Contribution of hypercapnia and trigeminal stimulation to cerebrovascular dilation during simulated diving

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Ollenberger, G. P., and N. H. West. Contribution of hypercapnia and trigeminal stimulation to cerebrovascular dilation during simulated diving. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R921–R930, 1998.—We investigated the relative contribution of humoral (carbon dioxide) and neural (trigeminal stimulation) inputs in the cerebrovasodilatory response to simulated diving in the rat. The cerebral hemodynamic profile of rats was determined using the brain blood flow tracer N-[14C]isopropyl-p-iodoamphetamine. During a simulated dive response, cerebral vascular resistance (CVR) decreased 63.1%, resulting in a 1.5-fold increase in cerebral blood flow (CBF). To investigate the contribution of hypercapnia to the decrease in CVR during simulated diving, we measured CBF during simulated diving in rats with preexisting hypocapnia. To investigate the contribution of trigeminal input, we measured CBF during periods of trigeminal stimulation alone with continued ventilation. Preexisting hypocapnia abolished the cerebrovasodilatory response to simulated diving. Trigeminal stimulation alone did not produce a significant increase in CBF from control values in any brain region, suggesting that trigeminal input does not contribute to the cerebrovascular response to simulated diving in rats. These results suggest that the cerebrovascular response observed during diving in small mammals is driven primarily by progressive hypercapnia associated with asphyxia.

Laboratory rats exhibit a marked redistribution of cardiac output (CO) in response to both voluntarily initiated diving (29) and forced head immersion (21). A redistribution of CO during diving preferentially perfuses tissues sensitive to hypoxic challenge, such as the heart and brain, at the expense of decreasing perfusion to tissues relatively insensitive to hypoxia, such as skeletal muscle and viscera (17, 18, 41). Although previous studies suggest that the brain is perfused continuously, few studies have examined the pattern of blood flow in brain regions during diving. In a recent study, we determined the cerebral hemodynamic profile of conscious, voluntarily diving rats (30). We found regional cerebral blood flow (rCBF) increased markedly during diving due primarily to a corresponding decrease in cerebral vascular resistance (CVR). Only some regions of the basal ganglia (caudate putamen and globus pallidus) and limbic areas (hippocampus and amygdala) did not increase rCBF significantly during diving. Because some brain regions did not participate in the intracerebral increase in blood flow, we suggested that the cerebrovasodilatory response to diving in rats may have both a humoral component, mediating a global fall in CVR, and a neural component, mediating differential changes in CVR.

The objective of the present study was to expand on the results of Ollenberger and West (30) concerning the contribution of humoral and neural inputs producing the cerebrovasodilatory response to diving in the rat. We first investigated carbon dioxide (CO2) as a potential humoral input in cerebrovasodilatory response to diving because CO2 has been implicated as a possible mediator of cerebral vasomotion during diving in sea lions (6), seals (1), and ducks (19, 37). Furthermore, numerous studies have demonstrated that CBF increases steeply in response to increased CO2 (for review, see Ref. 8). Therefore, our primary hypothesis was that the progressive increase in arterial CO2 mediates a global fall in CVR during diving in rats.

Second, we investigated trigeminal stimulation as a potential neural input to the cerebrovasculature during diving, because this input has been demonstrated to be necessary in the cardiac response to diving in small mammals (24) and has been shown to elicit regional alterations in CBF (10). Moreover, the neural connections of the trigeminal system with cerebral blood vessels are so numerous that the concept of a trigemino-cerebrovascular system has arisen (27). Therefore, our secondary hypothesis was that trigeminal afferent input differentially modulates the global cerebrovasodilatory response to diving in rats.

To investigate these hypotheses, we used a simulated diving model that enabled us to differentiate between the effects of carbon dioxide and trigeminal stimulation on the cerebrovasculature during diving in the rat (25). In the simulated diving model, the diving response was initiated by flowing water through the nasal passages (trigeminal stimulation) during expiratory apnea in anesthetized, paralyzed, artificially ventilated rats. To investigate the first hypothesis, we measured rCBF during simulated diving in rats with preexisting hypocapnia to remove the CO2 stimulus (hypocapnic simulated diving). To test the second hypothesis, we measured rCBF during periods of trigeminal stimulation alone with continued ventilation (trigeminal stimulation). We compared the results from the hypocapnic simulated diving group and trigeminal stimulation alone group to the control (anesthetized, paralyzed, artificially ventilated) and normocapnic simulated diving (nasal water flow plus apnea) groups.

