The Journal of Experimental Biology 201, 549–558 (1998)
Printed in Great Britain © The Company of Biologists Limited 1998
JEB1276

DISTRIBUTION OF REGIONAL CEREBRAL BLOOD FLOW IN VOLUNTARILY DIVING RATS

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Accepted 24 November 1997: published on WWW 26 January 1998

Summary

The distribution of regional cerebral blood flow (rCBF) was examined in conscious, voluntarily diving rats using the brain blood flow tracer *N*-[^{14}C]isopropyl-*p*-iodoamphetamine and quantitative autoradiography. A detailed examination of the regional distribution of cerebral blood flow revealed that almost all brain regions were hyperperfused during diving. During diving, rCBF increased by an average of 1.7-fold in 29 of the 33 brain regions examined, despite a 69.2 % decrease in cardiac output. 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. We determined that the increase in rCBF during diving is primarily due to a corresponding 20.9 % decrease in cerebrovascular resistance. A significant increase in perfusion pressure during diving also potentially contributed to the increase in rCBF. Because some brain regions did not increase flow significantly during diving, these results suggest that not all brain regions participate equally in the global cerebrovascular response to diving. This study provides evidence to support the view that the brain is preferentially perfused during conscious voluntary diving in the rat. The mechanism(s) that probably produce the cerebrovascular changes during diving are discussed.

Key words: voluntary diving, cerebral blood flow, rCBF, bradycardia, cardiac output, rat.

Introduction

The ability of mammals to dive under water and extend underwater dive duration is dependent upon the utilization of internal oxygen stores. Maximal oxygen utilization is achieved by a cardiovascular adaptation known as the dive response. The dive response redistributes a decreased cardiac output ($\dot{Q}$) away from tissues with a capacity for anaerobic metabolism, such as skeletal muscle, towards tissues sensitive to hypoxia, such as the heart and brain (Irving, 1934; Johansen, 1964; Zapol *et al.* 1979). Although previous studies suggest that the brain is perfused continuously during diving, few studies have examined the pattern of blood flow in the cerebral circulation during diving. Zapol *et al.* (1979) and Blix *et al.* (1983) examined cerebral blood flow (CBF) in major brain divisions, such as the pons and cerebellum, during forced diving in seals. Zapol *et al.* (1979) reported that brain blood flow remained relatively constant during diving, whereas Blix *et al.* (1983) reported a differential brain blood flow pattern, which changed with dive duration. This latter study provided evidence to suggest that there is a differential perfusion pattern within the brain itself during diving in specialized divers. A differential pattern of CBF during diving could potentially indicate a control of CBF in response to regional variations in oxygen demand within the brain. Moreover, it is possible that some brain regions are more susceptible to hypoxic damage and, therefore, are preferentially perfused during periods of asphyxia. Previous studies, however, measured CBF globally or in major brain divisions, such as the pons and the cerebral cortex, and did not quantify CBF in smaller brain regions, such as the spinal trigeminal nucleus and the hippocampus. Variations in CBF on a smaller scale could occur during diving that would not be exposed by examining CBF changes in larger brain divisions.

In pursuit of this idea, Stephenson *et al.* (1994) utilized the brain blood flow tracer *N*-[^{14}C]isopropyl-*p*-iodoamphetamine (IMP) and quantitative autoradiography to examine rCBF during forced head immersion in Pekin ducks. They found that rCBF increased approximately twofold during diving in all seventeen brain regions examined. Therefore, they concluded there is no selective intracerebral redistribution of blood flow during diving in the Pekin duck.

The objectives of the present study were to reinvestigate the hypothesis of Stephenson *et al.* (1994) concerning the possibility of a selective intracerebral vascular response during diving. We tested this hypothesis by evaluating rCBF in a conscious, voluntarily diving small mammal, the rat. Conscious rats exhibit a marked redistribution of cardiac output in response to diving similar to that of small semi-aquatic mammals such as muskrat and mink (Lin and Baker,

1975; Ollenberger *et al.* 1997). We also evaluated rCBF during surface swimming without submersion to determine whether locomotor activity associated with diving had any effect on rCBF. We compared the results with the distribution of rCBF in rats at rest.

Materials and methods

Experiments were performed on 20 male Sprague-Dawley rats (397.6±20.2 g). All experimental interventions were approved by the Animal Care Committee of the University of Saskatchewan and were performed in accordance with guidelines of the Canadian Council on Animal Care.

Surgical procedures

Rats were anaesthetized with Innovar-Vet (MTC Pharmaceuticals, Cambridge, Ontario; 0.l5–0.2 ml kg^{-1} intramuscularly, diluted to a 10 % solution in saline), after initial inhalation induction with methoxyflurane (Metofane, MTC Pharmaceuticals). Buprenorphin-hydrochloride analgaesic, 0.06 mg intramuscularly (Temgesic, Reckitt and Coleman Pharmaceuticals, Hull, England) was given after surgery. All surgical instruments were sterilized in an alcohol–iodine solution. The right and left femoral arteries were cannulated with micro-renethane tubing (Braintree Scientific, Braintree, MA; i.d. 0.355 mm, o.d. 0.836 mm). Cannulae were advanced 2.0 cm towards the abdominal aorta. The right and left femoral veins were cannulated with silicone tubing (Baxter Scientific, McGaw Park, IL, USA; i.d. 0.635 mm, o.d. 1.194 mm) connected to micro-renethane tubing (Braintree Scientific, Braintree, MA, USA). Cannulae were advanced 6 cm into the inferior vena cava. All the cannulae were filled with heparinized sterile saline (100 i.u. ml^{-1}, Hepalean, Organon Teknika Inc., Toronto, Ontario) and fed subcutaneously to the nape of the neck, where they were connected to hypodermic tubing (23 gauge, i.d. 0.3302 mm, o.d. 0.635 mm; Small Parts, Miami Lakes, FL, USA). The tubing was previously attached to a patch of polypropylene screen cloth (Small Parts, 500 μm), using dental acrylic. 1 cm of metal tubing was exteriorized through the skin and connected to a short length of polyethylene tubing (PE 50; i.d. 0.58 mm, o.d. 0.965 mm; Clay Adams, Parsippany, NJ, USA) which was kinked off with a piece of larger tubing (PE 205, Clay Adams). The incisions were closed with wound clips (Autoclip, Clay Adams), and the rats were left to recover for 4–5 days. Experiments were only performed on animals that had full recovery of hindlimb function, indicating that collateral blood flow was sufficient to maintain hindlimb perfusion.

