**Effect of Hindlimb Isolation Procedure on Isogravimetric Capillary Pressure and Transcapillary Fluid Dynamics in Dogs**

**ROBERT A. BRACE, PH.D., AND ARTHUR C. GUYTON, M.D.**

**SUMMARY** We measured isogravimetric capillary pressure ($P_{ci}$) and plasma colloid osmotic pressure ($\pi_p$) in isolated dog hindlimbs. A very rapid isolation technique and a perfusion technique involving no weight change were developed in order to compare the effects of different isolation procedures. Also, a “previous isolation procedure” (PIP) was used to approximate isolation procedures previously reported; this procedure included (A) anesthesia for 1 to 1½ hours before limb isolation, (B) ½ hour of denervation before isolation, and (C) perfusion after isolation for ¼ hour at an arterial pressure of 100 mm Hg and a venous pressure of 6 mm Hg. These different procedures altered average (± SE) capillary pressure and fluid dynamics in the hindlimb as shown in the following table:

<table>
<thead>
<tr>
<th>Isolation procedure</th>
<th>$P_{ci}$ (mm Hg)</th>
<th>$\pi_p - P_{ci}$ (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIP</td>
<td>16.6 ± 1.1</td>
<td>1.4 ± 1.1</td>
</tr>
<tr>
<td>PIP without C</td>
<td>15.2 ± 1.3</td>
<td>2.5 ± 1.4</td>
</tr>
<tr>
<td>PIP without B and C</td>
<td>13.1 ± 0.4</td>
<td>4.4 ± 0.8</td>
</tr>
<tr>
<td>PIP without A, B, and C (immediate isolation with no overperfusion)</td>
<td>8.8 ± 0.6</td>
<td>8.2 ± 0.6</td>
</tr>
</tbody>
</table>

These data indicate that average isogravimetric capillary pressure in the intact resting dog hindlimb may be 8 mm Hg below plasma colloid osmotic pressure, or about 9 mm Hg, and that many reported values have been heavily influenced by the isolation technique.

Sodium heparin (10,000 units) then was administered and allowed to circulate for 2-3 minutes. The femoral artery was ligated and cannulated immediately after commencement of bleeding from the carotid artery. This was followed immediately by ligation and cannulation of the femoral vein. An adjustable clamp then was tightened around the upper leg as close to the hip joint as possible. The leg was rapidly severed proximal to the clamp at the hip joint, and perfusion was initiated. Bleeding usually required 1-4 minutes. The time interval between initiation of bleeding and totally clamping the leg was about 5 minutes, and leg removal with the scalp required only 1 minute. Thus, the total preparation time required was between 10 and 20 minutes, less than one-quarter of the time required in any other reports of which we are aware.

The perfusion apparatus has been described previously. In brief, the isolated perfused leg was suspended in a weighing pan. The venous blood leaving the leg entered a venous reservoir and then was pumped into an arterial reservoir in which a constant pressure head was maintained. The arterial and venous pressures were controlled by adjusting the heights of the two reservoirs. The zero control pressure was considered to be at the midlevel of the leg. The blood was oxygenated by bubbling a mixture of 95% oxygen and 5% carbon dioxide through the venous reservoir. The blood was warmed to 37°C prior to its entering the leg.

Another important feature of the procedure was that special care was taken to prevent fluid shifts either into or out of the interstitial spaces when the isolated leg was first perfused. This was accomplished by adjusting the arterial pressure to precisely the level at which the leg neither gained nor lost weight. As was to be expected, because of the denervated and vasodilated state of the tissues, the arterial pressure level that maintained an isogravimetric condition

---

From the Department of Physiology and Biophysics, University of Mississippi Medical Center, Jackson, Mississippi.

Supported by Grants HL 11678 and GM 00316 from the National Institutes of Health.

Received August 15, 1975; accepted for publication December 3, 1975.
was much lower than the normal arterial pressure of the animal; this will be discussed in more detail in the section on Results.

Arterial pressure, venous pressure, blood flow rate, and change in leg weight were recorded continuously. To measure plasma colloid osmotic pressures we used an electronic colloid osmometer described by Prather et al. 

