Hemorrhage-Induced Cerebral Vasoconstriction in Dogs

WILLIAM J. PEARCE, PH.D. AND LOUIS G. D'ALECY, D.M.D., PH.D.

SUMMARY Cerebrovascular responses to a 20% volume hemorrhage were studied in chloralose-anesthetized dogs with the Doppler cerebral venous outflow method. Arterial PCO₂, PO₂, and pH were held constant by servocontrol of ventilation. The experimental results were divided into 2 groups as determined by the spontaneous responses of mean arterial pressure (MAP) to hemorrhage. In Group 1 (n = 11), steady state MAP decreased 25%, cerebral blood flow (CBF) decreased 15%, and cerebrovascular resistance (CVR) decreased 13% (autoregulatory vasodilatation). In group 2 (n = 23), MAP changed less than 10 mm Hg, CBF decreased 13%, and CVR increased 15%. The hemorrhage-induced cerebral vasocostriction in Group 2 was characterized by the following: phenoxybenzamine (2 mg/kg i.v., n = 3) reduced post-hemorrhage CVR from 116% to 95% of prehemorrhage CVR (cCVR); phenolamine (2 mg/kg i.v., n = 5) reduced post-hemorrhage CVR from 114% to 91% of cCVR; and verified local anesthetization of both superior cervical ganglia (n = 5) reduced post-hemorrhage CVR from 116% to 94% of cCVR. Thus in Group 2, sympathetic vasocostriction contributed approximately 5% of cCVR; following normotensive hemorrhage, it accounted for up to 20% of post-hemorrhage CVR. In combination with previous studies, these data suggest that cerebrovascular responses to hemorrhage balance between autoregulatory vasodilatation and sympathetic vasocostriction.

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DESPITE the widely accepted existence of a sympathetic cerebrovascular innervation, the functional significance of this innervation remains unclear. Methodological controversy complicates interpretation of the results of electrical stimulation of this innervation. Reflex stimulation, however, as produced during studies of cerebral blood flow (CBF) autoregulation, has suggested a physiological role for these nerves. When arterial hypotension was produced by graded hemorrhage, the lower limit of CBF autoregulation was approximately 70 mm Hg. When arterial hypotension was produced by pharmacological ganglionic blockade, however, the lower limit of CBF autoregulation was approximately 40 mm Hg. In other words, the maximal cerebral vasodilatation observed during hypotension resulting from ganglionic blockade was greater than that observed during hemorrhagic hypotension. Since sympathetic outflow is interrupted by ganglionic blockade and is augmented by hemorrhage, these results may be interpreted to suggest reflex sympathetic cerebral vasocostriction during hemorrhage. Consistent with this interpretation are the findings that pretreatment with alpha-receptor blockade (ARB) or acute cervical sympathectomy (CSx) extends the lower limit of CBF autoregulation.

A common problem complicating demonstrations of reflex sympathetic cerebral vasocostriction has been the simultaneous vasocostriction or vasodilatation attributable to metabolic or autoregulatory mechanisms. For example, the sympathetic cerebral vasocostriction suggested to occur during hemorrhagic hypotension has been concealed in an overall cerebral vasodilatation (in response to lowered cerebral perfusion pressure). Thus, the best way to confirm reflex sympathetic cerebral vasocostriction would be to demonstrate this vasocostriction in the absence of "intrinsic" autoregulatory or metabolic responses. Such an approach would require a stimulus which would produce no steady state changes in arterial pressure or blood gases. The stimulus of normotensive hemorrhage fulfills these criteria, particularly since compensatory sympathetic vasocostriction is often heavily recruited prior to any hypotensive response.

Methods

Preparation

In all experiments, male dogs weighing between 20 and 30 kg were anesthetized with 120 mg/kg (60 mg/ml) intravenous alpha-chloralose in aqueous solution. Depth of anesthesia was maintained by continuous intravenous infusion (6.5 ml/kg/h) of an isotonic solution (pH: 7.44) containing 6.18 mg/ml sodium chloride and 30 kg were anesthetized with 120 mg/kg (60 mg/ml) intravenous alpha-chloralose in aqueous solution. Depth of anesthesia was maintained by continuous intravenous infusion (6.5 ml/kg/h) of an isotonic solution (pH: 7.44) containing 6.18 mg/ml sodium chloride and 40 millimolar sodium bicarbonate and 97.4 millimolar sodium chloride. Small adjustments of the infusion rate were made for each animal to maintain a standard depth of anesthesia, such that orthostatic cardiovascualr reflexes were preserved, but corneal and pedal withdrawal reflexes were completely depressed. Orthostatic reflexes were assumed intact when an increase in heart rate was produced by lifting a rear limb from a horizontal to a vertical position.

