An autoperfusion technique for nonsteady-state vascular pressure-flow analysis in the rat

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Summary

A technique was developed to measure pressure-flow relationships in the autoperfused intact subclavian vascular bed of areflexic rats. Changes in blood flow and perfusion pressure were produced by occlusion of the aortic arch. This perfusion technique is suitable for evaluating vascular distensibility and responsiveness in the rat.

Vascular pressure-flow relationships of an organ or limb can be estimated by changing perfusion pressure and measuring corresponding changes in blood flow (Green et al., 1963). The expression of this pressure-flow relationship as a function curve might be used to evaluate pharmacological or physiological alterations in the vasculature (Stainsby, 1973). It has been argued that perfusion techniques which employ an extracorporeal circuit and pump could alter the blood in such a way that the vasculature itself may become affected (Folkow, 1952); and while plasma substitution avoids this difficulty, it is relatively nonphysiological (Hysell and Bohr, 1970). This report details a simple method for autoperfusion of the intact subclavian circulation in the rat to permit evaluation of vascular resistance and responsiveness by non steady-state pressure-flow analysis.

Methods

Procedure

Male Sprague-Dawley rats, with an average body weight of 0.192 kg and age of 47 d, were used in these studies. Under light ether anaesthesia tracheae were cannulated and the animals were artificially ventilated (Harvard rodent respirator). Rate and tidal volume were selected on the basis of body weight according to the Harvard Ventilation Graph. After transection of the spinal cord at the base of the skull, the right common carotid artery was ligated and cannulated with polyethylene tubing for pressure measurement (Statham pressure transducer, P23DC). The chest was entered through a left thoracotomy, the aortic arch was dissected free, and the left subclavian and left common carotid arteries were ligated and severed at their origins. Since under these conditions the rats displayed no signs of consciousness (e.g., twitching of the nasal vibrissae, or movements of the mouth, tongue, or eyes) and since there was no response to touch, pressure, or painful stimuli, ether administration was withdrawn and all surgical procedures were confined below the level of spinal transection.

A diagram of the experimental preparation is shown in Fig. 1. The innominate artery was dis-
spheres has been described (Nishiyama et al., 1973). Infusion of water into the occluder produced a gradual but complete occlusion of the arch with a consequent gradual shunting of cardiac output into the subclavian vascular bed via the innominate artery. In this manner pressure and flow could be increased, reflecting the pressure-flow relationship. In preliminary studies central venous pressure was monitored in either the superior or inferior venae cavae via catheters introduced into the jugular or femoral veins, respectively. Under resting conditions central venous pressure was between 0.1 and 0.9 kPa and did not change significantly during aortic arch occlusion until the peak subclavian pressure response was achieved and maintained; at this point central venous pressure increased by 0.5 or 0.7 kPa. Since central venous pressures were low and did not change during periods of increasing blood pressure and flow, they were not monitored in the majority of the experiments.

In order to ensure a proper phase relationship between pressure and flow recordings the frequency responses of the pressure and flow transducers were set at 0.5 Hz, so that arterial pulsations in flow or pressure could still be detected but were minimal. Pressure-flow values under these conditions were similar to higher-frequency pulsate pressure (60 Hz) and flow (3 Hz) recordings. When the aortic arch was occluded gradually and the rate of change of pressure was small (0.2 Hz, 2.66 to 4.66 kPa/s), it was possible to ensure an adequate phase relationship between pressure and flow recordings.

**RATE OF PRESSURE RISE AND SUBCLAVIAN BLOOD FLOW DISTRIBUTION**

In order to determine the rate of pressure rise on the pressure-flow relationship, the rate of rise was varied from 1.6 to 21 kPa/s in each of 5 rats. In 4 of these same rats, before terminating the experiment, 50 000 141Ce-labelled (100 000 cpm) 15 μm diameter microspheres were injected into the subclavian artery after complete ligation of the aortic arch. The details for quantitating the distribution of blood flow in the rat using radiolabelled microspheres has been described (Nishiyama et al., 1976).

Tissues above and below the point of ligation were sampled for radioactivity measurements.

**PRESSURE-FLOW RELATIONSHIPS**

After the surgical preparations were complete, 10 min were allowed for stabilisation. Studies were performed in 10 rats to evaluate the pressure-flow relationship in the subclavian vascular bed, and to determine the reproducibility and stability of the preparation during a 30 to 40 min period. The aortic arch was occluded in each rat 5 times within 60 s and with each occlusion lasting approximately 10 s. This manoeuvre was repeated 4 more times at 10 min intervals to test the viability of the preparation.

**RENAL, ADRENAL, AND SPINAL NEURONAL INFLUENCES**

In a group of 5 rats pressure-flow analysis was performed as described with the addition that the renal veins and arteries had been ligated. In another group of 5 rats pressure-flow analysis was performed after the spinal cord was pithed using a metal rod.

