Regional cerebral blood flow during submergence asphyxia in Pekin duck

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Stephenson, Richard, David R. Jones, and Robert M. Bryan, Jr. Regional cerebral blood flow during submergence asphyxia in Pekin duck. *Am. J. Physiol.* 266 (*Regulatory Integrative Comp. Physiol.* 35): R1162–R1168, 1994.—The cerebrovascular response to submergence asphyxia was studied in the Pekin duck (*Anas platyrhynchos* var.) by use of the cerebral blood flow (CBF) tracer [^{14}C]isopropyliodoamphetamine and quantitative autoradiography. Blood flow of the whole brain was 158 ± 14 (SE) ml·min^{-1}·100 g^{-1} (n = 7) in control animals. There was a doubling of flow to 320 ± 61 ml·min^{-1}·100 g^{-1} (n = 6) during submergence asphyxia. The hypothesis that CBF is redistributed within the brain during asphyxia was not supported. There were no regional reductions in CBF during submergence asphyxia. Mean arterial blood pressure was similar (~140 mmHg), but heart rate, arterial blood gas tensions, and arterial pH were significantly different in control and submerged ducks at the time CBF was measured. The differences in CBF among submerged animals correlated strongly with arterial P_{CO_2} and mean arterial blood pressure. The smallest proportional difference in regional CBF between control and submerged ducks occurred in the ectostriatum (+141%) and the largest in the locus ceruleus (+241%). The largest absolute difference in regional CBF was in the nucleus ruber (+322 ml·min^{-1}·100 g^{-1}). These are the first measurements of blood flow in discrete nuclei and regions of the avian brain.

quantitative autoradiography; forced dive; functional brain mapping

THE ABILITY OF BIRDS and mammals to survive prolonged periods of submergence is dependent on their capacity to conserve oxygen for the brain (5, 6). Sparing of endogenous oxygen stores is effected by a profound cardiovascular reflex in which most tissues, particularly the skeletal muscles and gut, are rendered ischemic (3, 21, 36).

If the brain is a major contributor to the depletion of oxygen during submergence, significant prolongation of underwater survival time might be achieved by selective redistribution of cerebral blood flow (CBF) so that any hypoxia-tolerant regions of the brain are rendered partially or completely ischemic. Such a mechanism would effectively reduce the rate of oxygen consumption by reducing the mass of perfused, and therefore aerobic, tissue. In support of this idea, radioactive microsphere studies in seals showed that there may be considerable variability between brain regions in the cerebrovascular response to submergence (3, 36). However, the results differed considerably between studies, probably because of species and protocol differences. There are also reports of heterogeneous regional CBF (rCBF) responses to asphyxia in fetal and newborn terrestrial mammals (20, 29, 34), including regional reductions in flow in asphyxic newborn dogs and piglets (13, 19).

There have been few studies of avian CBF. Blood flow in normoxic brain of domestic duck and bar-headed goose increased dramatically during hypercapnia (2, 9, 16), and the cerebrovascular sensitivity to arterial P_{CO_2} (Pa_{CO_2}) was greater than that in mammals (7, 29). Moreover, Faraci and Fedde (9) found higher CBF-Pa_{CO_2} sensitivity in the cerebral hemispheres than in the cerebellum and brain stem in the goose. CBF of whole duck and goose brains also increased during normocapnic and hypocapnic hypoxia, the increase in perfusion being better related to oxygen delivery than to arterial P_{O_2} (Pa_{O_2}) (10, 14, 15).

Global CBF increased progressively during forced immersion in mallard and Pekin ducks, reaching 8.6 times the "predive" value at 144–250 s of submergence (21). Jones et al. (21) concluded that the separate effects of hypoxia and hypercapnia, as reported by Grubb et al. (14, 16), summate during submergence. However, the predive CBF values measured by Jones et al. were lower than those in other studies, which would impact on the validity of their calculation.

All techniques used so far in avian CBF studies (inert gas clearance, organ trapping of radioactive microspheres, optical methods, and H_2 clearance) are open to criticism (1), although many of the potential methodological problems were explicitly avoided in some of the studies cited above. We used a different approach, quantitative autoradiography, to measure the effects of submergence asphyxia on global and regional cerebral blood flow in awake Pekin ducks. We tested the hypothesis that there is a redistribution of blood flow between brain regions during prolonged submergence in ducks. A secondary aim of the study was to assess the potential usefulness of the technique as a means of functional mapping of the avian brain. We exploited the high spatial resolution of the technique to identify specific brain nuclei that may be involved in cardiorespiratory control during submergence.

