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Methods of blood flow measurement in the arterial circulatory system

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Abstract

The most commonly employed techniques for the in vivo measurement of arterial blood flow to individual organs involve the use of flow probes or sensors. Commercially available systems for the measurement of in vivo blood flow can be divided into two categories: ultrasonic and electromagnetic. Two types of ultrasonic probes are used. The first type of flow probe measures blood flow-mediated Doppler shifts (Doppler flowmetry) in a vessel. The second type of flow probe measures the ''transit time'' required by an emitted ultrasound wave to traverse the vessel and are transit-time volume flow sensors. Measurement of blood flow in any vessel requires that the flow probe or sensor be highly accurate and exhibit signal linearity over the flow range in the vessel of interest. Moreover, additional desirable features include compact design, size, and weight. An additional important feature for flow probes is that they exhibit good biocompatability; it is imperative for the sensor to behave in an inert manner towards the biological system. A sensitive and reliable method to assess blood flow in individual organs in the body, other than by the use of probes/sensors, is the reference sample method that utilizes hematogeneously delivered microspheres. This method has been utilized to a large extend to assess regional blood flow in the entire body. Obviously, the purpose of measuring blood flow is to determine the amount of blood delivered to a given region per unit time (milliliters per minute) and it is desirable to achieve this goal by noninvasive methodologies. This, however, is not always possible. This review attempts to offer an overview of some of the techniques available for the assessment of regional blood flow in the arterial circulatory system and discusses advantages and disadvantages of these common techniques.

Keywords: Blood flow; Flow probes; Radioactive microspheres; Arterial circulation; Rats

1. Introduction

In the late ninth century, Adolph Fick, a physiologist, was the first to formulate the idea that blood flow in the body could be measured. The ''Fick Principle'' as it is now known, stipulated that the amount of a substance taken up by an organ is the product of blood flow to the organ and the concentration difference of the substance between the arterial and venous systems (Wright, 1971). This important physiological concept was used later by Zuntz and Hagemann in 1898 to measure equine cardiac output (Wright, 1971). The other most notable event in the history of blood flow measurement was the introduction of the electromagnetic flow meter by Kolin in the late 1930s (Kolin, 1937). Currently, a variety of techniques are routinely employed for the measurement of beat-to-beat cardiac output. The same techniques have also been routinely employed to assess blood flow to individual organs or regional blood flow in a specified area of a tissue/organ.

Presently, the most common techniques for the in vivo measurement of blood flow to individual organs employ the use of flow probes or sensors. Commercially available systems for the measurement of in vivo blood flow include Doppler (Bachelard & Pitre, 1995; Barfod, Akgoren, Fabricius, Dirnagl, & Lauritzen, 1997; Bitterman, Brod, Weisz, Kushnir, & Bitterman, 1996), electromagnetic flowmetry (Iida, 1995, 1999), and ultrasonic transit-time flow sensors (Elhawary & Pang, 1995; He & Tabrizchi, 1997). Obviously, measurement of blood flow in any vessel requires that the flow probe or sensor is accurate and linear over the flow range in the vessel of interest. Moreover, additional desirable features include, design, size and weight. Furthermore, an additional feature is biocompatability in that it is

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imperative for the sensor to behave in an inert fashion towards the biological system.

In addition, a sensitive and reliable method to assess blood flow in individual organs in the body, other than by the use of probes/sensors, is the reference sample method (Malik, Kaplan, & Saba, 1976). This method has been utilized under a variety of experimental settings to assess regional blood flow in the body (Nekooeian & Tabrizchi, 1996; Pang, 1983; Tabrizchi & Pang, 1993; Tabrizchi, Pang, & Walker, 1989). In most cases, radioactively labeled microspheres are utilized to measure blood flow since regional blood flow is proportionate to the number of microspheres trapped in the organ of interest (Pang, 1983; Tabrizchi & Pang, 1987). This technique offers an efficient and cost effective method of measuring blood flow to organs in the body of both large and small animals (Hoffbrand & Forsyth, 1969; Kaihara, Van Heerden, Migita, & Wagner, 1968; Pang, 1983; Tabrizchi & Pang, 1987).

Clearly, the purpose of measuring blood flow is to determine the amount of blood delivered to a given region per unit time. Obviously, it is desirable to achieve this goal by a noninvasive method. This, however, is not always possible. In this review, we have attempted to offer an overview of some of the current techniques currently available for the measurement of arterial blood flow in vivo. In this context, a comparison will be made between transit-time flow measurements to the radioactively labeled microsphere technique for blood flow in the gastrointestinal tract of anaesthetized rats. In addition, a comparison will also be made between electromagnetic flowmetry and that of radiolabelled microspheres in gastrointestinal tract blood flow of conscious rats. Furthermore, a brief assessment of laser Doppler flowmetry will be made in assessing cerebral blood flow.

