). Deconvolution of the measured arterial input curves with the IRF changed the measured curve only minimally. Whole blood radioactivity concentration derived from manual and on-line sampling were in excellent agreement. The curves derived from on-line sampling were of high statistical quality. In conclusion, a femoral a-v shunt allows multiple manipulations such as measurement of the arterial whole blood activity, continuous blood pressure monitoring, injection of the tracer and collection of blood samples if necessary. It is not associated with blood loss if the collection of blood samples is not required. It is more convenient to use than manual sampling, the peak of the input curve is never missed and the input curves are of high statistical quality.

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Introduction

Many in vivo experiments using radioactive tracers require knowledge of the arterial tracer concentration for quantification. One example is in vivo autoradiography, which is widely used to investigate physiological processes or to test the suitability of new tracers. One method first described by Sokoloff et al. [1] allows the quantitative measurement of glucose metabolism in the brain using carbon-14 deoxyglucose. Since then the method has been adapted to other glucose analogues such as fluorine-18 fluorodeoxyglucose (FDG) [2]. This tracer is of special importance since it is one of the most widely used radioligands in positron emission tomography (PET). The use of the relatively short-lived radionuclide ^{18}F (half-life 110 min) has certain advantages compared with ^{14}C (half-life 5,730 years). One of them is that contamination is much less of a problem. New possibilities for physiological experiments were opened up by the introduction of animal PET scanners. In PET and autoradiography the quantification of the investigated processes often requires knowledge of the time course of authentic tracer in arterial plasma, i.e. the input function. This is commonly obtained from arterial blood samples, which in rats are mostly drawn from a catheter in the femoral artery. This blood sampling, and the subsequent analysis in a well counter, is labour intensive and associated with a loss of blood, which may lead to haemodynamic instability. These factors make it difficult to perform multiple experiments in one session, for instance in an animal PET scanner.

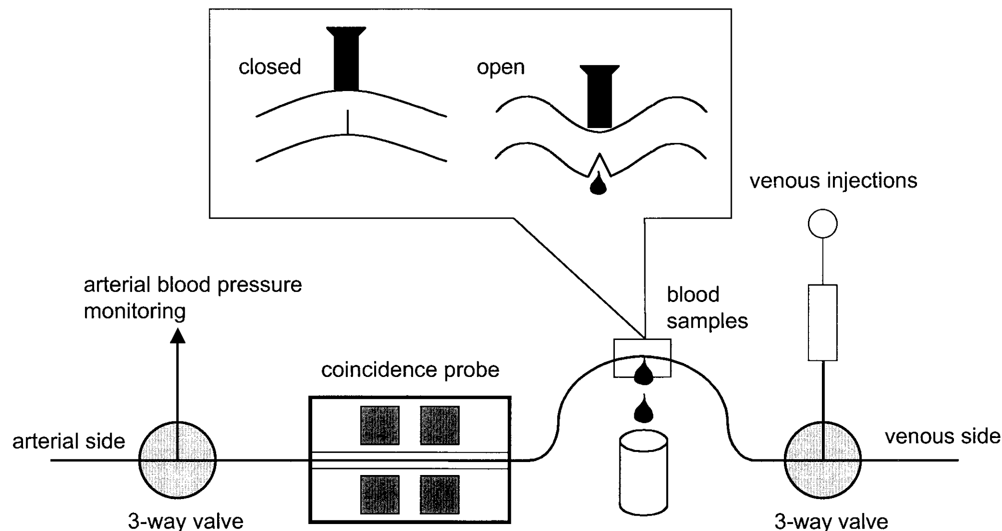

Fig. 1. Schematic drawing of the shunt system. The *insert* depicts the incision in the shunt for the withdrawal of discrete blood samples. The incision opens when slight pressure is applied from the top

In this study we evaluated an alternative method for determination of the input function which relies on counting the radioactivity in arterial whole blood flowing through an arteriovenous (a-v) shunt running from the femoral artery to the ipsilateral femoral vein. The shunt system was evaluated in rats using ^{18}F-FDG. Similar shunt systems have been proposed previously [3, 4].

Materials and methods

The shunt system is depicted in Fig. 1. It consists of 40 cm polyethylene tubing (PE 50, inner diameter 0.58 mm). Four centimetres from the arterial end, a first three-way valve is inserted for continuous blood pressure monitoring. The following 20 cm is guided through a probe (GE Medical Systems) consisting of two pairs of BGO crystals operating in coincidence mode. The probe allows data to be written out at intervals as short as 0.1 s. After the probe, the tubing is bent upwards through a groove in a block of polyethylene. An incision at the top of the curvature in the downward side of the catheter can be opened by pressing the catheter slightly down to allow for the collection of blood samples. If pressure is removed the incision closes again and the pressure in the catheter wall caused by the curvature seals the opening (insert, Fig. 1). A second three-way valve is inserted for the intravenous injection of tracer and pharmaceuticals as required.