Experimental protocol

Before surgery, rats were divided into three groups for the measurement of rCBF. In the resting control group (N=7), rats were left undisturbed in a cage. In the surface-swimming group (N=7), rats trod water in a large plastic cylinder. In the diving group (N=6), rats were trained to swim underwater through a maze constructed of Plexiglas. Water temperature was the same as room temperature (22–24 °C) in both swimming groups.

A detailed description of the dive training procedure has been presented previously (Ollenberger *et al.* 1997; McCulloch *et al.* 1997). Briefly, the dive training started with rats diving under a single piece of Plexiglas in order to swim the rest of the maze on the surface. The diving distance was gradually increased by adding horizontal subsurface pieces to the maze during training sessions. To extend underwater duration, the exit to the surface was blocked, trapping the rat under water. A similar procedure has been used to extend dive duration in tufted ducks (Stephenson *et al.* 1986). Experimental dives were also voluntarily initiated, but were terminated by lethal injection of sodium pentobarbital (Somnotol, MTC Pharmaceuticals) after 50 s.

One arterial cannula was attached to a pressure transducer (type 4-327-C, Beckman Instruments, Sciller Park, IL, USA) and the pulsatile signal was connected to a cardiotachometer (Beckman, type 9857B) to monitor heart rate. Pulsatile arterial pressure and heart rate were recorded on a chart recorder writing on rectilinear coordinates (Beckman R511A). The second arterial cannula was connected to a preweighed heparinized 5.0 ml syringe attached to an infusion/withdrawal pump (Harvard Syringe Infusion Pump 22, Ealing Scientific, St Laurent, PQ; 0.4 ml min^{-1}). One venous cannula was connected to a 1.0 ml syringe containing the radioactive blood flow tracer, the other to a syringe containing 1.0 ml of pentobarbitol (Somnotol, MTC Pharmaceuticals; 65 mg ml^{-1}).

Measurement of rCBF

The brain blood flow tracer *N*-[^{14}C]isopropyl-*p*-iodoamphetamine (IMP) (NEN, Boston, MA, USA) was used to quantify rCBF. The specific activity was 1.65 GBq $mmol^{-1}$. IMP is 100 % extracted during a first pass through the brain capillaries with a brain washout half-time ($t_{1/2}$) of 318 s (Winchell *et al.* 1980). Therefore, the experiment was performed using a modification of the indicator-fractionation technique, first described by Goldman and Sapirstein (1973). IMP is ideally suited for studies in which rCBF is expected to be in the high range, since the extraction rate of IMP remains linear even at high rates of CBF (Lear *et al.* 1982; Bryan *et al.* 1988). In all protocols, a reference blood sample was withdrawn at a steady rate of 0.4 ml min^{-1}, which provided a reference flow rate (R in equation 2) necessary to determine rCBF.

Rats were given at least 1 h to stabilize after all the cannula connections had been made. In all groups, the arterial withdrawal was started first, followed by injection of IMP into the femoral vein cannula. In the resting control and surface-swimming groups, the IMP was allowed to circulate for 40 s, after which pentobarbitol was rapidly infused to cause cardiac arrest and stop the circulation of the tracer. In the diving group, the 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 $\dot{Q}$ associated with diving

bradycardia. These tracer distribution times were chosen on the basis of arterial radioisotope-dilution curves that have previously been determined in conscious rats (Lin and Baker, 1975). Lin and Baker (1975) determined that the peak of the radioisotope-dilution curve is reached within 15 s in control rats (heart rate 411±11 beats min^{-1}), whereas the peak of the dilution curve is not reached until approximately 30 s during forced diving in rats (heart rate 118±14 beats min^{-1}). Therefore, we adjusted the circulation time of the radioisotope in the diving group from 30 to 50 s to reflect the cardiovascular changes that occur as a result of diving bradycardia. On the basis of the above arterial circulation times for radioisotopes, the rCBF values in the current experiments reflect a limited time frame after injection of the tracer and not a 'smeared' measurement of the entire experimental period. For the resting control and surface-swimming groups, the values in the current experiment are indicative of rCBF in the time frame 5–15 s after injection, whereas for the dive group, the rCBF values are indicative of rCBF in the time frame 15–35 s into the dive.

