A Simplified Method for Measuring Regional Blood Flow
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SUMMARY A method of measuring regional blood flow (RBF) that is simple in procedure and calculations is described. By arresting flow promptly after a short pulse of diffusible tracer, it is feasible to equate the tracer retained in the tissue (Ci) with that delivered by the blood. If the arterial pulse is characterized by its mean concentration (Ca) over a known duration (Δt), RBF can be estimated from Ci/CaΔt. The error involved is relatively small and can be corrected for. If the amount of tracer injected is known, this procedure also provides an estimate of cardiac output and its fractional distribution to the regions sampled. The values obtained for RBF in 4 regions of brain were similar to those previously reported.

MEASUREMENT OF regional blood flow (RBF) has been greatly facilitated by the use of tracers that are biologically inert and sufficiently diffusible to approach equilibrium with the tissue water as they pass through the capillaries. The calculation of flow rates has remained difficult, however, because it has required rapid serial sampling of arterial blood to define the changing concentration of tracer (e.g. Ref. 1) and the use of complicated equations requiring a computer for their solution (e.g. Ref. 1, 2).

We describe here a method of measuring RBF in which the tissue is sampled promptly after a short arterial pulse of diffusible tracer. By keeping the time during which the tissue is exposed to the tracer sufficiently short, it is possible to use the amount of tracer retained by the tissue as a reasonably accurate measure of the amount that had been delivered by the arterial blood. RBF can then be calculated by dividing tissue concentration of tracer by mean arterial concentration and time. The measurements and calculations are considerably simpler than those commonly used, and the relatively small errors involved can be estimated, and corrected for if desired.

General Approach

The experimental procedure involves only:
1. Introducing a bolus of diffusible tracer into the circulating blood. (Though the bolus should be short, it must be well mixed with that portion of the moving column of blood that it occupies. We injected the tracer into the pulmonary artery, though other sites between vena cava and left atrium might have served as well.)
2. Waiting just long enough to be certain that the blood that contained the bolus of tracer has passed through the capillaries of the tissue being studied; then arresting the circulation and measuring the concentration of tracer in the tissue.
3. Meanwhile, withdrawing a sample of arterial blood at a constant rate over a measured period that includes the passage of the bolus; and measuring the mean concentration of tracer in the sample.

RBF is then calculated from:

\[ k' = \frac{C_i}{C_a\Delta t} \]

in which \( k' \) is the approximate flow rate, expressed as volume per unit volume of tissue per unit time; \( C_i \) is the concentration of tracer found in a unit volume of tissue at the time of harvest; and \( C_a \) is the mean concentration of tracer in the sample of arterial blood withdrawn at constant rate during sampling time \( \Delta t \).

As indicated above, equation 1 is an approximation and the correct relationship is

\[ k = \frac{C_i}{C_a\Delta t} \]

in which \( k \) is the actual flow rate and \( C_i \) represents the tracer that is delivered to the tissue by the arterial inflow. \( C_i \) is less than \( C_i' \) because of tracer removed in the venous blood, as shown by

\[ C_i = C_i' + k \int_{t_0}^{t_1} C_a dt + k \int_{t_0}^{t_2} C_a dt \]

in which it is assumed that flow remains constant during the measurement and that the partition coefficient of tracer between blood and tissue is 1 (cf. Ref. 1). The interval \((t_1-t_0)\) represents the period during which the bolus is perfusing the capillaries and \((t_2-t_1)\) represents the period between the end of the bolus and arrest of circulation during which blood without tracer is entering the capillaries. Since the concentration of tracer in the tissue \( (C_i) \) will rise from 0 to a value slightly greater than \( C_i' \) during \((t_1-t_0)\) and then fall slightly to \( C_i \) during \((t_2-t_1)\), equation 3 can be rewritten as the following approximation.

\[ C_i' \approx C_i' + \frac{1}{2} C_i' k(t_1-t_0) + C_i k(t_2-t_1) \]