In vitro characterization of the shunt system. This characterization included the measurement of blood flow at various pressure gradients and the assessment of the response of the probe to inputs such as a true impulse (impulse response function, IRF) or a step function. The IRF is characterized by a dispersion and a delay. It is an important parameter because it determines how the true time course of arterial activity (input function) gets delayed and dispersed when measured in the shunt. Mathematically the output of the probe, i.e. the measured input function, is the convolution of the true arterial input function with the IRF. The test arrangement consisted of a pressure bag attached to 1 m of polyethylene tubing of 3 mm diameter. This system simulated the arterial side of the animals. It was connected to a reservoir consisting of 20 cm of polyethylene tubing, also with a 3 mm inner diameter. The shunt system was then connected to this reservoir. All tests were performed with heparinized human venous blood kept at 37°C. For each test the reservoir and the shunt were filled with blood. To simulate an impulse-shaped input, blood mixed with ^{18}F-FDG (1 MBq/cc) was put into the first 2 cm of the shunt. To create a step input function, the whole reservoir was filled with radioactive blood. The system was tested at pressure gradients of 160, 120 and 100 mmHg. Probe data were written out at 0.1-s intervals. Blood flow in the shunt was measured by collecting the blood at the free (venous) end in a vial for 1 min.

Animals. Arterial input curves in the a-v shunt were measured in eight normal adult male Sprague-Dawley rats, weighing 280–350 g. The experiments were approved by the local veterinary authorities.

The probe yields whole blood data. However, quantification most often requires knowledge of the tracer concentration in plasma. The time course of the ratio of FDG in plasma and whole blood was determined in three animals by manual blood sampling in addition to using the shunt. This also allowed a direct comparison of manual and on-line blood sampling.

Experimental procedures. The animals were anaesthetized with halothane, and polyethylene catheters (PE 50) were inserted into the femoral artery and vein. Tracheotomy was performed for mechanical ventilation. The two three-way valves were added to the catheters and the shunt was closed and guided through the probe. For radiation protection, lead blocks were put around the animal. In five animals, data were acquired over 40 min following the injection of 140–180 MBq ^{18}F-FDG in 0.8 ml saline with a dedicated injection pump over 35 s. At the end of the experiment, 0.5 ml of arterial blood was collected to determine the ratio of ^{18}F activity in plasma and whole blood. The animals were then sacrificed by injecting pentobarbital into the shunt. In three additional animals, manual sampling was performed in parallel to on-line sampling. During the first 2 min, a sample was taken every 6–9 s through a catheter placed in the femoral artery contralateral to the shunt, then the intervals were progressively prolonged. Whole blood activity was determined in a well counter. In addition, the cell and plasma fraction were counted separately following centrifugation and the time course of the ratio ($^{18}F_{plasma}/^{18}F_{whole\ blood}$) was calculated. Well counter and coincidence probe were calibrat-