Isogravimetric pressures were determined by the standard method of Pappenheimer and Soto-Rivera: arterial perfusion pressure was reduced and venous pressure increased to provide four to five isogravimetric states; we then determined $P_c$ by linearly extrapolating to zero flow the curve relating isogravimetric venous pressure to blood flow rate. Each isogravimetric state was maintained for 2-4 minutes.

**Statistical**

To analyze the data, it was necessary to find the best linear fit for the data. Standard least squares regression analysis identifies the slope ($A$) and intercept ($B$) of the expression $Y = AX + B$ by minimizing the sum of the square of the errors ($E$):

$$E = \sum_{i=1}^{n} (Y_i - AX_i - B)^2.$$

Use of this method assumes that the independent variable, $X_i$, is accurately known and that the only source of error is in the measurement of the $Y_i$ variable. The results are such that the values of the slope and intercept are dependent upon the choice of which variable is selected as the independent variable. In fact, analysis of each variable as the independent variable yields two lines that differ in slope by the square of the correlation coefficient. In order to obtain a best linear fit that was not dependent upon which parameter had been selected as the $X$ variable, we defined the total error of fit as

$$E = \sum_{i=1}^{n} (Y_i - AX_i - B)^2 + \sum_{i=1}^{n} (X_i - \bar{X})^2.$$ 

The best slope and intercept then were found by minimizing this error function. This expression provides a better fit of data, when unknown errors are present in both of the measured variables. In comparison, the slope ($A$) found through standard linear regression analysis is approximately equal to the correlation coefficient times the slope found with the above method.

Data were analyzed for significance through use of Student's $t$-test.

**Results**

**"Previous isolation procedure"**

To simulate the isolation procedures previously used, a standardized "previous isolation procedure" (PIP) was used for the first series of dogs. We anesthetized the dogs 1 hour to 1½ hours before very rapidly isolating the leg. One-half hour before isolation the limb was denervated by severing the sciatic and femoral nerves close to the hip. Once isolated, the limb was perfused for ½ hour at arterial and venous pressures of 100 mm Hg and 6 mm Hg, respectively. For five hindlimbs, weight increased by 1-3% after blood flow had been reestablished. $P_c$ averaged at the end of the ½-hour period of perfusion averaged ($\pm$se) 16.6 ± 0.8 mm Hg while $\pi_v$ averaged 18.0 ± 1.0 mm Hg. The average value for $\pi_v - P_c$ was 1.4 ± 1.1 mm Hg. These values are all in close agreement with those most frequently reported for other studies. 

**Effect of perfusing the isolated limb at normal arterial and venous pressure**

Perfusion of the isolated limb at norma arterial and venous pressures (100 mm Hg and 6 mm Hg, respectively) caused leg weight (and thus interstitial fluid volume) to increase. In an attempt to determine whether this increase in volume altered fluid dynamics in the hindlimb, we isolated six limbs by the standard previous procedures described above, but instead of perfusing these limbs at normal arterial and venous pressures, we repeatedly adjusted perfusion pressures so that no change in weight occurred after blood flow had been established (discussed below). In these limbs $P_c$ averaged 15.2 ± 1.3 mm Hg; $\pi_v$ was 17.6 ± 0.5 mm Hg; and $\pi_v - P_c$ was 2.5 ± 1.4 mm Hg. The average value for $\pi_v - P_c$ in these limbs was slightly but not significantly lower than $\pi_v - P_c$ measured in the limbs that were perfused at normal arterial and venous pressures for ½ hour.

**Effect of denervation on hindlimb fluid dynamics**

In the studies described thus far the hindlimbs were denervated during the isolation procedure while the legs were still autoperfused. The effects of the denervation were determined by isolating hindlimbs 1½ hours after anesthesia but with no prior denervation. Once the limb had been isolated, perfusion pressures were adjusted so that there was no slow change in weight. $P_c$ averaged 13.1 ± 0.4 mm Hg ($n = 8$) and $\pi_v$ averaged 17.4 ± 0.6 mm Hg. The difference between $\pi_v$ and $P_c$ (4.4 ± 0.8 mm Hg) was significantly greater ($P = 0.05$) than the values found when the standardized previous isolation procedures were used.