**DRUG EFFECTS**

The effects of drug infusions on pressure-flow relationships were evaluated in a group of 10 rats. Before each drug infusion a set of control pressure-flow responses were obtained during 0.9% saline infusion. Norepinephrine HCL (2.4 × 10⁻⁹ mol·kg⁻¹·min⁻¹), angiotensin II amide (8.5 × 10⁻¹⁰ mol·kg⁻¹·min⁻¹), histamine diphosphate (8.14 × 10⁻⁷ mol·kg⁻¹·min⁻¹), and nitroglycerine (2.86 × 10⁻⁷ mol·kg⁻¹·min⁻¹) were infused into the right jugular vein of each rat in random order at a volume flow rate of 0.0001 litre/min. These doses were selected on the ability of each drug to change mean arterial pressure by 2.7 to 5.3 kPa during intravenous infusion into pentobarbital anaesthetised (1.81 × 10⁻² mol·kg⁻¹·i.p.), and otherwise intact rats. Differences between mean values were analysed statistically by Student's t test or paired t test (Snecador, 1956). The international system of units was used for all values (Kappagoda and Linden, 1976).

**Results**

After postoperative homeostasis was assured as indicated by stabilisation of haemodynamic values, basal subclavian arterial blood flows and pressures were recorded. Resting flows and pressures averaged 0.0045 litre/min and 9.2 kPa, respectively, and remained at these levels throughout the experiment.

**RATE OF PRESSURE RISE AND SUBCLAVIAN BLOOD FLOW DISTRIBUTION**

The rate of pressure rise had no significant effect on
Fig. 2a. Pressure (MAP) and flow responses of the subclavian vascular bed to the first and fifth consecutive aortic occlusions. Shaded area indicates approximate reduction in blood flow, compared with the first occlusion response.

Fig. 2b. Regression lines calculated from individual data points for first (○) and fifth (x) occlusion flow responses. *The slope of the fifth occlusion regression line was significantly reduced compared with the first occlusion slope, \( P < 0.02 \).
the pressure flow curves. At 16.6 kPa blood flows \((\times 10^{-4})\) were 15.04 (1.63, SE), 15.9 (1.59), 16.9 (2.14), and 13.2 (1.4) litre/min at 1.3 to 2.0, 2.7 to 4.7, 6.7 to 9.3, and 13 to 21 kPa/s, respectively. The flow values are means of 3 to 5 observations. As an estimate of error within rats, the ratio of the standard error to the mean flow value obtained in each rat was calculated by averaging flows over the range of rates of pressure rise. Although the range of rates was large, the average error within each rat was 2.45% (0.22) at 13.3 kPa and 3.12% (0.57) at 16.6 kPa. However, the error among rats at a pressure rise of 2.7-4.7 kPa/s was 10% at both 13.7 and 16.6 kPa. The greatest source of error or variation was therefore among rats, and not in different rates of pressure rise within a rat.

Analysis of radioactive microspheres injected into the subclavian artery during complete aortic arch occlusion showed that 36% (1.7) of injected radioactivity was in the right forelimb and shoulder. The neck contained 8.6% (1.9) and the brain 0.81% (0.24). In areas below the level of occlusion there was 14% (1.3) in the right thorax and 6.45% (0.86) in the lungs. In the left thorax, kidneys, and right hindlimb there was very little radioactivity (0.24%, 0.12% and 0.16%, respectively).

**PRESSURE-FLOW RELATIONSHIPS**

With brief occlusion of the aortic arch, increasing subclavian arterial pressure was accompanied by rising subclavian blood flow (Fig. 2a). If several occlusions were made in succession, the relationship between pressure and flow was altered. By the fifth consecutive occlusion, pressure responses were normal, but flow was reduced, as indicated by the shaded area in Fig. 2a. Second, third, and fourth occlusion flow responses were intermediate between the first and fifth occlusions, and further reductions in flow beyond 5 occlusions did not occur. Calculated pressure-flow curves of the first and fifth occlusions (Fig. 2b) demonstrated that during the first occlusion the pressure-flow curve was closer to the flow-axis, whereas during the fifth occlusion the curve was closer to the pressure axis. Individual flow values were plotted for several 1.3 kPa (10 mmHg) increment changes in perfusion pressure in order to calculate linear regression equations for fifth occlusion pressure-flow relations (Fig. 2b). The slope of the fifth occlusion regression line was reduced 28% \((P < 0.02)\) compared with the slope of the first occlusion regression line. Recovery from this shift in the pressure-flow curve occurred within 5 min after releasing the occluder.

In order for this technique to be useful for measuring drug responsiveness, the pressure-flow relationship should be stable and reproducible over a sufficient time period. These pressure-flow relationships of the subclavian vascular bed remained stable and reproducible throughout the duration of the experiment as indicated by similar pressure flow curves obtained at the beginning and end of a 45 min test period (Fig. 3). Intermediate pressure-flow curves obtained at 10, 20, and 30 min intervals were also similar to the initial pressure-flow curve. Thus it was possible to obtain the same pressure-flow curves at the end of the experiment as at the beginning even though several occlusions were repeated at 10 min intervals.