Each animal was mechanically ventilated without paralysis (Harvard, Model 607). Tidal volume was adjusted to yield a calibrated end-tidal percent carbon dioxide (ETCO₂, Beckman LB-2) of 4.5% at a rate of 15 breaths per minutes. During periods of data collection, respiratory rate was adjusted to minimize changes in ETCO₂. Esophageal temperature, measured at the level of the heart, was maintained at 39°C by a proportionally controlled heating pad. Pulsatile arterial pressure, mean arterial pressure, and...
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heart rate were obtained from a calibrated pressure transducer (Statham 23BB) connected to a catheter threaded through the left femoral artery into the descending aorta. Arterial PCO₂, PO₂, and pH were determined on anaerobically drawn samples with an Instrumentation Laboratories blood gas analyzer (Model 113/127). Cuvette reading times were standardized and all electrodes were calibrated prior to and immediately following each blood gas determination. Maximum corrections for calibration drift were .005 pH units for the pH electrode and 1.0 mm Hg for the oxygen and carbon dioxide electrodes. For each estimate of venous hematocrit, 4 heparinized microhematocrit tubes (Clay Adams, Yankee 1020) were filled from a single sample of femoral venous blood. The resulting 4 hematocrit values were averaged together.

Measurement of Cerebral Blood Flow

Cerebral blood flow was measured continuously with the Doppler cerebral venous outflow (DCVO) method. For this measurement, the entire venous effluent from the anterior cranial fossa was diverted through the left temporal sinus by occluding the occipital emissary veins, both sigmoid sinuses and the right temporal sinus. Cerebral venous outflow from the anterior cranial fossa was then measured by a Doppler flow transducer secured in a fixed position over the left temporal sinus. Upon conclusion of each experiment, the left retroglenoid vein was dissected and cannulated so that the retroglenoid catheter exclusively drained the left temporal sinus. The cerebral venous effluent from this catheter was then collected in graduated cylinders during timed intervals over a range of flows which encompassed the maximum and minimum observed in each preparation. A linear regression of millivolt output as a function of flow was calculated for each preparation. The mean r² for 34 experiments was .9878 ± .0051 SEM.

Following calibration, each animal was sacrificed and the source of the measured outflow was defined by retrograde perfusion of fast cure acrylic through the retroglenoid catheter. The presence of hardened acrylic within the cavernous sinus (20 preparations) was assumed to invalidate the preparation. In the 34 validated preparations, the contents of the anterior cranial fossa were weighed in order to express cerebral blood flow values in ml/min/100 g.

Recently, the degree of separation between the cerebral and non-cerebral venous systems in validated DCVO preparations has been assessed. In that study, the amount of blood sodium extracted by the contents of the anterior cranial fossa was measured and compared to the amount extracted by non-cerebral tissues. Cerebral venous blood samples were taken simultaneously from the dorsal sagittal sinus and the left temporal sinus. The calculated cerebral sodium extractions were identical (4.9 percent) at both cerebral venous sampling sites. Cephalic muscle and skin tissues, however, extracted up to 60 percent of delivered sodium. Because single pass cerebral sodium extraction has been reported to be 1.4%, these data demonstrate that non-cerebral tissues contribute less than 9% of the measured outflow in the verified DCVO preparation.

Protocol

The protocol followed in our experiments is shown in figure 1. After a 13-minute pre-hemorrhage period we opened a valve connecting the right femoral arterial catheter to a volume calibrated bottle. During the following 3 minutes, 1.6% of each animal's body weight (estimated 20% of blood volume) was withdrawn. After passive blood withdrawal was completed, 10 minutes (pre-blockade period) were allowed for cardiovascular compensation. At the end of this period, experiments were categorized according to whether mean arterial pressure (MAP) had returned (normotensive experiments) or had not returned (hypotensive experiments) to within 10 mm Hg of its average prehemorrhage value. Hypotensive experiments were discontinued after the pre-blockade period. In normotensive experiments either alpha-blockade or acute cervical ganglionic blockade was carried out during the subsequent 3-minute blockade period (fig. 1).