2. Methods of blood flow measurement

2.1. The transit-time method for blood flow measurement

This particular technique involves the use of an ultrasound signal that is emitted from a transducer, deflected and then captured by an ultrasonic transducer to determine blood flow in the blood vessel(s) of interest. This type of flow probe consists of a probe body containing ultrasonic transducers as well as a fixed acoustic reflector (Fig. 1). Upon excitation, the transducer(s) emit a wave of ultrasound that traverses the vessel and rebounds off the acoustic reflector. The reflected wave traverses the vessel, in the opposite direction, back to the transducer where it is captured and converted into an electrical signal by the ultrasonic transducer. The illumination signals gathered are used to derive (integrate the received velocities over the width of the vessel) the volume flow within the lumen of the blood vessel. Essentially, ultrasonic signals traveling back and forth alternately intersect the flowing liquid in the upstream and downstream direction. During the upstream cycle the ultrasonic signal travels *against* flow, and the resulting transit time is increased by a factor dependent upon flow. However, during the downstream cycle, the ultrasonic wave travels *with* flow. The resulting transit time is therefore reduced to the same degree as during the upstream cycle as a result of flow. This dual-crystal reflective ultrasonic pathway subtracts the upstream from downstream integrative components of transit time providing an accurate measure of volume flow (Drost, 1978). This ultrasound transit time volume method (Transonic Systems, Ithaca, NY) provides the researcher with many sophisticated advantages including the ability to acquire precalibrated flow sensors for vessels between 0.25 and 0.32 mm in diameter; full automation and blood flow determination using dual and self-diagnostic crystal reflective ultrasound technology.

2.2. The electromagnetic method for blood flow measurement

The measurement of blood flow with electromagnetic flow probes uses the principle described by Faraday (1832) in his law of electromagnetic induction as it applies to liquids (i.e., blood). The Faraday law states that if you change a magnetic field ($d\phi_B/dt$) you produce an electric field ($\S E \times dl$). Accordingly, $\S E \times dl = d\phi_B/dt$ where the induced electromagnetic force is a uniform electric field

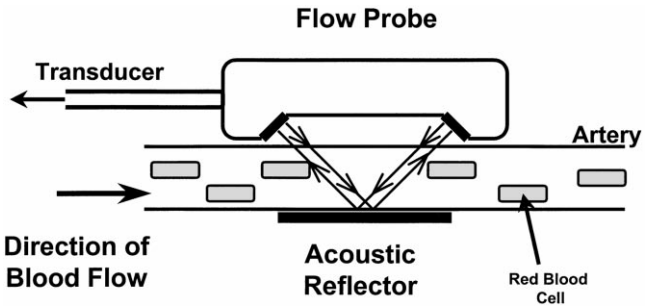

Fig. 1. An example of a flow probe used to determine blood flow in vessel. Depicted are the basic structural components of a Transonic ultrasonic volume flow probe. The transducers emit ultrasonic waves that traverse the blood vessel. The waves are reflected by a fixed acoustic reflector and are detected by sensor windows (not shown) in the body of the probe. The time taken by the ultrasonic waves to travel across the vessel is termed the "transit time." The integration of transit times provides an accurate measure of volume blood flow in the vessel.

filling a cylindrical volume of space, i.e., a vessel. Such a force results from the movement of blood (or specifically the displacement of ions within the blood), an electrically conductive liquid, through the generated field (Bevir, 1970). The electromagnetic force or potential difference that is generated can be used to calculate a volume of flow by estimating average velocity across the blood vessel.

2.3. The laser Doppler technique for blood flow measurement

This particular technique is not readily employed to measure absolute flow but rather determines relative changes in regional blood flow. The principles of the technique are adapted from the light wave theory of Christian Doppler (1842) where he showed that the sound (or color) of a body must change by the relative motion of the body and the observer. Thus, transmitted laser light from an optic fiber is scattered from the vessel of interest and transmitted back to a photodetector for measurement. The optic fibers used in laser Doppler studies transmit visible (633 nm) light that provides for its use in a range of research and clinical applications such as the assessment of cerebral blood flow (Bolognese, Miller, Heger, & Milhorat, 1993). For blood flow measurement, the moving object, blood, reflects back the laser light at a different wavelength than it is transmitted, i.e., produces a Doppler shift. Only the laser light that hits a moving object is changed; light hitting stationary tissue remains unchanged. The observed changes in frequency distribution and magnitude of the wavelength (scatter) are detected by a photosensitive optic fiber and the Doppler shift converted into flux values based on the velocity of the moving object. In the case of assessing blood flow in a region, the Doppler shift is created by the moving red blood cells, and changes in flow are determined based on the passage of red blood cells through a unit area in the blood vessel. Therefore flow velocities are determined as frequency changes and are not actual measured velocities. This technique has the advantage of allowing for an evaluation of microvascular perfusion in real time.

2.4. The reference sample method for blood flow measurement

This method will be elaborated upon in this issue of the Journal therefore we will present only a limited overview here. The principle that is used to determine regional blood flow involves the use of radioactive labeled microspheres.