METHODS

All procedures involving the use of live animals were done in compliance with the guidelines of the Canadian Council on Animal Care. The work was conducted at the Baylor College of Medicine (Houston, TX) with the approval of the Animal Protocol Review Committee.

Fourteen Pekin ducks of both sexes (body mass 2.1–2.8 kg) were used in the study. Before surgery, the ducks were housed as a group with free access to food and water for drinking and bathing. After surgery, they were kept singly or in pairs in

large dry cages with food and drinking water supplied ad libitum.

Animal preparation. Ducks underwent surgery ≥14 h before rCBF experiments. Anesthesia was induced with 3% isofluorane in 70% N_2O-30% O_2, after which the isofluorane was reduced to 1%. Catheters (PE-90 or PE-160) were implanted into two brachial (wing) arteries and veins. In the ducks to be subjected to submergence, an intravascular oxygen electrode (size F3 or F4, Orange Medical Instruments) with a built-in cannula was used instead of polyethylene tubing in one brachial artery. The catheters were filled with heparinized (200 IU/ml) saline solution, plugged or flame-sealed, and then coiled and taped to the underside of the wing. The ducks were given a broad-spectrum antibiotic (100 mg/kg im, ampicillin sodium, Ayerst Laboratories) and allowed to recover overnight.

Experimental protocol. Ducks were randomly divided into two groups: seven control and seven submerged. They were positioned in ventral recumbency on a padded bench. They were restrained with use of paper tape, and the head was held by a padded clamp in such a way that the bill pointed vertically downward into a glass beaker and the brain was at approximately the same level as the heart. The duck's neck lay over the blade of a guillotine.

One venous catheter was connected to a syringe containing the radioactive blood flow tracer, the other to a syringe containing concentrated pentobarbital sodium (500 mg/ml) to kill the animals. One arterial cannula was connected to a preweighed heparinized 2-ml glass syringe attached to an infusion/withdrawal pump (Harvard Apparatus Pump 22) for withdrawal of a reference blood sample. The second arterial catheter was attached via a four-way stopcock assembly to a pressure transducer (Statham Spectramed P23XL) and syringes for withdrawal of discrete (0.7-ml) blood samples. Bipolar subcutaneous electrocardiographic electrodes were attached at the right shoulder and left leg.

Arterial blood pressure, mean arterial blood pressure (MABP), electrocardiogram, and heart rate were recorded on a Grass model 7 polygraph with appropriate amplifiers. Gas tensions and pH of arterial blood samples were analyzed at 37°C with a Corning 178 pH/blood gas analyzer. Readings were corrected to the duck's body (axillary) temperature (~41°C) with use of the automatic facility built into the blood gas analyzer. In the submerged group, the intravascular oxygen electrode was connected to a Po_2 monitor and calibrated during steady-state rest by analysis of a blood sample withdrawn from the same artery.

Measurement of rCBF. The tracer *n*-isopropyl-*p*-iodoamphetamine [isopropyl *methyl*-1,3-^{14}C] (IPIA) was used. A preliminary test confirmed that IPIA is not sequestered by avian erythrocytes. Because IPIA is fully extracted from the blood during a single pass through the brain (7), the experiment was conducted as a conventional organ-trapping protocol. A reference blood sample was withdrawn at a constant known rate before, during, and after a bolus injection of the tracer. This provided a reference flow rate (0.8 ml/min) with which to calculate the rCBF from autoradiographic images.

After the apparatus was connected, the ducks were given ≥30 min to stabilize. In control ducks, an arterial blood sample was taken, and then the blood withdrawal pump was activated. After 5 s, the IPIA (40 μCi/kg) was injected into a brachial vein (0.6 ml in ~10 s). After 29 ± 8 (SD) s of circulation, pentobarbital sodium was rapidly injected to effect rapid cardiac arrest, hypotension, and muscular relaxation. The duck was then decapitated, and the arterial catheter connected to the withdrawal pump was simultaneously cut.

In submerged ducks, the beaker was filled with water (20°C) after withdrawal of an initial arterial blood sample. The head of the duck was submerged past eye level. Pa_{O_2}, measured by the intravascular oxygen electrode, was monitored continually, and a second blood sample was withdrawn when Pa_{O_2} decreased to 50 Torr. Blood withdrawal, IPIA injection, and experiment termination then followed in the same way as for control ducks, except circulation was allowed for 91 ± 7 s to accommodate the considerably lower cardiac output (and therefore longer circulation time) during submergence. The first submerged duck was allowed only 20 s of circulation time, and the quantity of IPIA in the brain was found to be extremely low. This duck was therefore not included in analysis, reducing sample size to six for the submerged group.