A detailed account of the determination of rCBF has been given elsewhere (Bryan *et al.* 1988; Stephenson *et al.* 1994). Briefly, rCBF is calculated according to the following relationship:

$$\mathrm{CBF} = C_\mathrm{b} \Big/ \int_0^T C_\mathrm{a}\mathrm{d}t \ , \qquad (1)$$

where C_b is the concentration of the tracer in a particular brain region and C_a is the concentration of the tracer in arterial blood at any time t. The evaluation of the numerator in equation 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 (T) (Sapirstein, 1958; Goldman and Sapirstein, 1973; Van Uitert and Levy, 1978). It is important that the arterial blood withdrawal is terminated at the same time that the animal is killed. However, timing errors are minimized by injecting IMP as a bolus, since the arterial concentration is very low towards the end of the experimental period (Patlak *et al.* 1984). The integrated arterial blood sample can be expressed in terms of rate of blood withdrawal, R (ml min^{-1}), and the total tracer activity in the withdrawn blood sample, Q_A (disints min^{-1}):

$$\int_0^T C_\mathrm{a}\mathrm{d}t = Q_\mathrm{A}/R \ . \qquad (2)$$

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

$$Q_\mathrm{A} = C_\mathrm{s} \times M_\mathrm{r}/M_\mathrm{s} \ , \qquad (3)$$

where C_s is the quantity of tracer in a sample of blood, M_s is the mass of the sample 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 °C. The brains were cut at 20 µm thickness and placed in contact with autoradiographic film (Kodak TMS-1 RA, Eastman Kodak Co., Rochester, NY, USA; 18 cm×24 cm) in a light-tight cassette. After a short exposure period (5–10 days), the film was developed and a grey level brain image with a spatial resolution of approximately 100 µm was produced (Greenberg, 1989). Densitometry was performed on autoradiographic images using a computer-based image-analysis system (Image 1, Universal Imaging Corp., West Chester, PA, USA). The autoradiograph grey level density was converted to tissue tracer concentration using calibrated ^{14}C standards (American Radiolabeled Chemicals Inc., St Louis, MI, USA), which were packed with the brain slices. Regional cerebral blood flow was calculated in absolute terms (ml min^{-1} 100 g^{-1}), and rates of blood flow were pseudocolour-coded and displayed as a colour image (Fig. 1). To identify brain nuclei positively, the 20 µm brain slices were stained with Neutral Red, which stains for Nissl bodies in neurones. The stained slides were then compared with a stereotaxic brain atlas (Paxinos and Watson, 1986) to identify specific brain regions.

Estimation of cardiac output

Cardiac output ($\dot{Q}$) was determined using the 'reference sample' technique. Blood was withdrawn from the femoral artery at a rate of 0.4 ml min^{-1} during the experimental protocol. Cardiac output was determined from the equation:

$$\dot{Q} = 0.4C_\mathrm{t} \Big/ \int_0^T C_\mathrm{a}\mathrm{d}t \, , \qquad (4)$$

where C_t is the total counts of tracer injected, determined by the principle described in equation 3. Therefore, $\dot{Q}$ was determined by dividing the withdrawal rate by the fraction of injected tracer in the 'reference sample'. A potential error in the determination of $\dot{Q}$ is the possibility that IMP is not extracted during the first pass through the peripheral tissues, which would result in an overestimation of $\dot{Q}$.

Statistical analyses

All values reported in the text and figures are grand means ± standard error (S.E.M.); Heart rate (f_H, beats min^{-1}) and mean arterial blood pressure ($\bar{P}$a, mmHg; 1 mmHg=0.133 kPa) were measured for each animal in all protocols. If only one of the two arterial cannulas was patent on the day of the experiment, a blood pressure tracing was taken before the experiment for determination of f_H and $\bar{P}$a. f_H and $\bar{P}$a were determined by calculating f_H and $\bar{P}$a at 5 s intervals and then averaging the values for the entire experimental period. $\bar{P}$a was calculated from pulsatile blood pressures traces (diastolic plus one-third pulse pressure). Stroke volume (V_S; ml) and total peripheral

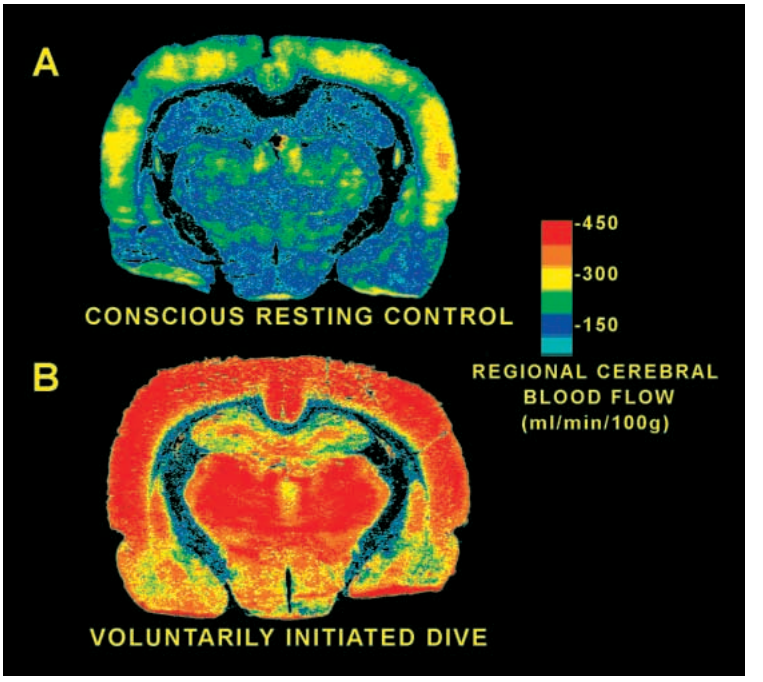

Fig. 1. Autoradiographic images of coronal sections through the rostral portion of the hippocampus, thalamus, hypothalamus and cerebral cortex in a resting control (A) and a voluntarily diving rat (B). Images have been pseudocolour-coded to calibrated regional cerebral blood flow (rCBF) values. During diving, rCBF increased to all brain regions except the globus pallidus, hypothalamus and amygdala.