Materials and Methods

Experiments were performed on 30 male Sprague-Dawley rats (434.9 ± 16.1 g). All experimental interventions were approved by the Animal Care Committee of the University of
Syringe containing a paralytic agent (D-tubocurare, Sigma, pump 22, Ealing Scientific, St. Laurent, Quebec; 0.4 ml/min) to an infusion/withdrawal pump (Harvard Syringe infusion connected to a preweighed heparinized 5.0-ml syringe attached 4–327-C, Beckman Instruments, Schiller Park, IL) and re-

Copper leads were inserted under the skin to record the cava. All the cannulas were filled with heparinized saline 1.194 mm) that was advanced 6 cm into the inferior vena that was advanced 2.0 cm toward the abdominal aorta. The polypropylene tubing (PE-50, Clay Adams, Parsippany, NJ) 33% solution in saline) was given to ensure analgesia was methoxyflurane (Metofane, MTC Pharmaceuticals). One-half-

10% solution in saline), after initial inhalation induction with icaltics, Cambridge, Ontario; 0.15–0.2 ml/kg im, diluted to a

been described previously (25). Briefly, rats were anesthe-

Physiological variables in control, trigeminal stimulation, hypocapnic dive, and simulated dive groups was withdrawn from the rostral-facing cannula at a rate comparable to the infusion rate.

Experimental protocol. Cardiovascular and cerebrovascular variables were determined in four groups of rats. The control group (n = 10) consisted of anesthetized, paralyzed, artificially ventilated rats [respiratory frequency (f) = 70 min⁻¹, tidal volume (Vₜ) = 2.6–3.8 ml]. The normocapnic simulated dive group (n = 7) consisted of anesthetized, paralyzed, artificially ventilated rats in which the dive response was elicited by flowing water into the nares (trigeminal stimulation) during concurrent apnea. We compared the results from the above groups to the results from flowing water into the nares with continuous ventilation (trigeminal stimulation alone group, n = 6) and to simulated diving after the arterial partial pressure of CO₂ (Paco₂) was reduced predive by hyperventilation (hypocapnic simulated dive, n = 7, f = 90, Vₜ = 4.5–5.8 ml). Arterial blood gases were determined before measurement of rCBF in all experimental groups (Table 1) to ensure blood gases were within the physiological range (BGM200 blood gas meter and BC202 blood gas cell; Cameron Instrument, Port Aransas, TX).

Measurement of rCBF. The brain blood flow tracer N-14C)sopropyl-p-ioidamphetamine (IMP) (NEN, Boston, MA) was used to quantitate rCBF. The specific activity was 44.7 mCi/mmol. IMP is extracted 100% during first pass in the brain capillaries, with a time to one-half brain washout (t½) of 318 s (40). Therefore, the experiment was performed using a modification of the indicator-fractionation technique first described by Goldman and Sapirstein (11). In all protocols the reference blood sample was withdrawn at a steady rate of 0.4 ml/min, which provided a reference flow rate (R in Eq. 2) that is necessary to determine rCBF.

In all groups the arterial withdrawal was started first, followed by injection of IMP into the femoral vein cannula. In trigeminal stimulation and simulated dive groups, the injection of IMP occurred within 2–3 s of a visible bradycardia on the ECG tracing (Figs. 3 and 4). In the control group, the IMP was allowed to circulate for 35 s, after which the rat was decapitated to stop the circulation of the tracer. In the other three experimental groups, IMP circulation time was extended to 50 s to ensure that the peak of the tracer concentration in the arterial blood had passed during the decreased CO associated with bradycardia. These tracer distribution times were chosen based on arterial radioisotope-dilution curves that were determined previously in control and simulated diving rats (Fig. 1). Briefly, we measured IMP concentration in arterial blood by dripping an open arterial cannula into scintillation vials during injection of IMP. The peak of the