Microspheres are small (submicron) particles composed of a variety of materials, many that are biologically inert such as silica, a hydrophilic compound that does not adsorb protein or polystyrene, a hydrophobic polymer that has a high degree of nonspecific protein absorption. In blood flow measures, biologically inactive microspheres are preferred.

The principles of microsphere use are simple: regional blood flow is simply proportional to the number of microspheres trapped in the region of interest. The reference blood flow sample is used to determine regional blood flow. The accuracy of this method is greatly dependent upon correct placement of the reference blood-withdrawal catheter that will properly sample mixed microspheres and blood. Therefore, at the time of microsphere injection a reference blood flow sample must be acquired. These principles are illustrated in the equation where reference sample flow (RSF, ml/min) ÷ radioactivity in reference sample (RRS) = organ flow (Q_{organ}) ÷ radioactivity in the organ (RO). This yields a measure of organ flow such that $Q_{organ} = RO \times (RSF \div RRS)$.

3. Experimental examples of blood flow measurement

3.1. Blood flow measurements using the transit-time volume method

Animals were anaesthetized with sodium pentobarbitone (65 mg/kg, ip) and catheters (polyethylene tubing I.D. 0.58 mm, O.D. 0.965 mm) were inserted into the iliac vein and artery for drug administration and blood pressure measurements, respectively. Body temperature was maintained at 37°C using a heating pad. All catheters were filled with heparinized (25 IU/ml) saline (0.9% NaCl). Arterial blood pressure was recorded with a pressure transducer (Gould Statham, USA; Model PD23D) connected to an amplifier (DA 100A) and coupled to universal interface module (UIM 100), which interfaced with the acquisition unit (MP 100).

Rats were placed in dorsal recumbence and the abdominal cavity opened through a ventral midline incision. The celiac artery and superior mesenteric artery were isolated and an approximate 1-cm length of each artery was dissected free of connective tissue. Any fatty tissue associated with, or in close proximity to, the arteries was removed to prevent a reduction in acoustic coupling. A Transonic ultrasonic volume flow probe (RB-Series, 1 mm, Transonic Systems) was placed around the blood vessels and blood flow was measured using a flowmeter (Model T106, Transonic Systems) coupled to the universal interface module (UIM 100). Heart rate was calculated from the blood pressure signal using the Acqknowledge III waveform acquisition and analysis software system (Biopac Systems, Santa Barbara, CA). All haemodynamic data was also collected using Acqknowledge III and stored online.

3.2. Blood flow measurements using electromagnetic flow probes

Iida (1995) has provided a detailed description of the measurement of blood flow in the celiac and superior mesenteric blood vessels in conscious rats. Essentially,

electromagnetic flow probes (Type FC, 1–2 mm, Nihon Kohden, Japan) were implanted around the blood vessels and animals were allowed to recover for 3–4 days before measurements of blood flow, blood pressure and heart rate were made (Iida, 1995).

3.3. The use of laser Doppler flowmetry to assess changes in cerebral artery flow

A detailed description of animal preparation for the measurement of relative changes in blood has been given by Smeda, Van Vliet, and King (1999). Briefly, Sprague–Dawley rats were anaesthetized with sodium pentobarbitone (65 mg/kg, ip). Catheters (polyethylene tubing; I.D. 0.58 mm, O.D. 0.965 mm) were inserted into the left and right iliac artery and vein, respectively. Animals were tracheotomized and ventilated (Harvard Apparatus, Model 665, Hollington, MA), at 1.1 ml/100 g body weight at 40 cycles per minute. Following catheterization, each rat was placed in a stereotaxic apparatus (Micropositioner Model 660, David Kopf Instruments, Tujunga, CA), and the cranium was exposed. A 2-mm hole was drilled into the cranium above the middle cerebral artery and a second hole drilled at the level of the middle and posterior cerebral artery. Two optic fiber flow probes (1.5 mm diameter and 80 mm in length; PF 403; Perimed, Stockholm, Sweden) were inserted into the holes in close proximity to the vessels and held in place using two micromanipulators. Changes in blood flow were then monitored using a solid-state laser Doppler flowmeter (PeriFlux PF 4001 Master, Perimed AB) and recorded, analyzed and stored using PeriSoft for Windows (PSW). In these animals the impact of increasing blood pressure (from 96 to 170 mmHg) by the continuous infusion of noradrenaline (beginning with a dose of 0.2 μg/kg/min and doubling the infusion rate until a maximal tolerable response was achieved) on the relative changes in blood flow in the middle and posterior cerebral artery were examined.

3.4. The radioactive microsphere technique for blood flow measurement

3.4.1. Preparation of anaesthetized animals

Sprague–Dawley rats were anaesthetized with pentobarbitone (65 mg/kg, ip). Catheters (polyethylene tubing; I.D. 0.58 mm, O.D. 0.965 mm) were inserted into the left and right iliac artery and vein. The left arterial and right venous catheters were used for the measurement of blood pressure and drug administration, respectively, while the right arterial catheter was used for blood withdrawal of radioactive-labeled microspheres. An additional catheter was inserted into the left ventricle via the right carotied artery for measurement of the left ventricular pressure and injection of radioactive-labeled microspheres. Blood pressure, heart rate and ventricular pressure were monitored continuously prior to injection of microspheres.