To produce ganglionic blockade, 4 ml of a commer-
cially prepared carbocaine solution (Mepivacaine, Cook-Waite, 3% solution) was injected into each superior cervical ganglion. Pupillary constriction was assumed to indicate ganglionic blockade. To enable postmortem verification of the site of carbocaine injection, 1 mg of Evans Blue dye was added to each syringe prior to injection. When dye was found within the ganglionic fascial sheaths, local ganglionic anesthetization was assumed. Carbocaine control experiments were those in which carbocaine was injected but pupillary constriction was absent and no dye was found in the ganglionic fascial sheaths.

To produce alpha-blockade, either phentolamine (Regitine, Ciba, 2 mg/ml in 0.9% NaCl, 2 mg/kg) or phenoxybenzamine (Smith, Kline & French, 2 mg/ml in 35 volumes-percent polyethylene glycol in distilled water, 2 mg/kg) was administered i.v. In control experiments, 1 ml/kg of the corresponding solvent vehicle was infused.

The effects of carbocaine injection and phentolamine (or saline) infusion were evaluated during the 10 minutes (post-blockade period) subsequent to the blockade period (fig. 1). Due to the longer time required for the onset of blockade by phenoxybenzamine, the post-blockade period in phenoxybenzamine and phenoxybenzamine control experiments was of 30 minutes’ duration. Of those 30 minutes, the final 10 were used to evaluate the effects of infusion of phenoxybenzamine or its solvent vehicle.

When all protocol measurements were completed, the hemorrhaged blood was reinfused and 24 minutes were allowed to re-establish cardiovascular equilibrium. Blood volume was then measured with the radio-iodinated serum albumin (indicator dilution) method (Mallinckrodt IHSA I-125) to determine the exact magnitude of each hemorrhage.

Statistics

For each variable, the response produced by blood withdrawal was defined by the means of the paired differences between the individual means of the pre-hemorrhage and pre-blockade periods. The effects of each method of blockade were defined by the means of the paired differences between the individual means of the pre-blockade and post-blockade periods. Within a given period, differences between groups were evaluated by a one-tailed Student’s t-test. P values less than .05 were considered significant for all tests.

Results

Patterns of Cardiovascular Response to Hemorrhage

Experiments were categorized into two groups (fig. 2). In 23 (68%) of the experiments (the normotensive group), blood withdrawal produced changes in steady state MAP between 0 and 10 mm Hg (average change less than 1.0 mm Hg). In the remaining 11 experiments (the hypertensive group), hemorrhage produced decreases in steady state MAP between 16 and 48 mm Hg (average decrease of 26 mm Hg). The average magnitudes of hemorrhage in the 2 groups were 20.5 ± .9 and 19.5 ± 1.5 percent of measured blood volume, respectively; these values were not significantly different. The pre-hemorrhage value of MAP was significantly greater in the normotensive group (123.2 ± 2.9) than in the hypertensive group (104.5 ± 6.4).

The pre-hemorrhage values of cerebral blood flow were not significantly different between groups and in both groups blood withdrawal produced a significant decrease in CBF of 5.9 ml/min/100 g (table 1). In the normotensive group the CBF decrease was associated with a significant increase in cerebrovascular resistance (CVR, calculated as the ratio of MAP/CBF) of .45 resistance units (mm Hg/ml/min/100 g). Conversely, the CBF decrease observed in the hypertensive group was associated with a significant decrease in CVR of .35 units.