Table 1. Physiological variables in control, trigeminal stimulation, hypocapnic dive, and simulated dive groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n = 10)</th>
<th>Trigeminal Stimulation (n = 6)</th>
<th>Hypocapnic Dive (n = 7)</th>
<th>Normocapnic Dive (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>427.6 ± 19.0</td>
<td>257.3 ± 36.9*</td>
<td>141.5 ± 15.6†</td>
<td>107.3 ± 7.5†</td>
</tr>
<tr>
<td>MABP, mmHg</td>
<td>94.3 ± 4.0</td>
<td>117.0 ± 6.4*</td>
<td>60.2 ± 4.6</td>
<td>53.9 ± 3.1†</td>
</tr>
<tr>
<td>CO, ml/min</td>
<td>164.7 ± 14.1</td>
<td>102.4 ± 15.4*</td>
<td>60.5 ± 4.9</td>
<td>64.8 ± 5.4*</td>
</tr>
<tr>
<td>TPR, mmHg·min⁻¹·ml⁻¹</td>
<td>0.61 ± 0.06</td>
<td>1.26 ± 0.19*</td>
<td>1.02 ± 0.09*</td>
<td>0.79 ± 0.05</td>
</tr>
<tr>
<td>SV, ml</td>
<td>3.06 ± 0.03</td>
<td>0.43 ± 0.06</td>
<td>0.47 ± 0.07</td>
<td>0.64 ± 0.02†</td>
</tr>
<tr>
<td>10% CO₂ accumulation (%)</td>
<td>7.41 ± 0.04</td>
<td>7.36 ± 0.07</td>
<td>7.57 ± 0.03†</td>
<td>7.42 ± 0.04†</td>
</tr>
<tr>
<td>P⁰₂, mmHg</td>
<td>105.3 ± 4.3</td>
<td>118.9 ± 5.5</td>
<td>108.1 ± 2.6</td>
<td>96.7 ± 7.0</td>
</tr>
<tr>
<td>P⁰₂, mmHg</td>
<td>34.7 ± 0.9</td>
<td>35.8 ± 1.7</td>
<td>20.5 ± 0.7</td>
<td>36.8 ± 1.0</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals. Arterial blood gases were determined prior to experimental protocol. Cardiovascular variables reflect approximately the same period as regional cerebral blood flow (rCBF) measurements (control, 10–20 s; dive, 25–35 s; see Measurement of rCBF). HR, heart rate; MABP, mean arterial blood pressure; CO, cardiac output; TPR, total peripheral resistance; SV, stroke volume; P⁰₂ and P⁰₂, arterial partial pressure of O₂ and CO₂, respectively. *Response significantly different from control; †response significantly different from all other groups; ‡response significantly different from control and trigeminal stimulation; P < 0.05.
arterial curve occurred \( \sim 10-20 \) s after injection in the control rat (HR \( \approx 427 \) beats/min), whereas the peak did not appear until \( \sim 25-35 \) s after injection in simulated diving rats (HR \( \approx 104 \) beats/min). Therefore, we adjusted the circulation time of IMP in both simulated diving groups from 35 to 50 s to reflect the cardiovascular changes associated with diving bradycardia. We also adjusted the circulation time of IMP to 50 s in the trigeminal stimulation alone group due to the occurrence of a significant bradycardia (Table 1). Based on the above arterial circulation times for IMP, rCBF values in the current experiment reflect a limited time frame after injection of IMP and not a “smeared” measurement of the entire experimental period. For the control group, the values in the current experiment are indicative of rCBF in the time frame of \( \sim 10-20 \) s after injection, whereas for the dive groups, the rCBF values are indicative of rCBF in the time frame of \( \sim 25-35 \) s into the dive.

A detailed account of the determination of rCBF has been described in detail elsewhere (3, 37). Briefly, rCBF is calculated according to the following relationship

\[
\text{CBF} = C_0 \int_0^T C_a \, dt
\]

where \( C_0 \) is concentration of the tracer in a particular brain region, and \( C_a \) is concentration of the tracer in arterial blood at any time \( t \). The evaluation of the numerator in Eq. 1 is determined by quantitative autoradiography, and the denominator can be determined by measuring the tracer contained in the withdrawn blood during the experimental period (11, 34, 38). It is important that the arterial blood withdrawal is terminated at the same time the animal is killed. However, timing errors are minimized by injecting IMP as a bolus, because the arterial concentration is very low toward the end of the experimental period (31). The integrated arterial blood sample can be expressed in terms of rate of blood withdrawal (R) and the total tracer activity in the withdrawn blood sample (\( Q_A \))

\[
\int_0^T C_a \, dt = \frac{Q_A}{R}
\]

\( Q_A \) was determined by analyzing four 20-µl aliquots of blood from the reference blood sample that were weighed, solubilized (NCS II tissue solubilizer, Amersham, Oakville, Ontario), decolorized with 30% hydrogen peroxide, and counted in a liquid scintillation counter (Beckman LS 9800). \( Q_A \) was obtained as follows

\[
Q_A = C_s \times \frac{M_r}{M_s}
\]

where \( C_s \) is the quantity of tracer in an aliquot of blood, \( M_s \) is the mass of the aliquot, and \( M_r \) is the mass of the entire reference blood sample. A mean value of the four estimates was used for subsequent calculations.