**Effect of delay in isolation on isogravimetric capillary pressure**

Many previous techniques have required surgical procedures lasting for 1-2 hours to remove the hindlimb. By use of the clamping technique in the present experiments, only 10-20 minutes were required. In 11 limbs isolated immediately after anesthesia and not overperfused after they had been isolated, $P_c$ averaged 8.8 ± 0.6 mm Hg while $\pi_v$ was 17.0 ± 0.9 mm Hg. The difference between $\pi_v$ and $P_c$ averaged 8.2 ± 0.6 mm Hg and was significantly greater than the value of $\pi_v - P_c$ in limbs isolated 1½ hours after anesthesia ($P < 0.01$) as well as in the limbs isolated by the standardized previous isolation procedures ($P < 0.01$).

**Relationship between isogravimetric capillary pressure and the plasma colloid osmotic pressure**

It was found that in the control limbs isolated immediately after anesthetization, $P_c$ varied approximately in proportion to the variation in $\pi_v$. $P_c$ ranged between 6.5 mm Hg and 13.8 mm Hg and $\pi_v$ between 13.1 mm Hg and
21.8 mm Hg. Figure 1 is a plot of the respective isogravimetric pressures and colloid osmotic pressures in the separate dogs, and a regression analysis fit of this data is given by $P_{cl} = 0.77\pi_p - 4.25$. The correlation coefficient ($r$) is 0.705. A linear relationship ($r = 0.540$) also was found for the eight linear relationship

LEVEL OF ARTERIAL PERFUSION PRESSURE REQUIRED TO PREVENT FILTRATION DURING INITIAL PERFUSION

When the isolated leg was first perfused, its weight was continuously measured from the very beginning of the experiment. In all experiments except those using the standardized previous isolation procedures, the venous pressure was set at a low value, usually about 1 mm Hg, and the arterial pressure repeatedly was adjusted so that the isolated leg underwent no slow gain or loss of weight once blood flow had been established. In the 11 control experiments in this study, it was found that an initial arterial perfusion pressure averaging only 55 mm Hg was required to prevent slow changes in weight. However, during the ensuing 5-25 minutes the resistance to blood flow increased progressively, the flow decreased, and the arterial pressure required to maintain the isogravimetric state increased. After about 30 minutes, the preparations stabilized and no further change in arterial pressure was necessary to maintain the isogravimetric state. This pressure then was considered to be the control arterial pressure.

Figure 2 illustrates a typical time course for the changes in arterial pressure required to maintain the isogravimetric state. Note the initial value of only 50 mm Hg required for the isogravimetric state and a final value of 81 mm Hg. Also, note the progressive decrease in blood flow during the early period of perfusion.

In the 11 control experiments of this study, the control arterial pressure (after stabilization) averaged (±SE) 77 ± 8 mm Hg. The venous pressure averaged 1.2 ± 0.2 mm Hg, and the blood flow, 57 ± 5 ml/min. The leg weight distal to the clamp was 1,170 ± 70 g.

**Test for Hindlimb Dehydration**

It must be considered whether the 11 control hindlimbs in the present experiments were dehydrated. The limbs may have been underperfused and thus fluid reabsorption may have occurred only during the brief bleeding period. Any dehydration that might have occurred during bleeding probably would be offset by the overperfusion during the first 5 minutes of perfusion which was used to reestablish blood flow. As a direct test of whether or not the hindlimbs in the control study were dehydrated, interstitial fluid pressure ($P_{it}$) was measured continuously by use of the capsule technique in six hindlimbs before, during, and after the rapid isolation procedure. In six hindlimbs, $P_{it}$ averaged −5.5 ± 1.1 mm Hg shortly after anesthesia. Isolation procedures were started approximately 1 hour after anesthesia. At this time $P_{it}$ averaged −3.3 ± 0.7 mm Hg. After rapid isolation the limbs were perfused so that no slow changes in weight occurred. After stabilization, $P_{it}$ averaged −1.9 ± 0.4 mm Hg. During isogravimetric determinations, $P_{it}$ increased slightly and averaged −1.0 ± 0.4 mm Hg upon completion of the determinations. $P_{it}$ averaged 13.5 ± 0.4 mm Hg, and $\pi_p$ was 18.1 ± 0.5 mm Hg. The value of colloid osmotic pressure of the interstitial fluid ($\pi_{it}$), calculated from these three Starling forces, averaged 3.7 ± 0.3 mm Hg. Thus it was found that $P_{it}$ increased by an average of 1.4 ± 0.6 mm Hg during the techniques of rapid isolation and of perfusion with no weight gain, and there was a further increase of 0.9 mm Hg during the isogravimetric determinations. Since all previous studies of capsule pressures have shown that dehydration decreases $P_{it}$, we have concluded that the limbs were not dehydrated.