3.4.2. Preparation of conscious animals

Rats were anaesthetized with halothane (induction 5% in 100% O_2; maintenance 1.5% in 100% O_2) and catheters (polyethylene tubing; I.D. 0.58 mm, O.D. 0.965 mm) were inserted into the left and right iliac arteries and veins. The left arterial and right venous catheters were used for the measurement of blood pressure, and drug/vehicle administration, respectively, while the right arterial catheter was used for blood withdrawal of radioactive-labeled microspheres. An additional catheter was inserted into the left ventricle via the right carotid artery for measurement of the left ventricular pressure and injection of radioactive-labeled microspheres. All catheters were filled with heparinized (25 IU/ml) saline (0.9% NaCl), and tunneled subcutaneously to the back of the neck, exteriorized and secured. Animals were then allowed to recover for 24 h. In conscious rats, blood pressure, heart rate and left ventricular pressure were monitored for 1 h prior to injections of microspheres.

3.4.3. The microsphere technique

Suspensions of microspheres (15 μm diameter; Mandel Instruments, Guelph, ON, Canada) labeled with either ^{57}CO or ^{113}Sn (20,000—22,000 in 150 μl) were injected into the left ventricle over a period of 10 s. Reference blood samples were withdrawn from the right femoral artery at the rate of 0.35 ml/min starting 15 s before microsphere injection using an infusion/withdrawal pump (Kd Scientific, USA; Model 120) for 1 min for the determination of blood flow. Ficoll 70 (10%) and Tween 80 (0.05%) were used to suspend the microspheres in the volume that was injected. After the injection of the microspheres and completion of the experiment animals were killed by an overdose of pentobarbitone. Blood samples and syringes used for injection of microspheres or withdrawal of blood were counted for radioactivity at 80—160 keV using a dual-channel automatic gamma counter. Organs (kidneys, stomach, large and small intestines and caecum) were removed and the radioactivity in each sample was counted using the gamma counter. In any instance where the blood flow to the left and right kidney differed from one another by more than 20%, the experiments were rejected based on the supposition that an inadequate mixing of the microsphere occurred during their injection into the left ventricle.

3.5. Experimental protocol

This study examined the influence of mibefradil, a T-type calcium channel blocker, on blood pressure, superior mesenteric blood flow (using the Transonic volume flow probe transit-time method) and heart rate in anesthetized animals. Following stabilization of the animal for 1 h after surgery each received a cumulative, continuous infusion of cirazoline, an α_1-adrenoceptor agonist. Cirazoline was infused at 0.4, 0.8, 1.6 and 3.2 μg/kg/min for 6–8 min. After the completion of the first dose–response curve, each animal was allowed to recover for 60 min. This period was

Table 1
Haemodynamic values of anaesthetized animals in which blood flow to the gastrointestinal tract was determined either using radioactively labeled microspheres or Transonic ultrasonic volume flow probes

Blood flow method	Blood flow (ml/min per 100 g)	Conductance (ml/min mmHg per 100 g)	Blood pressure (mmHg)	Heart rate (beats/min)
Microspheres	6.10 ± 0.39	0.059 ± 0.004	105 ± 5.0	345 ± 13
Transit-time	5.43 ± 0.20	0.051 ± 0.003	107 ± 4.0	362 ± 9.0

Each value represents the mean ± S.E. of six experiments.

found sufficient to allow blood pressure, blood flow and heart rate to return to the baseline. Each animal then received mibefradil (0.3 and 1.0 mg/kg) as a bolus intravenous injection, and 20 min was allowed to elapse before the second cumulative dose–response curve to cirazoline was constructed.

3.6. Statistical analysis

In the microsphere experiments, blood (or organ, Q_{organ}) flow (ml/min) was calculated as the rate of withdrawal of blood (reference sample flow, RSF) multiplied by the total injected radioactivity in the reference sample (RRS) divided by radioactivity in the withdrawn blood sample (RO). Arterial conductance ($G_{arterial}$, ml/min mmHg) was calculated by dividing blood flow by mean arterial blood pressure.

An unpaired t test was used to test for statistical significance in a comparison of the blood flow, vascular conductance, blood pressure and heart rate measured using the two different techniques. The haemodynamic impact of mibefradil on cirazoline-mediated vasoconstriction was analyzed by one-way analysis of variance with repeated measures for comparison. Duncan's multiple range test was used for a comparison between means. A difference of $P < .05$ was considered to be significant.