At the end of the experimental period, the brain was removed from the skull and rapidly frozen in isopentane (2-methyl-butane) at \(-50^\circ\text{C} \). The brains were cut at 20-µm thicknesses and placed in contact with autoradiographic film (Kodak TMS-1 RA, Eastman Kodak, Rochester, NY; \( 18 \times 24 \) cm) in a light-tight cassette. After a short exposure period (5–10 days), the film was developed and a gray level brain image was produced. Densitometry was performed on autoradiographic images by a computer-based image analysis system (Image 1, Universal Imaging, West Chester, PA). The autoradiograph gray level density was converted to tissue tracer concentration using calibrated \( ^{14}\text{C} \) standards (American Radiolabeled Chemicals, St. Louis, MO) that were packed with the brain slices. rCBF was calculated in absolute terms (\( \text{ml} \cdot \text{min}^{-1} \cdot \text{100 g}^{-1} \)), and rates of blood flow were pseudo-color coded and displayed as a color image. To positively identify brain nuclei, the 20-µm brain slices were stained with neutral red, which stains for Nissl bodies in neurons. The stained slides were then compared with a stereotaxic brain atlas (32) to identify specific brain regions.

Arterial blood gases during simulated diving. We were unable to determine the status of arterial blood gases during the injection of the radioisotope (IMP). Therefore, separate experiments were performed (\( n = 5 \)) to demonstrate that \( P_{\text{aCO}_2} \) was successfully eliminated as a potential humoral input on the cerebrovasculature during hypocapnic simulated diving. Arterial blood was withdrawn and analyzed [arterial partial pressure of \( O_2 \) (\( P_{\text{aO}_2} \)), \( P_{\text{aCO}_2} \), and \( P_{\text{H}_2} \)] predive and during a 10-s interval (25–35 s) into a dive response in normocapnic and hypocapnic rats (Fig. 2). In Fig. 2, 30 s was chosen to reflect the midpoint in the arterial withdrawal interval.

Estimation of CO. CO was determined using the “reference sample” technique. Blood was withdrawn from the femoral artery at a rate of 0.4 ml/min during the experimental protocol. CO was determined from the equation

\[
\text{CO} = \frac{0.4 \text{ ml/min}}{\frac{\int_0^T C_a \, dt}{C_1}}
\]

where \( C_1 \) is the total counts of tracer injected, determined by the principle described in Eq. 3. Therefore, CO was determined by dividing the withdrawal rate by the fraction of injected tracer in the reference sample. A potential source of error in the determination of CO is the possibility that IMP is not extracted during first pass in peripheral tissues, resulting in an overestimation of CO.

Statistical analysis. All values reported in the text and figures are grand means ± SE; HR (beats/min) and mean arterial blood pressure (MABP, mmHg) were measured for each animal in all protocols. HR and MABP were determined by calculating HR and MABP at 5-s intervals and then
averaging the values for the entire experimental period. MABP was calculated from pulsatile blood pressure traces (diastolic plus 1/3 pulse pressure). Stroke volume (SV, ml) and total peripheral resistance (TPR, mmHg·ml⁻¹·min⁻¹) were calculated by substituting MABP, CO, and HR into the equations MABP = CO × TPR and CO = HR × SV. rCBF (ml·min⁻¹·100 g wet brain tissue⁻¹) was determined in 32 brain regions. Grand means were calculated by averaging the rCBF values in brain regions from all animals in a protocol. Brain regions were grouped into divisions based on function (basal ganglia and thalamus, limbic system, and primary cortical regions) except for the hindbrain, which was grouped according to anatomic location, because the hindbrain consists of a multitude of smaller functional regions. Global CBF (ml/min) was estimated by using the equation CBF = average rCBF × brain weight, where average rCBF represents the nonweighted average of all 32 brain regions measured in each animal. We assumed an average of the 32 brain regions reflected flow throughout the whole brain, because the regions spanned from posterior to anterior. Because the brain had to be rapidly frozen on removal, brain weight was calculated by using a correlation (r² = 0.98) between rat body weight and rat brain weight from previously published data (41). CVR (mmHg·ml⁻¹·min⁻¹) was determined by substituting CBF and MABP into the equation MABP = CBF × CVR. Statistical analyses were performed with a computer package (Systat, Evanston, IL). The data were analyzed with one-way analysis of variance with significance reached when P < 0.05 (42). In the case of significant F values, Tukey's honest significant difference a posteriori tests were performed to determine differences among group means.