By combining (4) with (2)

\[ k = \frac{C_i}{C_a\Delta t - C_i'[\frac{1}{2} (t_1-t_0) + (t_2-t_1)]} \]

and (1) and (5) with

\[ %\text{error} = \left(1 - \frac{k'}{k}\right) \times 100 \]

one obtains
(7) \% \text{error} = k' \left[ \frac{1}{2} (t_1 - t_0) + (t_2 - t_1) \right] \times 100

As would be expected, the error increases with faster flows and longer times, with the time elapsing after the bolus introducing about twice as much error as the time during which it perfuses the tissue. The figure is a nomogram from equation 7 to show the relationship between the flow rate as estimated \((k')\), the time that elapses during and after perfusion by the bolus, and the error entailed in the simplified calculation.

If the short arterial pulse is treated as if it were a square wave, the error associated with the simplified calculation can also be calculated by the following more rigorous, if perhaps less illuminating, approach.

\[
(8) \quad \frac{C(t)}{C_a} = \left[1 - e^{-k(t_1 - t_0)}\right] e^{-k(t_2 - t_1)}
\]

Divide

\[
(9) \quad \frac{C(t)}{C_a} = k(t_1 - t_0)
\]

to obtain:

\[
(10) \quad \frac{C(t)}{C(t)} = \frac{\left[1 - e^{-k(t_1 - t_0)}\right] e^{-k(t_2 - t_1)}}{k(t_1 - t_0)}
\]

and

\[
(11) \quad \% \text{error} = \left\{ 1 - \frac{\left[1 - e^{-k(t_1 - t_0)}\right] e^{-k(t_2 - t_1)}}{k(t_1 - t_0)} \right\} \times 100
\]

If labelling times are short, the error is small. Consider, for example, an experiment in which a square wave of tracer perfuses the tissue for 3 secs and another 3 secs elapse before arrest of the circulation, i.e., \(t_1 - t_0\) and \(t_2 - t_1\) are both 0.05 min. In a region of moderate flow rate (0.2 ml/ml per min) such as CNS white matter, \(k'\) as calculated from equation 1 would be 0.197 min\(^{-1}\) or 1.5\% low. In a region of relatively fast flow (1 ml/ml per min) such as CNS gray matter, \(k'\) would be 0.928 min\(^{-1}\) with an error of -7.2\% as calculated from equation 11. The error calculated from equation 7 would be nearly the same, -7.0\%. (It will be noted that equation 11 calculates the error from \(k\) and equation 7 from \(k'\), but both express it as a percentage of \(k\).)

It may seem paradoxical that equations 1 and 2, used to calculate flow rate from the flux of tracer between blood and tissue, do not include a term for the time during which this flux was taking place, the term \(\Delta t\) in these equations being the rather arbitrarily determined period during which the arterial sample is withdrawn. The reason for this becomes apparent from the following derivation of equation 2 that says, in essence, that RBF is calculated as the product of cardiac output times the fraction of the output that passes through the tissue in question. These 2 quantities are measured at about the same time, and it is assumed that the system remains in steady state during the measurements.

If the amount of tracer (B) that was injected in the bolus is known, then the cardiac output (C.O.) can be estimated from

\[
(12) \quad \text{C.O.} = \frac{B}{C_a \Delta t}
\]

(The validity of this relationship becomes apparent if one considers that the concentration of tracer in the arterial sample will be independent of the volume of the sample, i.e. independent of the rate at which the sample was being constantly withdrawn during \(\Delta t\). Thus a value obtained experimentally for the product \(C_a \Delta t\) would not differ from the value that would have been obtained in the hypothetical, limiting case in which the sample was withdrawn from the base of the aorta at a rate that corresponded to the entire cardiac output. It is clear that in the latter case, the sample would contain all of B and that C.O. \(\times C_a \Delta t\) must equal B as in (12).)

The fraction, \(f\), of the cardiac output that is delivered to the sampled tissue is described by

\[
(13) \quad f = \frac{C(t)}{B}
\]

Multiplying 12 and 13

\[
(14) \quad \text{C.O.} \times f = \frac{C(t)}{B} = \frac{C(t)}{C_a \Delta t}
\]

Knowing the amount of tracer injected is not necessary for calculating RBF but does permit estimation of the cardiac output and its fractional distribution among the various tissues.