**Effect of Venous Resistance ($R_v$) on Isogravimetric Capillary Pressure**

Isogravimetric capillary pressures were determined for venous pressures ($P_v$) and blood flow rates ($F$) by linearly extrapolating to zero flow the equation $P_{cl} = P_v + R_v F$. The lowest flows used in this study were approximately 20% of those associated with the lowest venous pressures. $R_v$ was found to be constant over this range. As seen in the above equation, $P_{cl}$ was dependent on $R_v$. Thus the different values of $P_{cl}$ found in this study might have been due to different
Table 1  Venous Resistance of the Hindlimb with Various Isolation Procedures

<table>
<thead>
<tr>
<th>Isolation procedure*</th>
<th>Rv (mm Hg/ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIP</td>
<td>0.21 ± 0.01</td>
</tr>
<tr>
<td>PIP without C</td>
<td>0.24 ± 0.03</td>
</tr>
<tr>
<td>PIP without B and C</td>
<td>0.22 ± 0.02</td>
</tr>
<tr>
<td>PIP without A, B, C</td>
<td>0.21 ± 0.02</td>
</tr>
</tbody>
</table>

* Refer to Summary for explanation of the isolation procedures. Results are expressed as mean ± SE.

venous resistances. However, this was not the case, because, as shown in Table 1, Rv was the same in all studies.

Discussion

This study shows that the standard hindlimb isolation procedures used in many previous studies probably have had great effects on both the Pcl and the net absorptive force in the capillaries. Most previous investigators have found Pcl to average 15 to 17 mm Hg.1-4 while Pfr - Pcl averaged only 1-3 mm Hg.5-6 Similar values were found in our study when the leg was subjected to (1) a long delay after anesthesia, (2) denervation while autoperfused, and (3) perfusion at normal pressures once isolated. If the goal of a study is to relate the value of Pm, measured in the isolated preparation to that in the intact animal, every attempt must be made to prevent the other three Starling forces, besides Pcl, from changing because Pm is totally determined by the other three Starling forces. Since Pm is very sensitive to changes in interstitial fluid volume when Pcl is in its normal range,13 it is critical that transcapillary fluid shifts be prevented.

Our present findings actually are predictable from the study by Haddy et al.14 and that of the compliance curve of the hindleg.15 Haddy et al.14 found that the autoperfused, innervated forelimb gained weight after isolation. Subsequent denervation caused an increase in the rate of limb weight gain and the total weight gain was approximately 7% after isolation and 1/2 hour of denervation. An increase in weight of this amount has been shown previously to produce an elevated Pcl. Since this change is matically, the slope is equal to irp; when 7rp is extrapolated to zero, Pcl = Pfr - 7T|f. Since the intercept represents interstitial fluid pressure, Pfr must change in order to have isogravimetric conditions. Mathematically, the slope is equal to σ(1 - irf/irp), where σ is the reflection coefficient for the plasma proteins. For the present experiments, this implies, if σ = 1, that Pfr is equal to 23% of irp, which translates to an interstitium to plasma protein concentration ratio of approximately one-third.

In the present study, the circulating blood was oxygenated by bubbling with 95% oxygen and 5% carbon dioxide. This procedure often is avoided because of protein denaturation which would decrease irp. We have found that the maximum decrease in irp was 1.0 ± 0.3 mm Hg. Since this change is counterbalanced by the measured change in interstitial fluid pressure, there is little overall effect on Pcl. Thus, the present studies suggest that Pcl in the intact, resting dog may be only about 9 mm Hg while the net absorptive force in the capillaries may be 9-10 mm Hg. True capillary pressure would be somewhat higher than Pcl since normally there is a slight net filtration of fluid which is carried away by the lymphatic system.