RESULTS

Cardiovascular responses to normocapnic simulated diving. Normocapnic simulated diving resulted in an immediate bradycardia that was maintained throughout the entire 50-s stimulation period (Table 1, Fig. 3). The physiological variables in the four groups of rats are presented in Table 1. HR decreased an average of 74.9% during the normocapnic simulated dive period compared with control values (427.6 ± 19.0 to 107.3 ± 7.3 beats/min). Bradycardia resulted in a significant 55.9% decrease in CO compared with control values (164.7 ± 14.1 to 64.8 ± 5.4 ml/min). All estimated CO values were moderately higher than values previously determined by averaging the values for the entire experimental period. MABP was calculated from pulsatile blood pressure traces (diastolic plus 1/3 pulse pressure). Stroke volume (SV, ml) and total peripheral resistance (TPR, mmHg·ml⁻¹·min⁻¹) were calculated by substituting MABP, CO, and HR into the equations MABP = CO × TPR and CO = HR × SV. rCBF (ml·min⁻¹·100 g wet brain tissue⁻¹) was determined in 32 brain regions. Grand means were calculated by averaging the rCBF values in brain regions from all animals in a protocol. Brain regions were grouped into divisions based on function (basal ganglia and thalamus, limbic system, and primary cortical regions) except for the hindbrain, which was grouped according to anatomic location, because the hindbrain consists of a multitude of smaller functional regions. Global CBF (ml/min) was estimated by using the equation CBF = average rCBF × brain weight, where average rCBF represents the nonweighted average of all 32 brain regions measured in each animal. We assumed an average of the 32 brain regions reflected flow throughout the whole brain, because the regions spanned from posterior to anterior. Because the brain had to be rapidly frozen on removal, brain weight was calculated by using a correlation (r² = 0.98) between rat body weight and rat brain weight from previously published data (41). CVR (mmHg·ml⁻¹·min⁻¹) was determined by substituting CBF and MABP into the equation MABP = CBF × CVR. Statistical analyses were performed with a computer package (Systat, Evanston, IL). The data were analyzed with one-way analysis of variance with significance reached when P < 0.05 (42). In the case of significant F values, Tukey's honest significant difference a posteriori tests were performed to determine differences among group means.

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Arterial blood gases during simulated diving. Figure 2 shows the results of separate experiments to determine arterial blood gas changes during simulated diving in the normocapnic and hypocapnic dive groups. During simulated diving in the normocapnic group, $\text{PaCO}_2$ rose from a predive level of $38.6 \pm 1.0$ to $48.7 \pm 2.2 \text{ mmHg}$, 25–35 s into the dive response. During a dive response in the hypocapnic group, $\text{PaCO}_2$ rose from a predive level of $24.8 \pm 0.8$ to $35.7 \pm 1.0 \text{ mmHg}$ 25–35 s into the dive response. The changes in $\text{PaCO}_2$ during simulated diving were accompanied by corresponding changes in $\text{pH}_a$. $\text{PaO}_2$ was maintained within the normal physiological range predive in both simulated diving protocols and decreased to approximately the same level during simulated diving (Fig. 2).

Global cerebrovascular response to normocapnic simulated diving. Figure 5 shows the global cerebrovascular effects of normocapnic simulated diving. During normocapnic simulated diving, CBF increased significantly compared with control values (2.4 $\pm$ 0.2 to 6.9 $\pm$ 0.7%).