It is evident from the above that it is the product, \(C_a \Delta t\), that is critical for the calculations and that the time during which the arterial sample is withdrawn is rather arbitrary. It should include the bolus without significant recycling and should terminate at the same time that flow through the tissue is stopped. As \(\Delta t\) is lengthened or shortened to collect more or less blood before (or after) the bolus, \(C_a\) will tend to vary reciprocally so that their product will remain quite constant.

Though RBF can be calculated without knowing precisely when the bolus of tracer perfuses the tissue, some information about this is important in deciding when to stop flow. Furthermore, if an estimate can be made of the time of passage of the bolus, the error involved in the simplified calculation can be largely corrected for using equation 7 or 11.

The experimental procedure described above was tested by measuring RBF in the brains of rabbits.

Methods

New Zealand White rabbits, 1.5 to 2.5 kg, were anesthetized with pentobarbital (30 mg/kg), anticoagulated with heparin, paralyzed with succinylcholine (0.1 mg/kg) and ventilated by a
mechanical respirator adjusted to maintain arterial $\text{PCO}_2$ between 30 and 40 mm Hg. Catheters were introduced into both femoral arteries with their tips in the iliac arteries; one to monitor arterial pressure, the other to sample arterial blood. Some of the rabbits had normal arterial pressure (89-103 mm Hg) when their cerebral blood flow was measured; some had been made hypotensive (37-44 mm Hg) by withdrawal of venous blood.

A bolus of 250 $\mu$Ci of $^{14}$C-antipyrine dissolved in 0.3 ml of saline was injected rapidly (< 2 sec) into the pulmonary artery. After 8 or 12 sec (depending on whether the rabbit was normotensive or hypotensive) circulation was stopped abruptly by clamping the pedicle of the heart; and the brain was promptly removed from the calvarium, cut into coronal sections, and frozen. Beginning at the time of injection of the antipyrine and terminating with the arrest of circulation, a sample of arterial blood was withdrawn at constant rate from the right femoral-iliac catheter using a constant withdrawal syringe pump. The concentration of $^{14}$C-antipyrine was measured in the arterial blood sample and in samples of brain cut from the frozen coronal slices, and RBF was calculated from equation 1. The analytic methods are described in more detail in the accompanying paper.\textsuperscript{3}

The times between injecting the antipyrine and arresting the circulation (8 sec in the normotensive and 12 sec in the hypotensive rabbits) were selected on the basis of preliminary experiments in which brain vessels were observed while 0.1 or 0.2 ml of carbon suspension was injected into the pulmonary artery. In the normotensive animals, the transit time from pulmonary artery to brain averaged 3.4 ± 0.4 (S.E.) sec, and the time required for passage of the bolus was 3.5 ± 0.6 sec. In the hypotensive animals, these times were increased by about 50%. Thus, for both groups of animals, circulation was arrested 4 to 7 sec after the front of the bolus had entered the microvasculature and 1 to 2 sec after the tail had entered.

In other preliminary experiments, the distribution of $^{14}$C-antipyrine was measured between serum and an ultrafiltrate, and 17% of the antipyrine was found to be bound to serum proteins. In calculating RBF on the basis of a single passage of a bolus of $^{14}$C-antipyrine, the protein-bound tracer was assumed to be unavailable to the tissue.

**Results**

Measurements of cerebral RBF were made on 4 normotensive and 4 hypotensive rabbits, with flow rates calculated according to equation 1. The results obtained on 4 brain regions in the 2 groups of rabbits are shown on the right side of the table. Also shown (table, left) are measurements obtained by Freygang and Sokoloff\textsuperscript{3} for comparable areas in lightly anesthetized cats. These investigators sampled the brain after a 1 min intravenous injection of tracer during which they measured its changing arterial concentration. They calculated flow from the concentration of tracer in the brain and the arterial curve, using a well established\textsuperscript{4} mathematical analysis that involved convolution and required either a graphic solution or computer.