**RENAL, ADRENAL, AND SPINAL NEURONAL INFLUENCES**

The change in the pressure-flow relation in response to phasic increases in blood flow was not significantly altered by ligation of the renal arteries or destruc-
tion of the spinal cord. In control animals (n=10) flow was reduced an average of 26\% (± 5 SE) during the fifth occlusion compared with the first, measured at 16.6 kPa perfusion pressure. In the pithed animals (n=5) this average was 22\% (± 7) and in animals with renal vessels ligated (n=7) 19\% (± 3). These latter 2 averages were not different from control.

Both procedures appeared to shift the pressure-flow curve towards the flow axis, but without significance in pithed rats. Blood flow at 16.6 kPa in intact rats was 18.8 (1.68) × 10^{-3} litre/min during the first occlusion; in pithed rats this value was 23.36 (2.25) × 10^{-3} litre/min and in rats with renal vessels ligated 28.4 (2.49), P < 0.01.

**Drug Effects**

Comparing control and drug responses, pressure-flow curves were plotted for saline and drug i.v. infusions (Fig. 4). No differences in control pressure flow curves were observed over the duration of the study. Nitroglycerin and histamine, both vasodilators, shifted the pressure-flow curve towards the flow axis. In contrast, norepinephrine and angiotensin, both vasoconstrictors, shifted the pressure-flow curve towards the pressure axis. During either angiotensin or nitroglycerin infusion, the shift in the pressure flow curve towards the pressure axis during the fifth occlusion was similar to that obtained in saline controls. With angiotensin flow was reduced 14\% (± 4 SE) at 16.6 kPa, which was similar to the control value of 20\% (± 7.4); and with nitroglycerin, flow was reduced 26\% (± 2), comparable with the control response of 21\% (± 4). However, norepinephrine and histamine prevented this shift, since first and fifth occlusion pressure-flow curves were not different from each other during infusion of either of these drugs. Flow reduction was only 5\% (± 5) with norepinephrine and 10\% (± 3) with histamine, compared with control values, 20\% (± 5) and 26\% (± 5) respectively, P < 0.01.

**Discussion**

The non steady-state pressure flow relationship of the subclavian vascular bed of the rat was measured in an in vivo preparation in which aortic flow was directed into the right subclavian artery by occlusion
of the aortic arch. Most of the flow supplied areas above the level of occlusion, but some flow was directed into the right chest wall and bronchial circulations. Two types of vascular pressure-flow relations could be detected in this preparation: one type reflected an initial passive vascular response to increased blood flow and pressure generated by the first occlusion of the aortic arch; the second was characterised by increased vascular resistance secondary to successive occlusions of the aortic arch. The fact that vascular resistance changes in response to changes in perfusion pressure or flow is well established (Stainsby, 1973), but the nature of these changes is not understood.

Our data indicate that the increased resistance following several increases in blood flow was a physiological vascular adaptation to increased flow and/or pressure since the response always occurred following successive increases in blood flow and perfusion pressure, was characterised by increased vascular resistance, was reversible and did not deteriorate with time. In addition, the vascular adaptation was selectively altered by vasoactive drugs (histamine and norepinephrine, but not by angiotensin or nitroglycerin). This response was also independent of the central nervous system, kidneys, and adrenal glands. Although the exact mechanism of this vascular response is unknown, it resembled transient skeletal muscle vascular autoregulation described by Pohost et al. (1976). However, since the area was not completely denervated, the possibility remains that local vascular neuronal reflexes may have also participated in vascular adaptation.

Autoperfusion of the subclavian vasculature as described has certain inherent disadvantages. For example, sustained or steady-state increases in flow cannot be produced without causing rapid cardiovascular deterioration. The method requires some form of baroreceptor denervation and may not be suitable for study of neuronal control of the vasculature. Finally, collateral circulation could occur in the arterial tree of the chest wall because during occlusion pressure is low in arteries arising from intercostal branches but high in arteries from subclavian branches. However, the results indicate that collateral circulation across these vascular beds is extremely small, since only trace amounts of radioactivity from microspheres were detected in the left thorax, kidneys, and hindlimb during complete aortic occlusion.

On the other hand, there are several advantages in this perfusion technique. Analysis can be performed on relatively young rats, and no extracorporeal circuit is required, which circumvents problems of blood alterations produced by the mechanical action of pumps (Folkow, 1952). There is no evidence of microemboli formation, as suggested by Stainsby (1973), which may be reflected in the excellent reproducibility of results and stability of this preparation. If the central nervous system is destroyed, vascular function can be observed in the absence of general anaesthesia. Furthermore, vascular resistance or reactivity can be examined at more than one perfusion pressure.

References