Global cerebrovascular response to trigeminal stimulation and hypocapnic simulated diving. CBF did not change from control values during either trigeminal stimulation and hypocapnic simulated diving (Fig. 5). During hypocapnic simulated diving the brain's share of CO increased significantly compared with control values (2.4 $\pm$ 0.2 to 5.6 $\pm$ 0.4%). This corresponded to a significant reduction in CVR during hypocapnic simulated diving compared with control values (30.1 $\pm$ 3.4 to 18.4 $\pm$ 1.3 mmHg·ml$^{-1}$·min$^{-1}$).

$r\text{CBF}$ during normocapnic simulated diving. $r\text{CBF}$ was determined in 32 brain regions (Figs. 6–9). During normocapnic simulated diving, $r\text{CBF}$ increased significantly from control values (30.1 $\pm$ 3.4 to 18.4 $\pm$ 1.3 mmHg·ml$^{-1}$·min$^{-1}$) during normocapnic simulated diving. CBF increased significantly compared with control values (2.4 $\pm$ 0.2 to 6.9 $\pm$ 0.7%).

Global cerebrovascular response to normocapnic simulated diving. Figure 5 shows the global cerebrovascular effects of normocapnic simulated diving. During normocapnic simulated diving, CBF increased significantly compared with control values (2.4 $\pm$ 0.2 to 6.9 $\pm$ 0.7%).

Cardiovascular responses to hypoxic stimulation alone. Trigeminal stimulation alone produced an immediate and intense bradycardia that subsided toward the end of the stimulation period (Fig. 4). During the trigeminal stimulation period, HR significantly decreased by 39.8% (427.6 $\pm$ 20.0 to 257.3 $\pm$ 40.4 beats/min), producing a significant 37.8% decrease in CO (164.7 $\pm$ 14.1 to 102.4 $\pm$ 15.4 ml/min) compared with control values. To counteract a decreased CO, TPR increased significantly (0.6 $\pm$ 0.1 to 1.3 $\pm$ 0.2 mmHg·ml$^{-1}$·min$^{-1}$) that maintained MABP significantly higher than control values during the trigeminal stimulation period (94.3 $\pm$ 4.0 to 117.0 $\pm$ 6.4 mmHg). There was no change in SV during the trigeminal stimulation period. Blood gases, determined before trigeminal stimulation, were not significantly different from either the control or trigeminal stimulation groups (Table 1).

Cardiovascular responses to hypocapnic stimulation alone. Hyperventilation before simulated diving resulted in a significant increase in $\text{pH}_a$ (7.57 $\pm$ 0.02) and decrease in $\text{PaCO}_2$ (20.5 $\pm$ 0.7 mmHg) from control values (Table 1). During hypocapnic simulated diving, HR (427.6 $\pm$ 19.0 to 141.5 $\pm$ 15.6 beats/min) and CO (164.7 $\pm$ 14.1 to 60.5 $\pm$ 4.9 ml/min) decreased significantly from control values, similarly to normocapnic simulated diving. A significant increase in TPR occurred during hypocapnic diving (0.6 $\pm$ 0.1 to 1.0 $\pm$ 0.1 mmHg·ml$^{-1}$·min$^{-1}$) compared with control values, that maintained MABP at a slightly higher level than during normocapnic simulated diving (Table 1). There was no change in SV during the hypocapnic dive period (Table 1).
sificantly in 16 of the 32 brain regions examined, compared with control values (Figs. 6–9). Most regions of the hindbrain and thalamus increased rCBF during normocapnic simulated diving compared with all other groups (Figs. 6 and 7). Regional CBF did not increase significantly during normocapnic simulated diving to any region of the basal ganglia or limbic system (Figs. 7 and 8). Most primary cortical regions increased rCBF significantly from control values during normocapnic simulated diving (Fig. 9). The largest absolute difference in rCBF (normocapnic simulated diving and control) occurred in the habenular complex in the dorsomedial aspect of the thalamus (133 ml·min⁻¹·100 g⁻¹), whereas the largest decrease in rCBF occurred in the anteroventral thalamic nucleus (63 ml·min⁻¹·100 g⁻¹).

rCBF during trigeminal stimulation and hypocapnic simulated diving. Trigeminal stimulation alone did not produce a significant increase in rCBF from control values in any brain region, including the trigeminal nuclei themselves (Figs. 6–9). During simulated diving after preexisting hypocapnia, rCBF did not increase significantly in any of the brain regions examined (Figs. 6–9).