Both group’s responses to blood withdrawal included a significant increase in heart rate. The heart rate increase observed in the normotensive group (59
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<table>
<thead>
<tr>
<th></th>
<th>Normotensive (n = 23)</th>
<th>Hypotensive (n = 11)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>123.2 ± 2.9</td>
<td>123.1 ± 3.4</td>
</tr>
<tr>
<td>CBF (mm/min/100g)</td>
<td>44.1 ± 2.1</td>
<td>38.2 ± 1.8</td>
</tr>
<tr>
<td>CVR (mm Hg/ml/min/100g)</td>
<td>2.92 ± .15</td>
<td>3.37 ± .17</td>
</tr>
<tr>
<td>Heart Rate (beats/min)</td>
<td>106 ± 5</td>
<td>165 ± 6</td>
</tr>
<tr>
<td>PCO₂ (mm Hg)</td>
<td>38.6 ± .5</td>
<td>38.7 ± .4</td>
</tr>
<tr>
<td>PO₂ (mm Hg)</td>
<td>88.6 ± 1.5</td>
<td>85.3 ± 1.4</td>
</tr>
<tr>
<td>pH (units)</td>
<td>7.399 ± .007</td>
<td>7.388 ± .008</td>
</tr>
</tbody>
</table>

All variables are abbreviated as in the text. All values are means ± standard error. Beneath the "paired sig" heading, the probabilities of paired differences are indicated. One asterisk represents p < .05; 2 asterisks represent p < .005; 3 asterisks represent p < .0005. NS indicates not significant.

beats per minute) was significantly greater than that observed in the hypotensive group (21 beats per minute).
The normotensive group had a significant increase in venous hematocrit of 1.3 percent in response to hemorrhage. The corresponding hematocrit increase in the hypotensive group averaged .3 percent which was not significant by paired t analysis.

Due to manual regulation of ETCO₂, blood withdrawal produced no change in arterial PCO₂ in either group (table 1). In the normotensive group, blood withdrawal produced small but significant decreases in arterial PO₂ (3.3 mm Hg) and arterial pH (0.11 pH units). In the hypotensive group, blood withdrawal produced no significant changes in arterial PO₂ or pH.

In the normotensive group, average body temperature was 38.9 ± .2°C, blood volume averaged 7.81 ± .32 percent of body weight, and pre-hemorrhage venous hematocrit averaged 42.5 ± .5 percent. In the hypotensive group, average body temperature was 38.7 ± .3°C, blood volume averaged 8.82 ± .55 percent of body weight, and pre-hemorrhage hematocrit averaged 41.2 ± .7 percent. No significant differences between groups were observed for these variables.

In summary, our standardized hemorrhage stimulus produced 2 patterns of response. In the normotensive pattern, MAP decreased an average of 26 mm Hg, CBF decreased an average of 5.9 ml/min/100 g, and CVR decreased by .35 units. In the normotensive pattern, MAP did not change significantly, CBF decreased an average of 5.9 ml/min/100 g, and CVR increased .45 units.

**Normotensive Hemorrhage: Effects of Blockade**

Alpha-blockade by phentolamine produced a significant 21% decrease in post-hemorrhage CVR of .60 units, significantly decreased MAP by 42.3 mm Hg, and produced a significant 17% decrease in CBF of 7.2 ml/min/100 g (table 3). Following phentolamine, arterial PCO₂ increased significantly by 2.8 mm Hg, arterial PO₂ decreased significantly by 3.8 mm Hg, and arterial pH decreased significantly by .260 pH units (table 2). Heart rate increase was insignificant. In phentolamine control experiments, vehicle infusion produced no significant changes in any variable.

Alpha-blockade by phenoxybenzamine produced a significant (18%) decrease in post-hemorrhage CVR of .53 units, coincident with a significant decrease in MAP of 25 mm Hg and no significant change in CBF (table 3). Following phenoxybenzamine, heart rate, arterial PCO₂, and arterial PO₂ did not change significantly. Arterial pH demonstrated an insignificant tendency to decrease. In phenoxybenzamine control experiments, vehicle infusion produced no significant changes in any variable.

Local anesthetization of the superior cervical ganglia produced a significant 19% decrease in post-hemorrhage CVR but did not significantly change MAP. After ganglionic anesthetization, CBF showed an insignificant tendency to increase; heart rate, arterial PCO₂, and arterial PO₂ were unchanged; and arterial pH showed an insignificant tendency to decrease (tables 2 and 3). In carbocaine control experiments, injection of carbocaine adjacent to the ganglia had no significant effect.