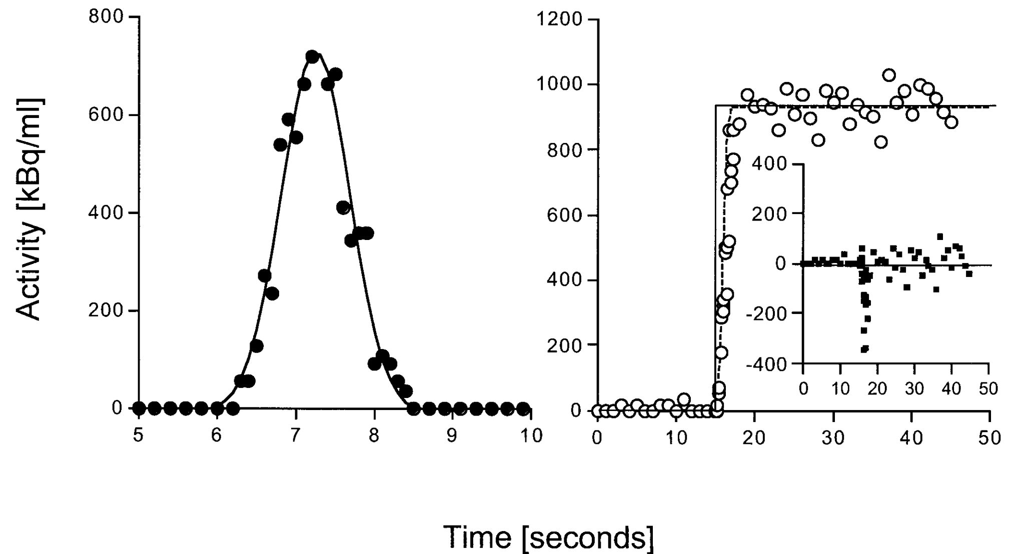

Fig. 2. The *left panel* depicts a measured impulse response function (*IRF, filled circles*) and a least squares fit with a Gaussian. The *right panel* displays a measured response to a step function (*open circles*) and the result of the convolution of the true step function (*solid line*) with the IRF (*dotted line*). The *insert in the right panel* represents the residuals

ed to yield data in kBq/ml. All data were additionally corrected for physical decay of ^{18}F. Arterial blood pressure was monitored continuously through the first three-way valve. At the beginning and end of each experiment, arterial oxygen saturation, pH and the partial pressure of oxygen (PaO_2) and carbon dioxide ($PaCO_2$) were measured.

Results

In vitro assessment of the shunt system

Blood flow in the shunt was 23.6, 29.2 and 42.8 ml/h at 100, 120 and 160 mmHg, respectively. A typical IRF measured at a pressure of 120 mmHg is demonstrated in the left panel of Fig. 2. It is characterized by a delay of 8 s and a dispersion of 1.1 s full-width at half-maximum (FWHM). The latter was determined by least squares fitting a Gaussian to the data (σ=0.43 s). The dispersions at 100 and 160 mmHg were calculated at 1.4 and 0.6 s FWHM. To validate the measured IRF, a true step input function was convolved with the measured IRF and the result compared with the measured response to a step input. The result is demonstrated in the right panel of Fig. 2. There is good agreement of the convolution and the measured response, although the measured values are somewhat overestimated by the convolution in the upslope, as demonstrated by the residuals.

In vivo experiments

The values for PaO_2, $PaCO_2$, oxygen saturation and pH were in the physiological range in all experiments. Haematocrit values in the three animals in which manual and on-line sampling was performed were 0.45, 0.45 and 0.46. In the five experiments without manual blood sampling, blood pressure was stable throughout the experiments (range 100–120 mmHg) and haematocrit values were 0.44, 0.45, 0.45, 0.46 and 0.46. The drop in pressure from the artery to the point of measurement in the first three-way valve was between 15 and 20 mmHg. In the three animals with additional manual sampling, the haemodynamic situation was less stable, with temporary pressure drops of up to 15 mmHg.

A direct comparison of manual and on-line blood sampling in one animal is demonstrated in Fig. 3. The whole blood values of both methods are in excellent agreement. The same res ult was observed in the other two animals.

The mean of five measured input curves in whole blood is shown by the dark line in the main graph of Fig. 4. To create the mean, all curves were normalized to the mean activity of the last minute of each experiment. The relatively small standard deviation demonstrates that the normalized curves were very similar. In fact, the coefficient of variation (SD/mean) of the integral from 0 to 40 min was 9%.

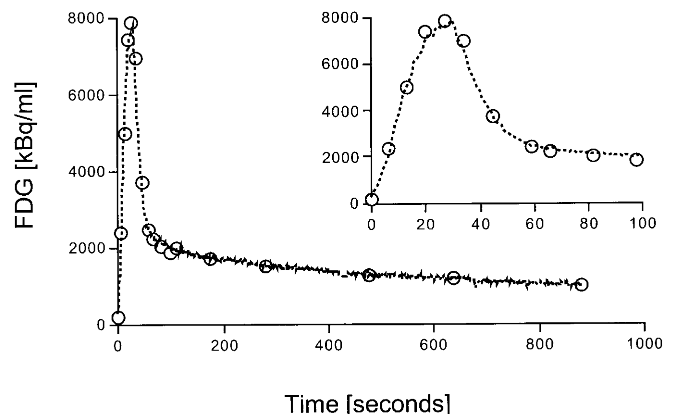

Fig. 3. Comparison of manual (*circles*) and on-line (*dashed line*) whole blood sampling following the injection of 150 MBq ^{18}F-FDG in one animal. The *insert* depicts the comparison for the first 100 s following injection