resistance (R_{TP}; mmHg ml^{-1} min^{-1}) were calculated by substituting $\bar{P}$a, $\dot{Q}$ and f_H into the equations:

$$\bar{P}a = \dot{Q} \times R_{TP} \quad (5)$$

and

$$\dot{Q} = f_H \times V_S . \quad (6)$$

All cardiovascular variables represent approximately the same time frame as the rCBF values in the experimental period, as described above. Regional cerebral blood flow (ml min^{-1} 100 g^{-1} wet brain tissue) was determined in 33 brain regions from two separate measurements. Grand means were calculated by averaging the rCBF values in specific brain regions from all animals in a protocol. Brain regions were grouped into divisions on the basis of function (basal ganglia and thalamus, limbic system and primary cortical regions) except for the hindbrain, which was grouped according to anatomical location since the hindbrain consists of a multitude of smaller functional regions. Global cerebral blood flow (CBF; ml min^{-1}) was estimated by using the equation:

$$CBF = \text{mean rCBF} \times \text{brain mass}, \quad (7)$$

where mean rCBF represents the non-weighted mean of all 33 brain regions measured in each animal. We assumed that a mean of the 33 brain regions reflected flow throughout the whole brain, since the regions spanned from posterior to anterior. Since the brain had to be rapidly frozen upon removal, brain mass was calculated by using a correlation (r^2=0.98) between rat body mass and rat brain mass from previously published data (Zeman and Maitland Innes, 1963). Cerebral vascular resistance (R_{CV}; mmHg ml^{-1} min^{-1}) was determined by substituting CBF and $\bar{P}$a into the equation:

$$\bar{P}a = CBF \times R_{CV} . \quad (8)$$

Statistical analyses were performed using a computer package (SYSTAT, Systat, Evanston, IL, USA). The data were analyzed using one-way analysis of variance (ANOVA) with significance reached when P<0.05 (Zar, 1984). In the case of significant F-values, Tukey's honestly significant difference *a posteriori* tests were performed to determine differences among group means.

Results

Cardiovascular response to surface swimming and diving

Heart rate decreased immediately upon voluntary submersion and remained at that level throughout the dive period (Fig. 2). Injection of the tracer during submersion did not alter blood pressure or heart rate. Cardiovascular variables measured in the three groups of rats are presented in Table 1. During diving, there was a significant fall in f_H (from 408.4±16.7 to 116.6±6.7 beats min^{-1}), which decreased $\dot{Q}$ by 69.2 % (from 181.4±13.2 to 55.8±8.0 ml min^{-1}) from resting control values. All estimated $\dot{Q}$ values were moderately higher than values previously determined using microspheres or the thermodilution techniques (Coleman *et al.* 1984). R_{TP} increased (from 0.68±0.1 to 2.98±0.4 mmHg ml^{-1} min^{-1}) by over fourfold during diving, leading to a significant increase in $\bar{P}$a (from 120.1±6.7 to 157.4 4.3 beats min^{-1}) from both resting control and surface swimming. There was no significant difference in V_S between the three groups of rats (Table 1). During surface swimming, f_H and V_S increased slightly, but not significantly, resulting in a significant increase in $\dot{Q}$ from resting control (268.6±33.5 ml min^{-1} when swimming and 181.4±13.2 ml min^{-1} when resting).

Global cerebrovascular response to surface swimming and diving

Fig. 3 presents the global cerebrovascular variables for

Table 1. *Cardiovascular variables in resting control, surface-swimming and diving rats*

	Resting control (N=7)	Surface swimming (N=7)	Conscious diving (N=6)
Heart rate, f_H (beats min^{-1})	408.4±16.7	455.2±11.9	116.6±6.7**
Mean arterial blood pressure, $\bar{P}a$ (mmHg)	120.1±6.7	131.0±4.9	157.4±4.3**
Cardiac output, $\dot{Q}$ (ml min^{-1})	181.4±13.2	268.6±33.5*	55.8±8.0**
Total peripheral resistance, R_{TP} (mmHg ml^{-1} min^{-1})	0.68±0.1	0.57±0.1	2.98±0.4**
Stroke volume, V_S (ml)	0.45±0.0	0.55±0.1	0.51±0.1

Values are means ± S.E.M.; N, number of animals.
Values reflect approximately the same time frame as regional cerebral blood flow measurements.
**Response significantly different from resting control and surface swimming; *response significantly different from resting control (P<0.05).
1 mmHg = 0.133 kPa.

resting control, surface-swimming and diving rats. During diving, CBF increased significantly (to 7.9±0.8 ml min^{-1}) from both resting control (4.7±0.4 ml min^{-1}) and surface-swimming (5.4±0.3 ml min^{-1}) values. There was a significant 1.7-fold increase in CBF during diving compared with control values. R_{CV} decreased significantly during diving (to 20.5±1.3 mmHg ml^{-1} min^{-1}) from both resting control (25.9±1.5 mmHg ml^{-1} min^{-1}) and surface-swimming (to 26.4 1.3 mmHg ml^{-1} min^{-1}) values. Therefore, the increase in CBF during diving is due primarily to a corresponding 20.9 % decrease in R_{CV} compared with control values. The brain's share of $\dot{Q}$ increased over fivefold during diving (13.6±1.5 %) compared with both resting control (2.6±0.1 %) and surface-swimming (2.1±0.2 %) values.

Regional cerebrovascular response to surface swimming and diving

Regional cerebral blood flow was determined in 33 brain regions (Figs 4–7). During diving, rCBF increased significantly in 29 of the 33 brain regions examined, compared with both resting control and surface-swimming values (Figs 4–7). During diving, rCBF increased significantly from both resting control and surface-swimming values in all regions of the hindbrain and thalamus examined (Figs 4, 5). There was a slight, but insignificant, increase in flow to the caudate putamen-posterior (CPu-P), globus pallidus (GP), hypothalamus (H) and amygdala (A) during diving compared with both resting control and surface-swimming values (Figs 5, 6). In all primary cortical regions, rCBF was significantly increased during diving compared with resting control values, except in the inferior colliculus (IC), for which the increase was only significantly different from the surface-swimming value (Fig. 7). In surface-swimming rats, rCBF to the forelimb motor area (FL) of the cerebral cortex was the only significantly different brain region compared with resting control values (Fig. 7). The largest absolute difference in rCBF (diving minus resting control) occurred in the entorhinal cortex (230 ml min^{-1} 100 g^{-1}), whereas the smallest difference was in the hypothalamus (9 ml min^{-1} 100 g^{-1}).