DISCUSSION

This is the first study to investigate the relative contribution of humoral and neural inputs on the cerebrovasculature during a dive response in a small mammal. During simulated diving, CVR decreased by 63.1%, resulting in a 1.5-fold increase in CBF. Preexisting hypocapnia abolished the cerebrovasodilatory response to simulated diving. Trigeminal stimulation alone did not produce a significant increase in rCBF from control values in any brain region. These results support the primary hypothesis that the progressive rise in arterial CO₂ produces a global fall in CVR during diving in rats. However, these results do not support a role for trigeminal input in the cerebrovascular response to simulated diving in rats.

Role of carbon dioxide in the cerebrovasodilatory response to simulated diving. The major finding of this study is that the progressive rise in arterial CO₂ decreases CVR and increases CBF during simulated diving in the rat. Numerous studies have clearly demonstrated that hypercapnia elicits a marked vasodilation in the cerebral circulation (for review, see Ref. 8). In almost all studies, CBF increases steeply in response to increased CO₂. This suggests that progressive hypercapnia during diving possibly produces widespread cerebrovasodilation. The reactivity of CBF in response to PaCO₂ has been described by a sigmoid curve with the linear portion ranging from ~25 to 70 mmHg (14, 28, 33). Therefore, as PaCO₂ increases during asphyxia associated with the dive response, CBF increases linearly. Other investigators have also suggested that CO₂ is the primary stimulus that increases CBF during diving in both mammals (1, 6) and ducks (19, 37). In this study, PaCO₂ increased from a predive level of 36.8 ± 1.0 to 48.7 ± 2.2 mmHg during normocapnic simulated diving, a level within the linear portion of the CBF–CO₂ curve. With use of a previously published equation to estimate CBF reactivity in response to PaCO₂, the increase in PaCO₂ during normocapnic simulated diving is predicted to increase CBF by ~33% (15). We report here that CBF increased by nearly 50% during simulated diving in the regions we studied. However, our estimate for CBF included brain regions composed primarily of gray matter. Perfusion to white matter has been shown to be significantly less than perfusion to gray matter (8).
During asphyxic diving, however, as CO₂ is increasing, arterial oxygen is also decreasing. Therefore, it is possible that hypoxia also stimulates a decrease in CVR. The reactivity of the cerebrovasculature in response to hypoxia is significantly less than to hypercapnia (26). In fact, CBF has been shown to be virtually unchanged over a PaO₂ range of 55 to 140 mmHg, at constant PaCO₂ (26, 23). Below ~55 mmHg, however, CBF increases sharply. Therefore, the effect of hypoxemia on the cerebrovasculature most likely occurs later in the dive period, only after PaO₂ has fallen considerably below 55 mmHg. Furthermore, although PaO₂ decreased to the same level in both simulated dive groups, rCBF did not increase in the hypocapnic simulated dive group to the same magnitude as in the simulated dive group. This suggests that the majority of the increase in rCBF during simulated diving is due to hypercapnia during the dive period and not hypoxemia. Jones et al. (19) proposed that the effects of hypoxemia and hypercapnia summate to produce the marked increase in CBF observed during diving. If the additive effect of hypoxemia on CBF occurs after hypercapnia, the ability of hypoxemia to increase CBF is possibly reduced, because cerebral blood vessels may already be near maximal dilation in response to hypercapnia. Therefore, our results support hypercapnia as the primary stimulus that increases CBF during diving in the rat.
Blood pressure effect on CBF during simulated diving. Simulated diving resulted in an immediate bradycardia that was maintained throughout the entire stimulation period. However, the decrease in CO was not matched by an increase in peripheral resistance, resulting in a significant hypotension during simulated diving (Fig. 3, Table 1). Blood pressure is maintained above control levels in conscious, voluntarily diving rats (30). The hypotension is likely due to the effect of the paralytic agent d-tubocurare on sympathetic ganglia (13). During a mammalian diving response, an increase in sympathetic outflow produces peripheral vasoconstriction that maintains arterial blood pressure (for a review, see Ref. 2). After curare administration, vasoconstriction could be partially blocked, resulting in hypotension during simulated diving. Hypotension has been shown to diminish the responsiveness of the cerebrovasculature to hypercapnia (14, 39). The decreased responsiveness is likely due to the autoregulatory mechanism in the cerebral circulation. Autoregulation keeps CBF constant by vasodilating in response to decreased arterial pressure and vasoconstricting in response to increased arterial pressure (8). Therefore, cerebral vessels may have lost some of their capacity to dilate in response to hypercapnia if cerebral vessels were previously dilated in response to hypotension. This may explain why CBF did not increase in the same magnitude as during conscious diving in the rat (30).
Role of trigeminal stimulation in the cerebrovasodilatory response to simulated diving. We hypothesized that trigeminal input differentially modulated the global cerebrovasodilatory response to simulated diving in rats. Trigeminal stimulation alone produced profound cardiovascular changes that resulted in a decreased CO (Fig. 4, Table 1). However, trigeminal stimulation alone did not produce a significant increase in rCBF from control values in any brain region. Therefore, these results do not support a role for trigeminal input in the cerebrovascular response to simulated diving in rats. This result was somewhat unexpected due to a significant amount of evidence demonstrating that stimulation of the trigeminal nerve or a ganglion associated with the nerve produces regional variation in CBF (for review, see Ref. 16).