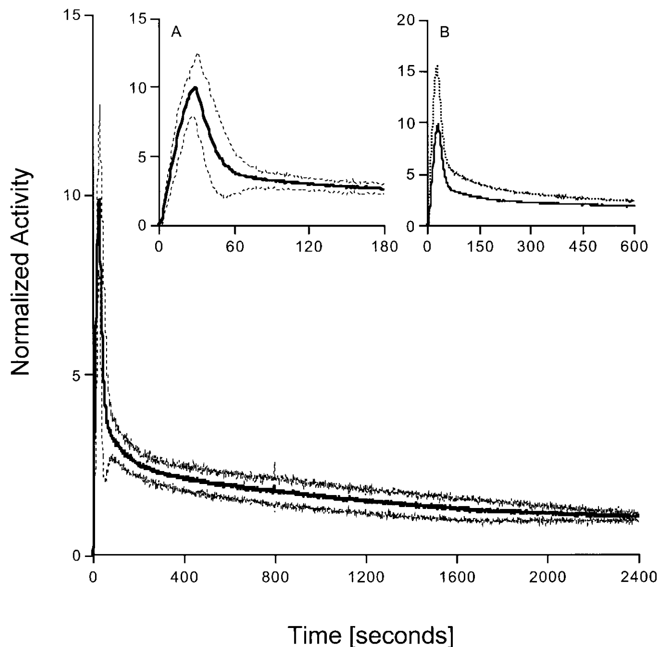

Fig. 4. In the main graph the *middle curve* represents the mean of five whole blood input curves as measured by the coincidence probe at 1-s intervals. The adjacent curves were drawn 2 SD above and below the mean. *Insert A* shows the same data focussed around the peak. *Insert B* demonstrates the time course of whole blood (*solid line*) and plasma (*dotted line*) activity. The latter was calculated using the correction function indicated in Fig. 5

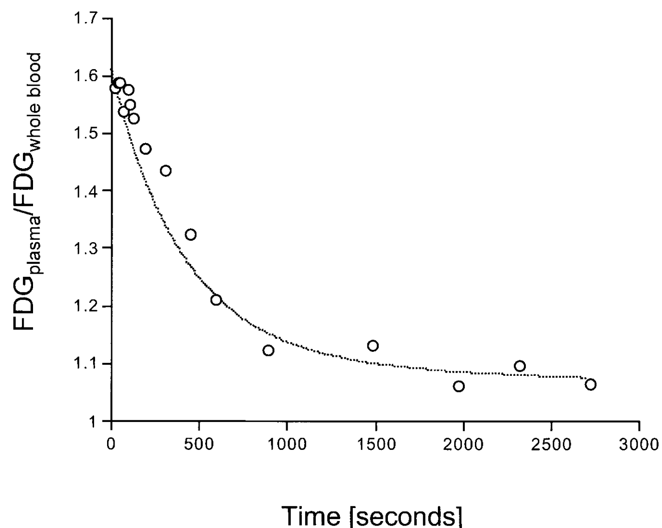

Fig. 5. Time course of the ratio of ^{18}F activity in plasma and whole blood. The *open circles* represent the pooled data of three animals. The *dotted line* is the result of the least squares fit using a function consisting of two exponentials and a constant. The function was calculated as: $^{18}F_{plasma}/^{18}F_{whole\ blood} = 0.51\ \exp(-\ln2/4.79\ t) + 0.3\ \exp(-\ln2/337\ t) + 0.8$. This corresponds to a decay half-time of 4.79 and 337 min for the fast and the slow exponential, respectively

The time course of the ratio of ^{18}F activity in plasma and whole blood established in three animals is shown in Fig. 5. The extrapolated ratio dropped from 1.61 at the start of the experiment to 1.08 at 40 min. The time course was adequately approximated by two exponentials and a constant. Using these to correct the mean whole blood curve shown in the main graph of Fig. 4 yields the curve shown in insert B of Fig. 4. The whole blood curve underestimates the plasma curve predominantly at the beginning of the experiment. The integral of the plasma curve from 0 to 40 min is 23% higher than that of the whole blood curve.