**Discussion**

In the majority of hemorrhage studies, arterial pressure response variation has been minimized by withdrawing blood until a standardized drop in arterial pressure was obtained. By contrast in the present study, the hemorrhage stimulus was standardized. In addition, our animal preparation was
Table 2  Summary of Effects of Blockade on Arterial Blood Gases

<table>
<thead>
<tr>
<th></th>
<th>(P_{CO_2})</th>
<th>(P_{O_2})</th>
<th>pH</th>
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<tbody>
<tr>
<td>Control (n = 10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre</td>
<td>33.3 ± .5</td>
<td>86.8 ± 1.8</td>
<td>7.399 ± .011</td>
</tr>
<tr>
<td>post</td>
<td>33.2 ± .5</td>
<td>87.1 ± 2.0</td>
<td>7.400 ± .011</td>
</tr>
<tr>
<td>sig</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Phentolamine (n = 5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre</td>
<td>34.0 ± .7</td>
<td>85.4 ± 2.8</td>
<td>7.396 ± .014</td>
</tr>
<tr>
<td>post</td>
<td>36.8 ± 1.0</td>
<td>81.6 ± 3.2</td>
<td>7.367 ± .022</td>
</tr>
<tr>
<td>sig</td>
<td>**</td>
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<td>*</td>
</tr>
<tr>
<td>Phenoxybenzamine (n = 3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre</td>
<td>35.4 ± .9</td>
<td>87.3 ± 4.7</td>
<td>7.331 ± .019</td>
</tr>
<tr>
<td>post</td>
<td>33.3 ± 2.2</td>
<td>87.8 ± 4.2</td>
<td>7.351 ± .037</td>
</tr>
<tr>
<td>sig</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Carbocaine (n = 5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre</td>
<td>33.2 ± 1.0</td>
<td>81.2 ± 3.4</td>
<td>7.394 ± .013</td>
</tr>
<tr>
<td>post</td>
<td>34.5 ± 1.5</td>
<td>82.1 ± 2.8</td>
<td>7.392 ± .014</td>
</tr>
<tr>
<td>sig</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

All values are means ± standard error. In the rows opposite "pre" and "post" the values of each variable during the pre-blockade and post-blockade period, respectively, are listed. Opposite "sig" the probabilities of paired differences between the pre- and post-blockade periods are indicated. One asterisk represents \(p < .05\); 2 asterisks represent \(p < .005\); 3 asterisks represent \(p < .0005\); NS indicates not significant. The data from all phentolamine, phenoxybenzamine, and carbocaine control experiments were pooled and are represented in the "control" group.

designed to optimize each animal's ability to compensate for hemorrhage: arterial blood gases were maintained at "normal conscious" values; variations in the standardized depth of anesthesia were minimized; acidosis was eliminated; and all animals were optimally hydrated. With this approach, our standardized hemorrhage stimulus produced changes in arterial pressure which ranged from an increase of 10 to a decrease of 48 mm Hg relative to control (fig. 2). Differences in ability to maintain arterial pressure were associated with differences in the pre-hemorrhage values of MAP and in the responses of heart rate and hematocrit to hemorrhage.

The hemorrhage-induced increase in venous

Table 3  Summary of Effects of Blockade on Mean Arterial Pressure, Cerebral Blood Flow and Cerebrovascular Resistance

<table>
<thead>
<tr>
<th></th>
<th>MAP</th>
<th>CBP</th>
<th>CVR</th>
</tr>
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<tbody>
<tr>
<td>Control (n = 10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre</td>
<td>127.8 ± 3.8</td>
<td>38.5 ± 2.3</td>
<td>3.45 ± .27</td>
</tr>
<tr>
<td>post</td>
<td>127.4 ± 4.0</td>
<td>39.2 ± 2.3</td>
<td>3.38 ± .27</td>
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<td>sig</td>
<td>NS</td>
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<tr>
<td>Phentolamine (n = 5)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>pre</td>
<td>123.8 ± 2.1</td>
<td>44.2 ± 4.4</td>
<td>2.90 ± .26</td>
</tr>
<tr>
<td>post</td>
<td>81.5 ± 3.7</td>
<td>36.9 ± 3.7</td>
<td>2.30 ± .25</td>
</tr>
<tr>
<td>sig</td>
<td>***</td>
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<td>**</td>
</tr>
<tr>
<td>Phenoxybenzamine (n = 3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre</td>
<td>115.4 ± 11.5</td>
<td>38.5 ± 4.0</td>
<td>2.96 ± .28</td>
</tr>
<tr>
<td>post</td>
<td>90.4 ± 14.4</td>
<td>37.2 ± 3.6</td>
<td>2.43 ± .27</td>
</tr>
<tr>
<td>sig</td>
<td>*</td>
<td>NS</td>
<td>*</td>
</tr>
<tr>
<td>Carbocaine (n = 5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre</td>
<td>117.4 ± 12.7</td>
<td>29.9 ± 1.5</td>
<td>3.92 ± .35</td>
</tr>
<tr>
<td>post</td>
<td>110.9 ± 19.7</td>
<td>34.1 ± 3.6</td>
<td>3.18 ± .33</td>
</tr>
<tr>
<td>sig</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
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</table>