4. Results of blood flow measurement

4.1. A comparison of blood flow measurement using transit-time flow probes and the microsphere reference technique in anaesthetized rats

Total blood flow to the stomach, small and large, intestines and caecum measured using the microsphere reference technique indicates a flow of 6.10 ± 0.39 ml/min per 100 g tissue ($n = 6$; mean ± S.E.). In comparison, the summation of blood flow to celiac and superior mesenteric arteries using the transit-time technique produced a value of 5.43 ± 0.2 ml/min per 100 g tissue ($n = 6$; mean ± S.E.). The values for blood flow to the gastrointestinal tract obtained using the microsphere technique was found not to differ significantly from those determine using transit-time technique (Table 1). This was also the case when conductance was calculated for the gastrointestinal tract using haemodynamic data from microspheres or transit-time flow probes (Table 1).

4.2. A comparison of blood flow measurement using electromagnetic flowmetry and the microsphere reference technique in conscious rats

A comparison between flow calculated using the microsphere technique and that of electromagnetic flow probes in the conscious rat indicates that values are 6.69 ± 0.67 ml/min per 100 g tissue, and 6.19 ± 0.26 (celiac + superior mesenteric flow) ml/min per 100 g tissue, respectively, ($n = 6$; mean ± S.E.). The flow values obtained in conscious rats using the two different techniques were found not to differ significantly from one another (Table 2). In addition, calculation of conductance for the entire mesenteric bed shows similar values between the microsphere reference technique and that of electromagnetic flowmetry values (Table 2).

4.3. Influence of mibefradil on α_1-adrenoceptor mediated vasoconstriction in the mesenteric bed of anaesthetized rats

Infusion with cirazoline increased blood pressure while reducing blood flow and arterial conductance in the mesenteric bed in a dose-dependent manner (Tables 3

Table 2
Haemodynamic values of conscious animals in which blood flow to the gastrointestinal tract was determined either using radioactively labeled microspheres or an electromagnetic flow probe

Blood flow method	Blood flow (ml/min per 100 g)	Conductance (ml/min mmHg per 100 g)	Blood pressure (mmHg)	Heart rate (beats/min)
Microspheres	6.69 ± 0.67	0.059 ± 0.006	113 ± 2.0	363 ± 9.0
Electromagnetic[a]	6.19 ± 0.26	0.054 ± 0.003	114 ± 2.0	377 ± 14

Each value represents the mean ± S.E. of six experiments

[a] Dr. Noriko Iida, University of Hiroshima, Japan provided the electromagnetic flow probe data as a personal communication.

Table 3
Haemodynamic effects of cirazoline in anaesthetized rats in the absence or presence of a low dose of mibefradil (0.3 mg/kg)

Dose (μ/kg/min)	Blood flow (ml/min per 100 g)	Conductance (ml/min mmHg per 100 g)	Blood pressure (mmHg)	Heart rate (beats/min)
Pre-mibefradil				
control	3.3 ± 0.29	0.029 ± 0.0027	107 ± 4	386 ± 9
0.4	3.1 ± 0.27[a]	0.027 ± 0.0028	114 ± 3[a]	386 ± 8
0.8	2.76 ± 0.27[a]	0.023 ± 0.0027[a]	122 ± 4[a]	374 ± 9
1.6	2.44 ± 0.27[a]	0.019 ± 0.0031[a]	131 ± 4[a]	355 ± 10[a]
3.2	1.79 ± 0.26[a]	0.012 ± 0.0019[a]	155 ± 5[a]	335 ± 8[a]
Post-mibefradil				
control	3.0 ± 0.25	0.031 ± 0.0028	99 ± 5	397 ± 13
0.4	0.87 ± 0.27[b]	0.028 ± 0.0026	103 ± 4[b,c]	391 ± 11
0.8	2.64 ± 0.27[b]	0.025 ± 0.0025[b]	107 ± 4[b,c]	385 ± 11
1.6	2.29 ± 0.27[b]	0.021 ± 0.0023[b]	112 ± 4[b,c]	364 ± 12[b]
3.2	1.81 ± 0.23[b]	0.013 ± 0.0021[b]	129 ± 5[b,c]	332 ± 14[b]

Each value represents the mean ± S.E. of seven experiments.
[a] Indicates a significant difference from pre-mibefradil control; $P < .05$.
[b] Indicates a significant difference from post-mibefradil control; $P < .05$.
[c] Indicates a significant difference from the corresponding dose of cirazoline, pre-mibefradil; $P < .05$.

and 4). The increase in blood pressure resulted in reflex bradycardia. While the administration of mibefradil did lower blood pressure of anaesthetized rats, it was found that the reduction was not significant. Treatment with mibefradil also did not appear to significantly affect mesenteric blood flow, conductance or heart rate. It was apparent that the administration of mibefradil at the lower dose (0.3 mg/kg) did not have a significant impact on cirazoline-mediated vasoconstriction in the mesenteric bed. Cirazoline-induced reduction in mesenteric blood flow and conductance were not significantly impaired by the lower dose of mibefradil (Table 3). In contrast, the higher administered dose of mibefradil (1.0 mg/kg) was able to significantly attenuate the reduction in blood flow and conductance in mesenteric bed that occurred due to the infusion of different doses of cirazoline (Table 4). The higher dose of mibefradil also attenuated the increase in blood pressure following infusion with cirazoline (Table 4).