Discussion

The main purpose of this study was to evaluate a shunt system to measure the arterial input function. The advantages of such a system are obvious. There is no loss of blood, which can potentially lead to haemodynamic instabilities, as also encountered with manual blood sampling in this study. The measured input curves are of high statistical quality and there is no risk of missing the initial peak. The latter may be crucial in experiments with tracers that have a fast kinetics, such as $H_2{}^{15}O$. It is clear that the presented shunt system, in the reported configuration, could be used for any tracer labelled with a positron-emitting radionuclide as well as for ^{18}F-FDG. In experiments with single-photon emitting radionuclides, the probe would be operated in singles mode. The counts in the available individual crystals could be averaged. In this case special attention would have to be paid to shielding the probe from background activity. Similar shunt systems have been proposed before [3, 4]. In comparison with those systems, we have introduced three important additional features. One is the possibility of sampling blood through an incision. The others are the monitoring of the arterial blood pressure and the injection of the radioligand in the shunt. Furthermore, this study included an in vitro evaluation of the system.

In vitro experiments

The in vitro evaluation of the system showed that the IRF of the shunt is very narrow. The validity of the direct measurement of the IRF is demonstrated by the finding that the convolution of the ideal step function with the measured IRF adequately fitted the measured response to the step-shaped input. A broad IRF would indicate a large distortion of the true input curve. In that case the true input curve would have to be calculated by deconvolution of the measured input curve with the IRF. This was actually performed: the mean input curve shown in Fig. 4 was deconvolved with the IRF of Fig. 2. The change was minimal. The integrated activities over the first minute, where the largest difference could be ex-

pected, were less than 1% different. This demonstrates that the measured values in the probe reflect the true values in the femoral artery up to a time shift, which is easily visualized by plotting the curve.

In vivo experiments

Flow of the viscous blood in the catheter causes a pressure drop from the femoral artery to the point of blood pressure measurement. This is not a problem since this drop can be easily measured by briefly clamping the catheter. In fact, a change in the pressure measurement from the open to the clamped condition indicates that the shunt is patent. We encountered rare instances of imminent shunt clogging which were easily recognized by an abrupt change in blood pressure values. Rinsing through the first three-way valve immediately restored patency. In experiments with a low bleeding risk, it may be advantageous to administer heparin systemically.

A disadvantage of the shunt-derived input function is that it reflects whole blood values whereas quantification often requires plasma values. For FDG a simple correction based on a standardized time course of the plasma to whole blood ratio is probably adequate under normal physiological conditions. A similar time course of the plasma to whole blood ratio was published by Ashworth et al. [3]. Their function yields slightly higher values during the first 25 min. However, if one corrects a whole blood curve with their and our functions, the difference in the integral between 0 and 45 min is only 2%. The method may fail in cases with altered haematocrit values. However, in such cases blood sampling could be performed through the shunt to measure the actual plasma values. Such sampling would be mandatory in cases that require the determination of metabolites. As opposed to the common method where blood samples are collected through a catheter that is clamped between samples, sampling through an incision in the shunt is characterized by zero dead space, meaning that blood loss is minimized.

Using the shunt also saves a considerable amount of time. To get all the functions of the shunt with standard catheters, one would have to puncture two arteries (for blood sampling and blood pressure monitoring) and two veins (for injection of tracer and pharmaceuticals). The shunt therefore cuts the preparation time by half. As demonstrated in Fig. 4, the quality of the measured input curves is high. The small standard deviation furthermore demonstrates a high similarity of the curves of different animals, given that a standardized injection protocol is used. This is in accordance with published results in humans [5]. Based on their results the authors validated a simplified approach to quantification using a standardized input curve. It is based on measuring a few samples during the experiment and then scaling the standardized curve so that it fits the measured samples. It might even be sufficient to take one sample at the end of the experiment. In this study we used FDG for practical reasons. While the shunt system facilitates blood sampling in FDG studies, it may reveal its true value with other tracers. For example, the introduction of animal PET scanners renders perfusion studies with $H_2{}^{15}O$ feasible. If blood flow is to be quantified, the arterial input function must be determined. Given the short half-life of ^{15}O and the fast kinetics of the tracer, manual blood sampling is impractical and a shunt system becomes mandatory. Furthermore, whole blood data are completely sufficient with $H_2{}^{15}O$ since the tracer is freely diffusible. Another point to consider is possible adsorption of radioligand to the catheter wall. This was not the case for FDG and the polyethylene used in this study. However, this possibility has to be excluded for each tracer.

Conclusion

Using a femoral a-v shunt allows continuous measurement of the arterial whole blood activity, blood pressure monitoring, the injection of the tracer and the collection of blood samples if necessary. It is not associated with blood loss if the collection of blood samples is not required. It is more convenient to use than manual sampling and the preparation time is markedly reduced. The input curves are of high statistical quality and their peak is never missed. The latter point may be of special importance in $H_2{}^{15}O$ studies in animal PET scanners.

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