Discussion

This study measures, for the first time, the detailed distribution of rCBF in a conscious, voluntarily diving mammal. In 29 of 33 brain regions examined, rCBF increased significantly during diving, despite a profound decrease in $\dot{Q}$ associated with diving bradycardia. Only some regions of the basal ganglia (CPu-P and GP) and limbic areas (H and A) did not increase rCBF significantly during diving compared with both resting control and surface swimming. Unequal regional participation in the global cerebrovascular response to diving suggests that there may be underlying cerebrovascular

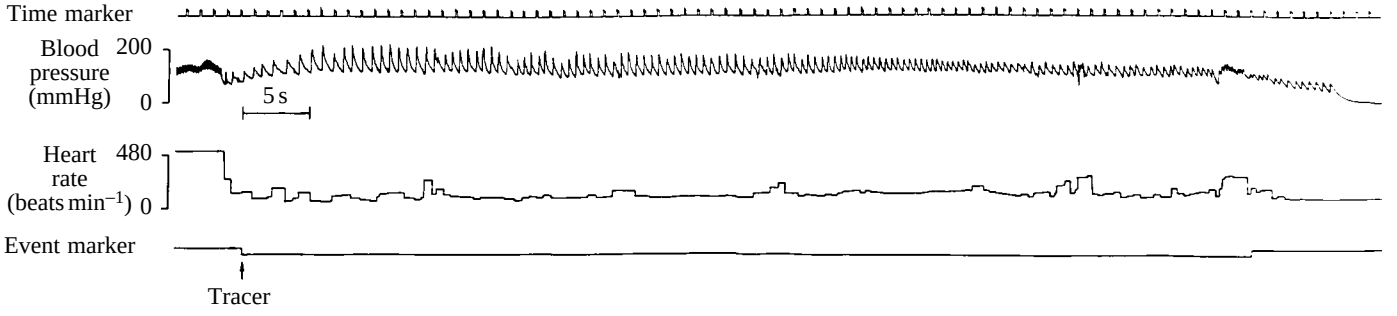

Fig. 2. Original recording of pulsatile blood pressure and heart rate in a conscious voluntarily diving rat swimming through an underwater maze. Downward deflection of the event marker signifies bolus injection of cerebral blood flow tracer and the start of arterial blood withdrawal. Injection of tracer and withdrawal of arterial blood did not interfere with cardiovascular variables throughout the dive. Upward deflection of the event marker signifies lethal injection of pentobarbitol. 1 mmHg=0.133 kPa.

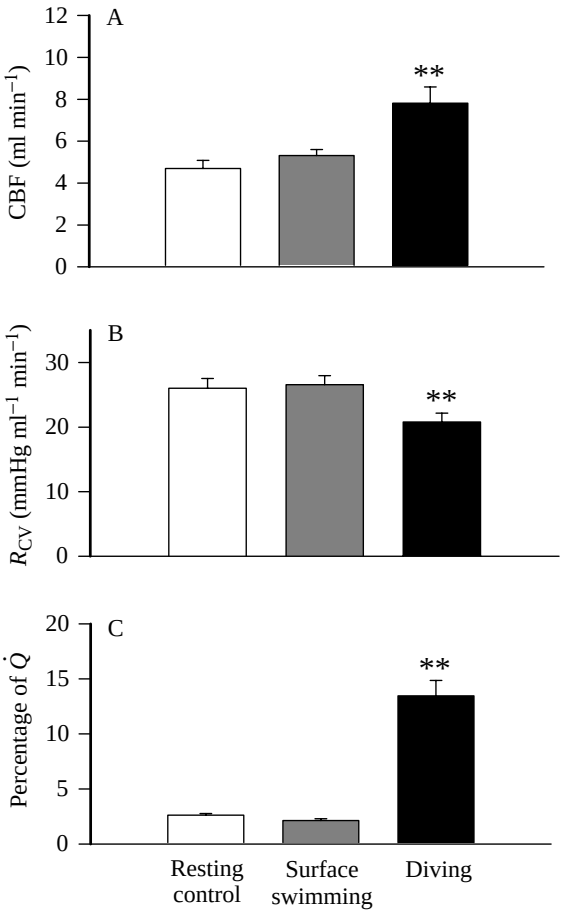

Fig. 3. Cerebral blood flow (CBF; A), cerebrovascular resistance (R_{CV}; B) and percentage of cardiac output to the brain (% of $\dot{Q}$; C) in resting control, surface-swimming and diving rats. Values are means + S.E.M. During diving, CBF (A), R_{CV} (B) and the percentage of $\dot{Q}$ to the brain (C) were all significantly different from both resting control and surface-swimming values. **Response significantly different from resting control and surface-swimming values (P<0.05). 1 mmHg=0.133 kPa.

mechanisms producing differential changes in R_{CV}. These results therefore differ somewhat from those of Stephenson *et al.* (1994), who found all brain regions increased flow significantly during forced diving in the Pekin duck.