4.4. The influence of infused noradrenaline on cerebral blood flow autoregulation

Increasing systemic blood pressure up to 135 mmHg by the infusion of noradrenaline did not appear to have an impact on cerebral blood flow. However, when the systemic blood pressure was elevated beyond 140 mmHg there appeared to be a ‘‘breaking point’’ in which there was a loss in autoregulation by the middle and posterior cerebral

Table 4
Haemodynamic effects of cirazoline in anaesthetized rats in the absence or presence of a high dose of mibefradil (1.0 mg/kg)

Dose (μg/kg/min)	Blood flow (ml/min per 100 g)	Conductance (ml/min mmHg per 100 g)	Blood pressure (mmHg)	Heart rate (beats/min)
Pre-mibefradil				
control	3.1 ± 0.19	0.029 ± 0.0018	104 ± 5	370 ± 9
0.4	2.86 ± 0.17[a]	0.026 ± 0.0015[a]	111 ± 4[a]	374 ± 8
0.8	2.56 ± 0.15[a]	0.022 ± 0.0012[a]	119 ± 4[a]	377 ± 10
1.6	2.13 ± 0.12[a]	0.017 ± 0.0017[a]	128 ± 5[a]	363 ± 12
3.2	1.67 ± 0.10[a]	0.012 ± 0.0018[a]	145 ± 6[a]	345 ± 11[a]
Post-mibefradil				
control	3.1 ± 0.24	0.032 ± 0.0017	95 ± 5	379 ± 9
0.4	2.93 ± 0.24[b]	0.029 ± 0.0018[b]	100 ± 4[b,c]	378 ± 8
0.8	2.86 ± 0.26[b,c]	0.026 ± 0.0017[b,c]	107 ± 4[b,c]	372 ± 9
1.6	2.51 ± 0.22[b,c]	0.022 ± 0.0014[b,c]	114 ± 4[b,c]	364 ± 10[c]
3.2	2.07 ± 0.18[b,c]	0.016 ± 0.0011[b,c]	127 ± 5[b,c]	337 ± 9[c]

Each value represents the mean ± S.E. of seven experiments.
[a] Indicates a significant difference from pre-mibefradil control; $P < .05$.
[b] Indicates a significant difference from the corresponding dose of cirazoline, pre-mibefradil; $P < .05$.
[c] Indicates a significant difference from post-mibefradil control; $P < .05$.

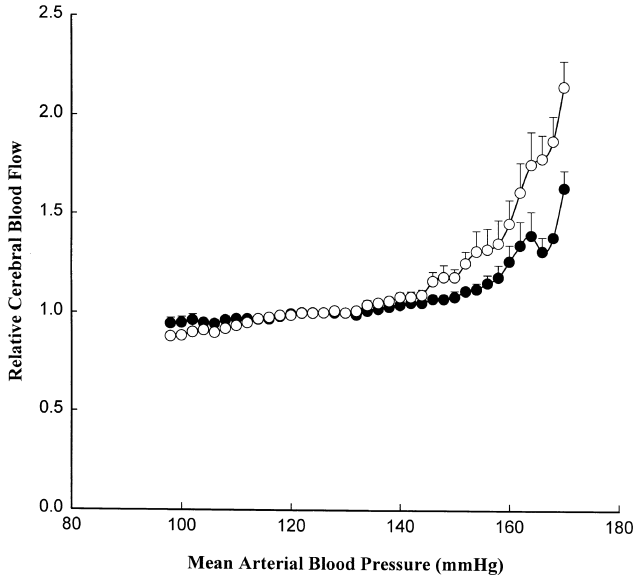

Fig. 2. Laser Doppler measured changes in relative cerebral blood flow associated with an increase in mean arterial blood pressure of the middle cerebral artery (closed circles) or posterior cerebral artery (open circles) in pentobarbitone anaesthetized rats. Mean arterial blood pressure was increased by a continuous infusion of 0.2 μg/kg/min noradrenaline. Each point represents a mean ± S.E. of six experiments. Data was provided as a courtesy by Dr. John S. Smeda.

arteries (Fig. 2). The loss in autoregulation resulted in an increase in relative cerebral blood flow in both arteries.