The mean values for our normotensive animals appear quite comparable to those reported by Freygang and Sokoloff. The largest difference was in caudate flow, and this may reflect the difficulty in excising caudate from the frozen coronal sections without contamination by adjacent white matter from internal capsule. When compared on the basis of standard deviations, the data obtained on the normotensive rabbits appear somewhat less variable than those obtained on the cats. The flow rates in the hypotensive rabbits were lower than in the normotensive rabbits (and more variable), presumably because their arterial pressure of 37-44 mm Hg was at or slightly below the lower limit of autoregulation in the anesthetized animal.

As indicated above, the rabbit measurements shown in the table must underestimate somewhat the actual flow. However, the error can be expected to be small. The measurements of circulation times (\textit{vide supra}) indicate that, in the normotensive animals, the bolus perfused the tissue for about 3½ sec ($t_1 - t_0 \approx 0.06$ min) and that the circulation was arrested about 1½ sec later ($t_2 - t_1 \approx 0.03$ min). The error can be estimated

<table>
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All animals were under light general anesthesia. Values are means ± s.e.; number of animals in parentheses. *Freygang and Sokoloff\textsuperscript{3} for our data.
In the procedure described here flow is measured from the rate that the tissue accumulates label from the blood, with the measurement restricted to the initial slope of the labeling curve before there is appreciable outflow of label. This offers several advantages. It eliminates much of the mathematics and precision in measurement that are required for calculating unidirectional uptake in the presence of significant backflow. It permits regions of markedly differing flows to be combined for analysis. Though resolution is, of course, lost, the result is a true average. It eliminates the partition coefficient of tracer (i.e., between tissue and blood at equilibrium) as an important factor in calculating flow.

The simplifying assumption — that initial net uptake of label by the tissue equals unidirectional uptake — introduces a systematic error in the direction of underestimating the flow. The error can be kept small (< 10%) by suitably restricting the labeling time, and it can be quite accurately predicted, and corrected for if the time course of the labeling is known — even approximately. Quite a precise correction might be made if a simultaneous measure of the labeling time was obtained by adding a nondiffusible gamma emitter to the diffusible tracer and monitoring its passage through the tissue with an external counter. Since the percent error varies directly with flow rate, its effect is to reduce, but not to eliminate, the difference observed in any comparison and it would not be expected to introduce a false positive.

There are several ways in which the procedure described above might be modified to meet particular requirements.

a. By using a measured amount of tracer and injecting into the pulmonary vein (to avoid loss in lung water), an estimate could be obtained of cardiac output (equation 12) and of the fractional flow to various regions (equation 13) in addition to RBF. An advantage of using a diffusible tracer for cardiac output is that recycled tracer would be markedly diluted.

b. To avoid having to expose the heart, the tracer might be introduced as a gas via the lungs or by catheter to the right atrium. Since a discrete arterial bolus could not be readily attained by either of these routes, a short continuous delivery terminating with the circulatory arrest would probably be preferable. Under this circumstance, the catheter sampling the arterial concentration of tracer should be so positioned that the blood passing its tip corresponds closely to the blood entering the microvasculature of the tissue where flow is being measured. In the equations to calculate the error, the term \( (t_2 - t_1) \) would be zero and \( (t_1 - t_0) \) would be the time from the tracer's first entry into tissue until circulatory arrest.

c. To measure reperfusion of a temporarily ischemic region, tracer might be injected into the general circulation during the ischemia in time to approach a steady state. Removal of the occlusion for a short period, \( \Delta t \), would then expose the previously ischemic region to a near "square wave" of tracer and permit flow to be calculated from

\[
(15) \quad k = \frac{1}{\Delta t} \ln \frac{C_a}{C_i} - \frac{C_a}{C_i}
\]

Note

Van Uitert and Levy (Stroke 9: 67-72, 1978) measured blood flow in gerbil brain by a method similar to that described here, using intravenously injected \(^{14}\)C-butanol as the tracer.

References

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