Global cerebrovascular response to diving

We have found a significant 1.7-fold increase in absolute CBF during diving compared with control values. This indicates that the share of $\dot{Q}$ going to the brain increased over fivefold during diving. However, our estimate for CBF included brain regions composed primarily of grey matter. Perfusion to white matter has been shown to be significantly less than to grey matter (Edvinsson *et al.* 1993). Therefore, it is possible that CBF overestimates blood flow since this value does not include blood flow to white matter in its derivation. These results agree with other studies on ducks (Jones *et al.* 1979) and beavers (McKean, 1982), which found CBF to increase significantly during forced head immersion. Our results differ from a previous study by Lin and Baker (1975), which determined the distribution of $\dot{Q}$ during forced head immersion in rats. They found that blood flow to the

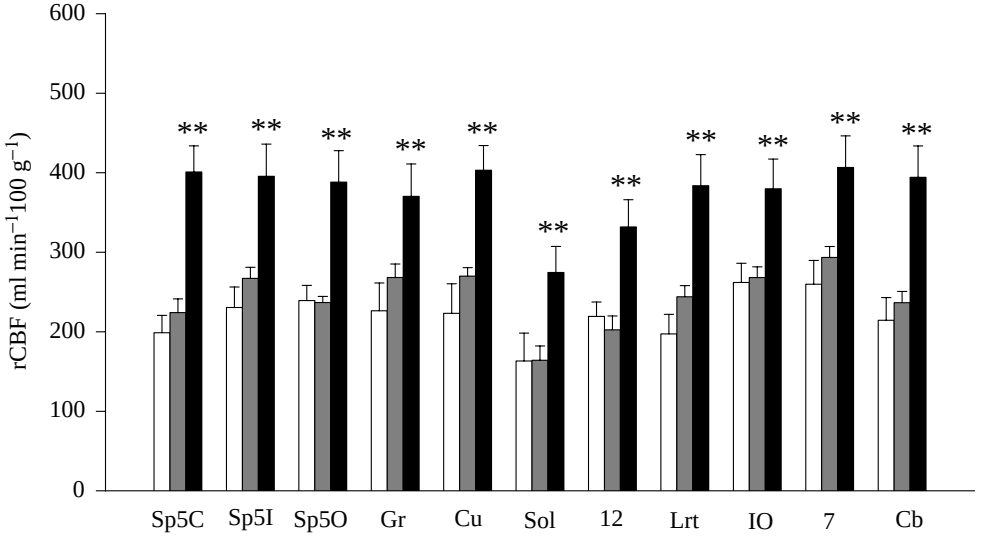

Fig. 4. Regional cerebral blood flow (rCBF) values in hindbrain regions of resting control (open columns), surface-swimming (hatched columns) and diving (filled columns) rats. Values are means ± S.E.M. During diving, rCBF increased significantly from both resting control and diving values in all brain regions. Sp5C, spinal trigeminal nucleus-caudal part; Sp5I, spinal trigeminal nucleus-interpolaris part; Sp5O, spinal trigeminal nucleus-oral part; Gr, gracile nucleus; Cu, cuneate nucleus; Sol, nucleus of the solitary tract; 12, hypoglossal nucleus; Lrt, lateral reticular nucleus; IO, inferior olive; 7, facial nucleus; Cb, cerebellum. **Response significantly different from resting control and surface-swimming values (P<0.05).

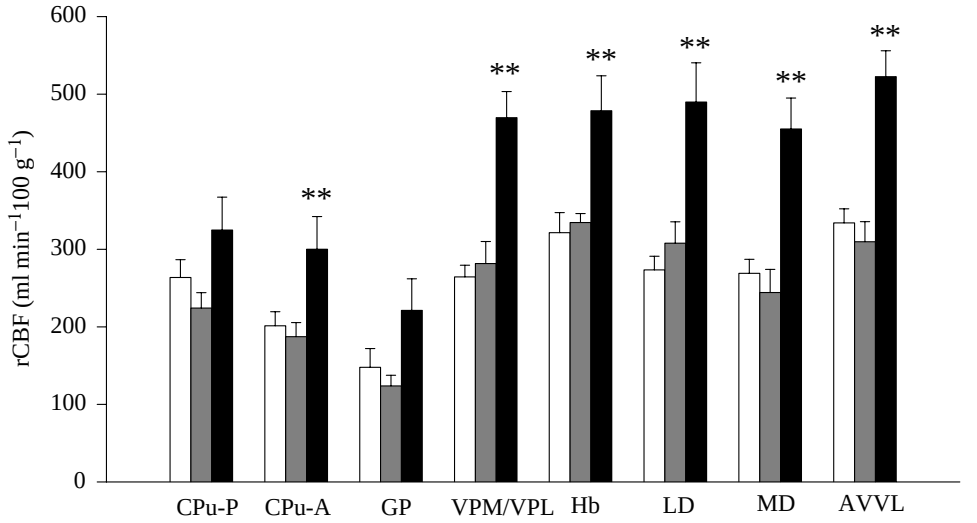

Fig. 5. Regional cerebral blood flow (rCBF) values in the basal ganglia and thalamic nuclei in resting control (open columns), surface-swimming (hatched columns) and diving (filled columns) rats. Values are means + S.E.M. During diving, rCBF did not increase significantly to the posterior portion of the caudate putamen (CPu-P) and the globus pallidus (GP), but increased significantly to all regions of the thalamus compared with both resting control and surface-swimming values. CPu-A, caudate putamen-anterior; VPM/VPL, ventral posteromedial and ventral posterolateral thalamic nuclei; Hb, habenular nucleus; LD, laterodorsal thalamic nulceus; MD, mediodorsal thalamic nucleus; AVVL, anteroventral thalamic nucleus. **Response significantly different from resting control and surface-swimming values (P<0.05).

brain remained at predive levels during forced head immersion, despite a marked peripheral redistribution of $\dot{Q}$ Lin and Baker (1975), however, stated that the radioisotope that they used, ^{137}caesium (^{137}Cs), is diffusion-limited across the blood–brain barrier and therefore potentially underestimates CBF values.