5. Discussion

5.1. Blood flow measurements using ultrasonic volume flow probes

The major obstacles associated with the measurement of blood flow using ultrasonic volume flow probes in vivo include sensitivity drift (i.e., error in precision), and zero flow baseline drift. However, it is possible to overcome these difficulties with recent technological advances. For example, with the use of Transonic transit-time flow probes, baseline value can be checked intermittently via built-in diagnostic circuitry that can be used to diagnose malfunctioning flow sensors and guard against excessive baseline drift. Thus, it would seem that transit-time flow probes are available that provide high accuracy, exhibit a linear response for vessels of all lengths and absence of zero-flow baseline drift and by internal identification of probe size can adjust calibration factors, flow ranges and gain automatically (Koenig et al., 1996). Another consideration with flow probes is that they do not damage the endothelium of the blood vessel during the measurement of blood flow. However, most probes appear to offer a range of reasonable physical characteristics such as small size to accommodate vessel diameter, improved accuracy of recording and non-constrictive construction that reduces the incidence of vessel spasm. Thus, available flow probes if implanted carefully result in minimal damage to blood vessels. One advantage of using flow probes such as electromagnetic or transit-time flowmetry is that both rapid (beat-to-beat) and long-term (over several weeks) changes in blood flow can be monitored in animals (Evans et al., 1997; Nelissen-Vrancken, Struijker-Boudier, & Smits, 1992; Sokol, Liechty, & Boyle, 1996).

One source of error that has been the subject of debate with the use of electromagnetic flow probes is changes in haematocrit (red blood cell volume) values. Since electromagnetic flow probes rely on the motion of electrically charged particles in blood, an increase in haematocrit may reduce charge and therefore reduce the accuracy of the recording method. However, it seems that this problem can be eliminated by use of probes with high impedance (>100 MΩ). Changes in haematocrit values from 0 to 0.677 appear not to have a significance influence on flow measurements (Roberts, 1967). Table 5 outlines some differences between the various flow probe methods used to measure arterial blood flow in organs and the whole body.

5.2. Blood flow measurement using the microsphere reference technique

The radioactive microsphere technique clearly provides an efficient method of estimating blood flow to various organs in the body. With the availability of different radioactive labeled microspheres, it is possible to measure regional blood flow repeatedly. In addition, this particular technique offers the opportunity for an investigator to measure blood flow in regions where the use of probes or

Table 5
A comparison of several flow probe methods commonly used to measure arterial blood flow

	Method		
	Ultrasonic	Electromagnetic	Doppler
Principle	Reflective ultrasound	Electromagnetic induction	Laser scatter photodetection
Probe type	Volume flow	Electromagnetic flow	Optic fiber
Vessel diameter (mm)	>0.25	>0.50	>0.040
Automated	Precalibrated automated	Sensor programmed	Programmed optics
Flow measure	Direct volume flow	Derived volume flow	Derived volume flow

sensors are impractical, i.e., where invasive surgical procedures may compromise the animal. Such regions include the entire muscular system as well as the dermatological and osteal systems (Tabrizchi, King, & Pang, 1986). In much smaller animals where very small probes are not available for the measurement of blood flow, the microsphere technique can be used to measure regional blood flow. The main disadvantage of this technique, compared to flow probe methods, is that it does not offer continuous monitoring of blood flow to a region, and rather only provides a "snapshot" of flow at a single point in time.

The application of microsphere techniques requires that a number of criteria be met for the measurement to be accurate. These are as follows: (a) microspheres must have inert properties in biological systems, (b) microspheres must behave uniformly like blood cells, (c) that essentially all microspheres are trapped in a single passage through the circulation, and (d) that the procedure does not measurably alter the regional haemodynamics of interest either during or after injection. In order to ensure that microspheres are mixed adequately and behave uniformly like blood cells it is a routine practice to monitor blood flow to the left and right kidneys and compare rates in those organs. In general, where there is a discrepancy between the blood flow to the kidneys of more 20% the experiment should be rejected based upon the assumption that inadequate mixing of microspheres occurred during injection (Pang, 1983). The idea that microspheres should not affect haemodynamics following their injection has been the subject a number of investigations. For example, in rats, it has long been considered that the injection of >100,000 microspheres in suspension interferes with haemodynamics (Tsuchiya, Walsh, & Forhlich, 1977). However, it has been reported that suspensions of up to 360,000 microspheres do not significantly affect haemodynamics in rats (Stanek, Smith, & Murphy, 1983). It is probable that, to some degree, the number of microspheres injected into rats depends upon body weight and the solution used to suspend microspheres for injection. Certainly, it would be prudent to suggest that injections of up to 180,000 microspheres would most likely not interfere with haemodynamics and adversely affect regional haemodynamics in rats (Stanek et al., 1983; Tuma, Vasthare, & Irion, 1986). Despite the actual number of microspheres, the amount of microspheres that are injected should be calculated so as to provide an accurate measure of heterogeneity and correlation coefficients. The number of microspheres per milliliter in suspension that should be injected can be calculated based upon the characteristics of the microsphere itself according to the information supplied by the manufacturer.