See footnote to table 2 for explanation.
hematocrit observed in the normotensive group suggests reflex splenic contraction. The absence of an increase in hematocrit in the hypotensive group may have been due to the increased capillary reabsorption of extravascular fluid which occurs during hypotension. Alternatively, reflex splenic contraction may not have occurred in the hypotensive group, perhaps because reflex recruitment was depressed. Depression of reflex recruitment in the hypotensive group was more strongly suggested by the fact that in response to hemorrhage, the heart rate increase observed in the normotensive group was significantly greater than that observed in the hypotensive group. Thus, the data suggest that overall autonomic reactivity was greater in the normotensive than in the hypotensive group. Similar differences, when observed by others, have been attributed to individual differences in animal constitution (nutrition, age, etc.).

Patterns of Cerebrovascular Response to Hemorrhage

Although a wide variety of methodological approaches have been employed in previous studies of the cerebrovascular effects of hemorrhage, the results of these studies can be conveniently divided into 4 general patterns of response. Each of the 4 patterns is consistent with the "dual effects hypothesis" which suggests that whole brain CVR is the sum of 2 separate components of resistance connected in series. The first component is attributed to neurogenic cerebral vasodilatation and is thought to have a predominant influence on the richly innervated extra-parenchymal arteries. The second resistance component is attributed to intrinsic (autoregulatory or metabolic) cerebral vasodilatation and is thought to have a predominant influence on the parenchymal arteries (which have greater accessibility to metabolites and are more sparsely innervated than the extra-parenchymal cerebral arteries).

In the first pattern of cerebrovascular response to hemorrhage, blood withdrawal produced decreases in MAP and CVR, but no detectable decrease in CBF. This pattern of response has been described as CBF autoregulation. In several studies, alpha-receptor blockade or acute cervical sympathectomy (ganglionic anesthetization) of the superior cervical ganglia, we interpret the slight effect of carbocaine on MAP as resulting from a decrease in total peripheral resistance. Consistent with this interpretation are the results that post-hemorrhage CVR decreased significantly to below pre-hemorrhage.
values and CBF tended to increase following ganglionic anesthetization.

Because MAP and arterial blood gases did not change significantly following carbocaine, autoregulation can not explain the statistically significant cerebral vasodilatation produced by ganglionic anesthetization. The carbocaine-induced cerebral vasodilatation also can not be attributed to direct action on or absorption into vasculature outside the cranium, since injection of carbocaine adjacent to the ganglia had no significant effect on CBF, MAP, or CVR in carbocaine control experiments. For these reasons, we attribute the cerebral vasodilatation produced by carbocaine to release of reflex sympathetic cerebral vasoconstriction.

Resting Sympathetic Cerebrovascular Tone

If it is assumed that the primary effect of carbocaine in our experiments was the release of sympathetic cerebrovascular tone (SCT), and that the "intrinsic" determinates of CVR were unaffected by carbocaine or normotensive hemorrhage, our carbocaine data estimate resting SCT to be approximately 5% of CVR during control conditions. Previous estimates of resting SCT have ranged from "undetectable" to approximately 10% of control CVR. The true value of resting SCT in our preparation would be greater than estimated by our carbocaine data due to cerebral vasoconstriction mediated by sympathetic fibers originating in the stellate ganglia; in other words, anesthetization of the superior cervical ganglia did not completely eliminate all cerebrovascular tone.