The increase in CBF during diving is primarily due to a corresponding 20.9 % decrease in R_{CV}. Coexisting with the large decrease in R_{CV} was a fourfold increase in R_{TP} that matched the decrease in $\dot{Q}$ associated with diving bradycardia. These results show that the cardiovascular and cerebrovascular changes associated with mammalian diving are opposite in direction. During diving, R_{TP} increases and R_{CV} decreases. The overall result is an absolute increase in CBF despite the profound decrease in $\dot{Q}$ associated with diving bradycardia.

The large increase in R_{TP} produced a significant increase in mean arterial blood pressure ($\bar{P}$a) during diving above both resting control and surface-swimming values. Changes in perfusion pressure do not normally result in alterations in CBF owing to the ability of the cerebral circulation to autoregulate (Kuschinsky, 1991). Generally, the autoregulatory mechanism keeps CBF constant by vasodilating in response to decreased arterial pressure and vasoconstricting in response to increased arterial pressure (Edvinsson *et al.* 1993). In the rat, the lower and upper limits of cerebrovascular autoregulation by $\bar{P}$a are approximately 50 and 150 mmHg, respectively (Hernandez *et al.* 1978). In the present study, $\bar{P}$a increased to a mean value of 157.4±4.3 mmHg during diving, a perfusion pressure just outside the autoregulatory limits. However, hypercapnia has been demonstrated to shift the CBF autoregulation curve to the

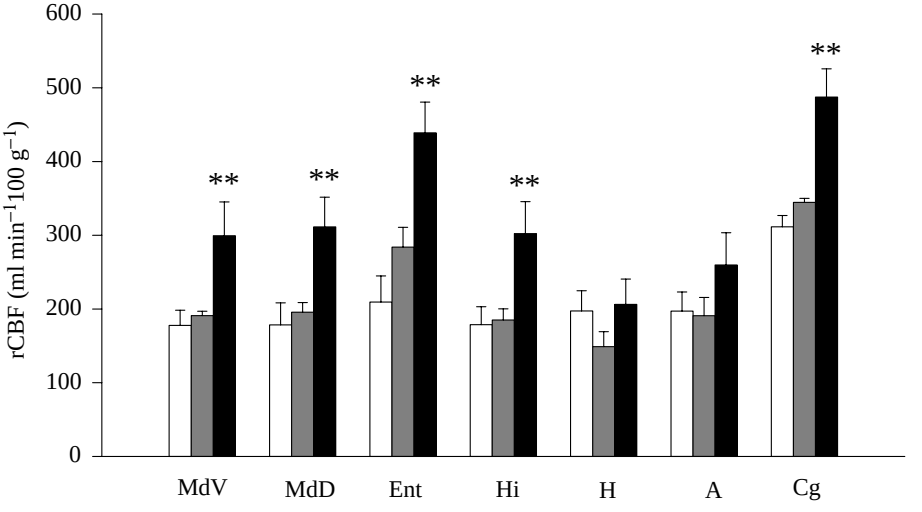

Fig. 6. Regional cerebral blood flow (rCBF) values in limbic brain regions of resting control (open columns), surface-swimming (hatched columns) and diving (filled columns) rats. Values are means + S.E.M. During diving, rCBF increased significantly from both resting control and surface-swimming values in all regions except the amygdala (A) and the hypothalamus (H). Mdv, medullary reticular nucleus, ventral part; MdD, medullary reticular nucleus, dorsal part; Ent, entorhinal cortex; Hi, hippocampus; Cg, cingulate cortex; H, hypothalamus. **Response significantly different from resting control and surface-swimming values (P<0.05).

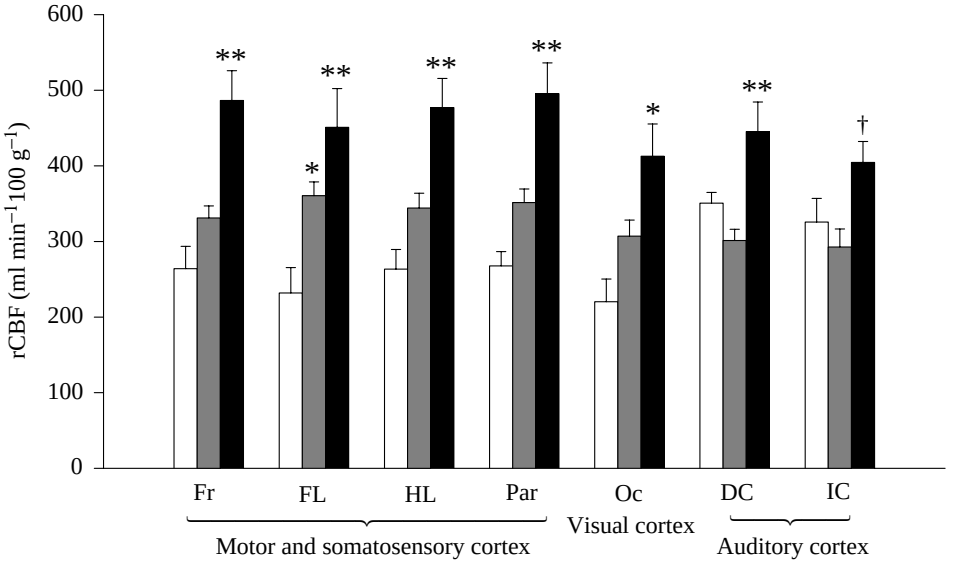

Fig. 7. Regional cerebral blood flow (rCBF) values in primary cortical regions of resting control (open columns), surface-swimming (hatched columns) and diving (filled columns) rats. Values are means + S.E.M. During diving, rCBF increased significantly from both resting control and surface-swimming values in all regions of the motor and somatosensory cortex. Fr, frontal cortex; FL, forelimb area of the cortex; HL, hindlimb area of the cortex; Par, parietal cortex; Oc, occipital cortex; DC, dorsal cochlear nucleus; IC, inferior colliculus. **Response significantly different from resting control and surface-swimming values; *response significantly different from resting control value; †response significantly different from surface-swimming value (P<0.05).

left (Raichle and Stone, 1972; Paulson *et al.* 1990). A curve shift to the left would increase CBF linearly over a lower range of blood pressures (<150 mmHg). This suggests that the ability of the cerebral vasculature to autoregulate may be partially lost during the dive, and increased perfusion pressure could make a minor contribution to the increase in CBF during diving in the rat.