Some possible sources of error associated with the use of radioactive microspheres include recirculation and leaching associated with chronic preparations. However, it has been reported that the estimated loss of microspheres (with a diameter of 15 μM) from the rat myocardium is only approximately 1.5% (including a "leach factor" in vivo) over a 1–3 week period (Medvedev, Martynova, Akchurin, & Khalatov, 1988). This essentially implies that this technique is stable with time and therefore can be used with reasonable accuracy for the measurement of regional blood flow under a number of circumstances. Briefly, another contentious issue relating to the use of microspheres (of a similar 15 μM diameter) involves discrepancies in the determination of regional blood flow distributions in the lungs of rats (Malik et al., 1976). However the observed differences in flow can be explained by the fact that ∽2% of the microspheres appear not to be trapped by the peripheral circulation but are instead found within the pulmonary circulation (Archie et al., 1973; Malik et al., 1976). Thus this factor should be considered when using microspheres to determine pulmonary blood flow. Essentially, the reference sample method of assessing blood flow allows an investigator to accurately and efficiently estimate regional blood flow in both anesthetized and conscious animals provided that one adheres to certain rules and standards and understands deficiencies associated with this method.

It is evident from our studies that when compared, the transit-time flow probe and microsphere reference techniques produce comparable values for the measurement of blood flow in the mesentery in anaesthetized rats. Furthermore, a comparison between electromagnetic flowmetry and microsphere reference technique also yielded comparable values for blood flow in the mesentery in conscious rats. These techniques offer investigators a multitude of ways in which to assess absolute blood flow in a given organ in either conscious or anaesthetized animals.

5.3. Use of transit-time flow probes to assess the impact of drugs on blood flow

In the present investigation, we found that the calcium channel antagonist, mibefradil, did not increase blood flow or alter vascular conductance in the mesenteric bed of the rat. However, it was able to impair cirazoline-mediated vasoconstriction in the mesentery. An examination of the impact of a series of calcium channel antagonists (such as verapamil, nifedipine and flunarizine) in anaesthetized rats has revealed that these compounds are able to increase vascular conductance in the gastrointestinal region (Waite, Pang, & Walker, 1990). In the rat, isolated perfused mesenteric blood vessels, vasoconstriction produced by noradrenaline has been reported to be attenuated by nifedipine (Criddle, De Moura, Greenwood, & Large, 1997) while the 1,4-dihydropyridine calcium antagonist, benidipine, has been found to produce vasodilatation of the arterioles in the rat mesenteric microcirculation (Nakayama et al., 1999).

Mibefradil is considered to be a selective inhibitor of T-type calcium channels (Mishra & Hermsmeyer, 1994). While it is possible that it is able to partially impair α_1-adrenoceptor-mediated vasoconstriction, it may not be able to substantially reduce blood pressure in normotensive animals. Recently, however, mibefradil has been found to

reduce blood pressure (Ernst & Kelly, 1998). In the present investigation, it was interesting to note that mibefradil, at the doses examined, did not exert a negative chronotropic effect on the heart, an action mediated by many L-type calcium channel blockers. Thus, it did not appear to produce a significant bradycardia. However, mibefradil as a putative selective T-type calcium channel antagonist appears to have a moderate negative inotropic effect at higher doses (Osterrieder & Holck, 1989; Triggle, 1996) and has been reported to exhibit a potent coronary artery vasodilating action in dogs (Clozel, Banken, & Osterrieder, 1989). It is evident from the present investigation that the use of transit-time volume flow probes offers an opportunity to assess the impact of investigative drugs on blood flow in regions such as the mesentery.

5.4. The use of the laser Doppler to assess changes in regional blood flow in cerebral arteries

The use of the optic fiber laser Doppler technique for the assessment of relative changes in blood flow has the advantage that flow can be measured without blood vessels being isolated or exposed. The relative changes in blood flow in cerebral arteries were made from vessels that are less than 40 μM in diameter. Essentially, this means that this technique allows for an assessment of blood-vessel function at the microcirculatory levels (Barfod et al., 1997). Obviously, it can also be used to assess blood-vessel function at a macrocirculatory level and therefore has good experimental and clinical utility. Clearly, the disadvantage of this technique is that it does not readily allow for the measurement of absolute blood flow in a region (Smits, Roman, & Lombard, 1986). However, the great advantage that this technique offers is that it allows for the assessment of relative changes in blood flow to be made in a noninvasive manner. The various optic fiber probes that are available allow for the investigation of vessels at different sites whether superficial or deep within tissue. It is apparent that in order for this technique to be used to assess blood-vessel function, it is not necessary to isolate the blood vessel. Thus it is possible for relative changes in blood flow to be recorded from that vessel as long as the laser light transverses the blood vessel. This clearly offers the advantage that for the assessment of blood-vessel function to be made the vessel does not have to be handled, thus preserving physiological function. However, this technique is intended to provide global indications of changes in blood flow rather than flow in isolated vessels.

6. Conclusion

Thus, a number of methods have been described that can be used in the determination of blood flow in the arterial circulatory system. While these methods have been briefly reviewed we present, by no means, a comprehensive overview of each method, rather many volumes of the Journal would be required to thoroughly evaluate, compare and contrast the methods outlined. Such a task is beyond the scope of this article. However, we hope that from this article readers may gain some understanding of the methods described and can use this information as a basis for establishing these methods in their own laboratories.

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