Conclusion

In agreement with the dual effects hypothesis, our data suggest that whole brain CVR is balanced between "intrinsic" (autoregulatory and/or metabolic) vasodilating influences and neurogenic vasoconstricting influences. As evidenced by many studies, the response to hemorrhage is variable in its recruitment of these opposing influences, but appears to be correlated with the individual animal's ability to maintain arterial pressure. When the ability to maintain arterial pressure is strong and not exceeded (as in our normotensive group), sympathetic cerebral vasoconstriction is the predominant influence on CVR and may increase from approximately 5% of CVR at rest to 20% of CVR following hemorrhage. When the ability to maintain MAP is weak or is exceeded (as in our hypotensive group), intrinsic cerebral vasodilator influences are predominant.

Acknowledgment

We wish to thank Ralph R. Sonnenschein for his editorial assistance in preparation of the final manuscript. We also wish to thank the Smith, Kline, & French Company for their donation of phenoxybenzamine, and the Upjohn Company for the heparin used in our studies.

References

12. D'Alecy LG, Manfredi J, Rose C, Sellers S: Cerebral sodium extraction in the dog: a test for extracerebral contamination. (Submitted for publication)
A Re-Examination of Physostigmine-Induced Cerebral Protection in the Hypoxic Mouse
A Critical Assessment of the Model
ALAN A. ARTRU, M.D. AND JOHN D. MICHENFELDER, M.D.

SUMMARY Using the hypoxic (FiO₂ = 0.05) mouse model as originally described, the survival time following pretreatment with physostigmine was examined. The maximum increase in survival time was 87% following a physostigmine dose of 0.4 mg/kg. This increase was considerably less than that previously reported for this drug in a hypoxic mouse study wherein the standard method for exposing mice to hypoxia was altered. We speculate that this alteration in methodology resulted in small variations in FiO₂ sufficient to account for the differences between these studies.

PHYSOSTIGMINE has recently been reported to provide cerebral protection in the hypoxic (FiO₂ = 0.05) mouse. At the maximally protective dose (0.3 mg/kg) survival was increased by 542% (survival time in controls was 4.3 min) and 4 of 14 animals survived more than one hour. These increases in survival are strikingly greater than any previously observed with this model for a variety of cerebral protective agents (barbiturates, anticonvulsants, anesthetics) or conditions (hypothermia, hypercarbia) either singly or in combination.

Though the hypoxic mouse model is based on a simple concept it is known to be quite sensitive to a number of variables, most importantly FiO₂. Accordingly, it is desirable for experimental animals to be tested simultaneously with control animals in the same hypoxic environment. By this means any unintended variation in FiO₂ will be recognized by abnormal survival times in the controls. In the aforementioned physostigmine study individual control or experimental mice were sequentially introduced into a single test chamber. This modification may have introduced unrecognized variations in FiO₂ resulting in altered survival times. The present study was designed to re-examine the increase in survival afforded by physostigmine using standard conditions for this model. We also examined the effect of both temperature and elapsed time from injection of the drug, two other conditions that may alter survival times.

Method
Subjects were 170 white male ARS HA/ICR albino mice (Sprague Dawley, Madison, WI) (weights 24-32 g) given free access to food pellets and tap water. Groups of 5 mice were weighed individually then injected intraperitoneally with either physostigmine (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, or 0.7 mg/kg), an equal volume of 0.9% saline (controls), or pentobarbital (PTB) 50 mg/kg (controls). The injected volume ranged from 0.24 cc to 0.64 cc. Ten minutes later one animal was placed in each of 5 inter-connected airtight compartments breathing room air supplied at 4 L/min for an additional 10 min of temperature equilibration.

The compartments were kept in a versa-range test chamber (Blue-M Engineering Co., Division of Blue-M Electric Co., Blue Island, IL) which, at an ambient temperature of 35°C, maintained mouse intraperitoneal temperature at 37.0 ± 0.2°C. At the onset of each test period the room air gas supply was replaced by a mixture of N₂ 8-8½ L/min and 5.22% O₂ (by Haldane analysis) in N₂ 15 L/min. Ten minutes later each compartment breathing room air supplied at 4 L/min for an additional 10 min of temperature equilibration.

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Hemorrhage-induced cerebral vasoconstriction in dogs.
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