The stimulus for the global decrease in cerebrovascular resistance during diving

Previous studies have implicated carbon dioxide (CO_2) as a potential mediator of cerebral vasomotion during diving in sea lions (Dormer *et al.* 1977), seals (Blix *et al.* 1983) and ducks (Jones *et al.* 1979; Stephenson *et al.* 1994). These investigators found that flow to the brain increased linearly with dive duration, suggesting a possible link between CBF and the progressive hypercapnia associated with diving. Numerous studies have clearly demonstrated that hypercapnia elicits a marked vasodilation in the cerebral circulation (for a review, see Edvinsson *et al.* 1993). In almost all studies, CBF increases steeply in response to increased levels of CO_2. This suggests that progressive hypercapnia during diving may produce widespread cerebrovasodilation. During asphyxic diving, however, as the CO_2 level is increasing, arterial oxygen concentration is also decreasing. Therefore, it is possible that hypoxia also stimulates a decrease in R_{CV}. The reactivity of the cerebrovasculature in response to hypoxia is significantly less than to hypercapnia (McDowall, 1966), indicating that the majority of the decrease in R_{CV} during diving is probably due to hypercapnia and not to hypoxaemia.

Stimuli producing differential cerebrovascular changes during diving

The results from this study raise the possibility that not all brain regions participate equally in the global cerebrovascular response to diving, suggesting that there may be underlying mechanisms producing differential changes in R_{CV} during diving. A number of stimuli could potentially modify the global cerebrovascular response to conscious diving. Hypercapnia itself could be the first of these stimuli. Regional alterations in CBF have been reported during hypercapnia and/or acute asphyxia (Shapiro *et al.* 1980; Goplerud *et al.* 1989). Therefore, it is possible that hypercapnia itself has the ability to produce a differential change in R_{CV} during diving.

Second, stimulation of the trigeminal nerve or a ganglion associated with the nerve, such as the sphenopalatine ganglion, has been shown to produce regional variations in CBF (Goadsby and Duckworth, 1987; Seylaz *et al.* 1988; Suzuki *et al.* 1990). In mammals, trigeminal stimulation is necessary for the manifestation of the cardiac response to diving (Drummond and Jones, 1979; McCulloch and West, 1992; McCulloch *et al.* 1997), whereas the role of trigeminal innervation in diving bradycardia varies considerably between bird species (Jones and Purves, 1970; Furilla and Jones, 1986). Thus, it is possible that stimulation of the trigeminal nerve during mammalian diving mediates regional variations in R_{CV}.

Third, the sympathetic component of the mammalian dive response normally produces peripheral vasoconstriction. This neural outflow could possibly act upon cerebral blood vessels. The cerebral circulation has a well-developed sympathetic innervation (for a review, see Edvinsson *et al.* 1993).

Furthermore, there is evidence of a differential distribution of sympathetic nerve fibres on the cerebrovasculature (Edvinsson and Owman, 1977). The caudate nucleus and hippocampus brain regions have been shown to have an extensive vascular sympathetic innervation (Edvinsson, 1975; Edvisson and Lindvall, 1978). There is evidence to suggest that innervation distribution in the cerebral vessels parallels the degree to which sympathetic nerve stimulation produces an alteration in cerebral perfusion (Sercombe *et al.* 1975). If efferent sympathetic outflow acts differentially upon the cerebrovasculature during diving, regional variations in CBF could result.

Lastly, the variability of the capillary density in different regions of the rat brain could constrain regional increases in CBF during diving (Klein *et al.* 1986). In the rat brain, capillary density has been shown to vary significantly between brain regions (Gobel *et al.* 1990). These investigators reported that the capillary density of the inferior colliculus (IC) and of some cortical areas was higher than the capillary density of the hippocampus (H) and caudate nucleus. This anatomical constraint may be responsible for the blood flow-limitation that was observed in some regions of the basal ganglia and limbic system during diving in this study.

In summary, we have demonstrated that the brain is preferentially perfused during conscious diving in the rat. We report an overall 1.7-fold increase in cerebral blood flow during diving, despite a profound decrease in cardiac output associated with the dive response. A detailed examination of the regional distribution of cerebral blood flow suggests that almost all brain regions are hyperperfused during diving. Some regions of the basal ganglia and limbic system did not increase flow significantly during diving, suggesting that not all brain regions participate equally in the global cerebrovascular response to diving. The stimuli producing the global cerebrovascular changes during conscious diving remain to be elucidated, although an obvious stimulus is progressive hypercapnia. We have also suggested a number of stimuli that could potentially modify the global cerebrovascular response to conscious diving. Of these stimuli, trigeminal input is a likely candidate, since this input has been demonstrated to be necessary for the cardiac response to diving in small mammals (McCulloch *et al.* 1997) and has been shown to elicit regional alterations in CBF (Goadsby and Duckworth, 1987). However, this input must operate within the powerful anatomical constraints of differential capillary density within the brain.

We thank Dr P. F. McCulloch and L. K. Bekar for their assistance. The research was supported by a research grant from the Natural Sciences and Engineering Research Council of Canada (NSERC) to N.H.W. G.P.O. was supported by a Heart and Stroke Foundation of Canada Research Traineeship.

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