REFERENCES

The Role of Angiotensin in the Canine Renal Vascular Response to Barbiturate Anesthesia

BRUNO M. BURGER, M.D., TIMOTHY HOPKINS, M.D., ALLISTAIR TULLOCH, M.D., AND NORMAN K. HOLLENBERG, M.D., PH.D.

SUMMARY The influence of barbiturate anesthesia on renal blood flow was assessed by the xenon washout method in trained dogs with catheters chronically implanted in the renal artery. Anesthesia induced with either thiopental sodium or pentobarbital sodium resulted in a striking reduction in renal blood flow (4.1 ± 0.1 vs. 2.6 ± 0.2 ml/g per min; P < 0.001) without a change in arterial pressure. The reduction in blood flow was prevented by a high salt intake and partially reversed by agents which interrupt the renin-angiotensin system (BPF 9; 1-Sar,8-Ala-angiotensin II; pranabol) but not by α-adrenergic blocking agents (phentolamine and phenoxybenzamine). Anesthesia blunted the renal vascular response to angiotensin II (P < 0.0005) whereas responsiveness to norepinephrine was increased (P < 0.05). We conclude that barbiturate anesthesia induces a major, angiotensin-mediated renal vascular response which must be considered in the interpretation of experiments performed under these conditions.

A MAJOR cardiovascular response including reduced renal blood flow is often induced by anesthesia in man and in experimental animals. Although the mechanisms for these are poorly defined, anesthesia-induced activation of the renin-angiotensin system is a possible cause, as the renal vasculature is especially sensitive to angiotensin II. However, there is considerable discrepancy between reports on the sensitivity of the renovascular bed to angiotensin. In man, for example, studies performed without anesthesia demonstrated a threshold response to angiotensin infused into the renal artery at doses generally below 10 ng/min, and the renal vessels were 100-fold more sensitive to angiotensin than to norepinephrine. In anesthetized animals, on the other hand, a number of studies have revealed a requirement for much higher doses of angiotensin, and it has been suggested that the renal vessels are more sensitive to norepinephrine than to angiotensin. These discrepant results may be due to the fact that anesthesia can blunt renal vascular responses to angiotensin II and perhaps reflect the frequent association of activation of the renin-angiotensin system and a reduction in vascular responsiveness to angiotensin II. It therefore seemed important to assess the role of renin-angiotensin system activation in the renal vascular response to anesthesia. We have performed such a study in dogs with catheters chronically implanted in the renal artery. Using this model, we assessed the effects of barbiturate anesthesia and of sodium intake on renal perfusion and responsiveness to vasoconstrictor agents and appropriate blocking drugs.

Methods

SURGICAL PREPARATION

Studies were performed on 25 healthy, mongrel dogs, weighing 21–35 kg and selected for gentleness. In experiments in which surgery was performed, or when the effects of anesthesia per se were to be studied, anesthesia was induced with an initial intravenous dose of sodium pentobarbital (30 mg/kg) and maintained with periodic additional doses of 3–5 mg/kg of the same agent, to prevent spontaneous movement. At the time of preparative surgery a period of at least 60 minutes was allowed after the completion of procedures before vascular studies were performed; a 30-minute period was allowed between the last supplemental dose of anesthesia and the hemodynamic study. On days during which surgery was not performed, the hemodynamic studies were performed 30 minutes after induction of anesthesia.

Several days prior to the surgical procedure an aortogram was obtained to define the renal vascular anatomy. On the day of preparative surgery the renal pedicle was approached through a flank incision by retroperitoneal dissection. The artery was exposed and its lateral margin cleaned suffi-
Effect of hindlimb isolation procedure on isogravimetric capillary pressure and transcapillary fluid dynamics in dogs.
R A Brace and A C Guyton

doi: 10.1161/01.RES.38.3.192

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1976 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/38/3/192

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation Research_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation Research_ is online at:
http://circres.ahajournals.org/subscriptions/