There are two possible explanations why our results suggest that trigeminal stimulation does not influence CBF. First, it is possible that during trigeminal stimulation alone, the neural pathway that increases CBF is potentially inhibited at the brain stem level. The trigeminal stimulation alone protocol required continuous ventilation of the rat, indicating that afferent input from pulmonary stretch receptors (PSR) was feeding back to an integration site within the medulla during the stimulatory period (9). Absence of PSR inputs, as during apnea, potentially “gates” information in the medulla during bradycardia (22). Therefore, it has been suggested that reduction of PSR afferent input is an important factor in the development of bradycardia (5). The neural pathway that increases CBF during trigeminal ganglion stimulation occurs via a reflex that traverses the brain stem (20). Therefore, during trigeminal stimulation alone, the neural pathway from the trigeminal ganglion that increases CBF is possibly inhibited at the brain stem level by PSR feedback. However, although trigeminal afferent input may be inhibited at the brain stem, this input would presumably increase local metabolic activity. Because neural metabolism and rCBF are normally tightly coupled, it would be expected that a metabolically driven increase in rCBF would occur to the trigeminal nuclei (23). Regional CBF did not increase significantly in any of the trigeminal nuclei during trigeminal stimulation. This raises the possibility that either metabolic activity in the trigeminal nuclei did not increase, appreciably increasing rCBF, or that the IMP tracer technique is insensitive to small metabolically driven changes in rCBF.

A second explanation why trigeminal stimulation did produce CBF changes is that the trigemino-cerebrovascular pathway was not activated during the trigeminal stimulation protocol. In summary, this study examined the contribution of humoral (carbon dioxide) and neural (trigeminal stimulation) inputs in the cerebrovasodilatory response to simulated diving in the rat. These results provide evidence to suggest that the decrease in CVR during diving in small mammals is driven primarily by progressive hypercapnia associated with asphyxia. Although trigeminal stimulation is necessary for the cardiac component of the mammalian dive response, we have found no evidence to suggest that this input has any role in differentially modulating the cerebrovascular response to diving.

**Perspectives**

There is little doubt that the brain is preferentially perfused during asphyxic diving in all vertebrates. Increased CBF maintains oxygen and substrate delivery to this crucial organ despite a reduced CO and a progressively falling arterial oxygen content. This study is the first attempt to investigate the mechanisms underlying the increase in CBF during the diving response in a small mammal. We used a simulated dive protocol that allowed us to investigate the role of the neural and humoral stimuli thought to be important in diving. Under circumstances in which ventilatory activity can acquire oxygen from the environment, rCBF is very sensitive to local changes in cerebral metabolism. Such local changes might reasonably be expected in simulated diving, because the central integration of input from cardiorespiratory receptors presumably results in some brain regions, such as the nucleus of the solitary tract and trigeminal nucleus, showing increased neural activity and metabolic rate. Carbon dioxide is a potent vasodilator in the cerebral circulation, suggesting that locally produced CO2 might be one important factor in the coupling of rCBF to local changes in aerobic metabolism in the brain. However, the largely homogeneous nature of the increase in rCBF during simulated diving suggests that any matching of rCBF to local metabolic activity, by whatever mechanism, is uncoupled by an overwhelming hypercapnic signal of systemic origin. The physiological outcome of this CO2-driven cerebrovasodilation is a prolonged maintenance of oxygen and glucose delivery, substrates critical for brain survival and therefore underwater endurance.

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**REFERENCES**