CBF was calculated according to the following relationship, the derivation of which was described in detail by Bryan et al. (7)

$$CBF = C_b / \int_0^T C_a \, dt \qquad (1)$$

where C_b is concentration of tracer in a particular brain region and C_a is concentration of tracer in arterial blood at any *time t*. The denominator of *Eq. 1* is the integral of arterial blood tracer concentration from the time of injection to the time the experiment was terminated (T). This quantity was obtained from analysis of the reference blood sample

$$\int_0^T C_a \, dt = C_r / F_r \qquad (2)$$

where C_r is the quantity of tracer in the reference sample and F_r is the reference blood flow rate. The reference blood sample was weighed and vortexed, and three 20-μl aliquots were each weighed, solubilized, decolorized, and counted in a liquid scintillation counter (Packard Minimax model B4430, United Technologies). C_r was obtained as follows

$$C_r = C_s \times M_r / M_s \qquad (3)$$

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 whole reference blood sample. A mean value of the three estimates of C_r was used in subsequent calculations.

After death, the brain was immediately removed from the skull and frozen at −50°C by immersion in isopentane (2-methylbutane). The brains were cut into 20-μm-thick sections with use of a refrigerated cryostat. Every 10th section was put on a glass slide and placed in contact with X-ray film (Dupont Chonix Microvision mammography film) in light-tight cassettes. Autoradiographic images were analyzed using a computer-based image analysis system (MCID, Imaging Research). The optical densities of the autoradiographs were converted to tissue IPIA concentrations by use of calibrated ^{14}C standards (American Radiolabeled Chemicals), which were packed in the cassettes with the tissue sections. Mean CBF for each region of interest was determined from an average of at least six bilateral measurements. Whole brain CBF was estimated by averaging the mean CBF of all exposed sections of the brain.

Specific brain nuclei were identified by reference to three stereotaxic atlases of avian brains (23, 24, 37). The brains were sliced in approximately the plane used by Kuenzel and Masson (24). Only clearly visible nuclei were analyzed.

Statistical analysis of the data. Data were analyzed using Data Desk software (Data Description, Ithaca, NY). Results are presented as means ± SE. Differences between control and submerged groups were tested using independent-samples *t*

test with the assumption of unequal variance. In data exhibiting significant heteroscedasticity, t tests were performed after logarithmic transformation. Comparisons among three or more brain regions within a group of ducks were made using one-way analysis of variance (ANOVA). Comparisons between physiological variables before and during immersion in the submerged group were made using paired-samples t test. Differences were considered significant at the 95% confidence level ($P < 0.05$).

RESULTS

Physiological variables measured in control and submerged ducks are presented in Table 1. The submerged group did not differ significantly from the control group in any of the variables measured before head immersion. Heart rate of submerged ducks decreased significantly to 28% of the presubmergence value after >4 min of apnea. Bradycardia was not accompanied by a significant change in MABP. Blood gas tensions and pH, measured immediately before injection of IPIA, were significantly different between control and submerged groups. Submergence asphyxia is not a steady-state condition, and blood gas tensions continued to change throughout the injection and tracer circulation period. Only Pa_{O_2} could be monitored continuously by means of the intravascular electrode. The minimum Pa_{O_2} reached just before decapitation (Table 1) was significantly lower (paired-samples t test, $P < 0.05$) than that at the start of IPIA injection in submerged ducks.

Average CBF of the whole brain and most major anatomic subdivisions more than doubled during submergence asphyxia (Table 2). An exception was the telencephalon, which increased by a statistically significant factor of 1.73. There were no brain regions in which CBF was lower in submerged ducks than in control ducks. There were no statistically significant differences in average CBF between major brain regions within either group of ducks (ANOVA, $P > 0.05$, Table 2). The data were variable, with coefficients of variation (SD × 100/mean) between 15 and 30% in the control group and between 45 and 60% in the submerged group (Table 2).

Within the submerged group, there were significant correlations between rCBF and all variables listed in Table 1, except body temperature. There were no significant correlations in the control group. The data for whole brain are illustrated in Fig. 1. The data for individual major brain regions (Table 2) are not shown but were qualitatively similar. The slope of the linear regression of CBF on preinjection Pa_{CO_2} in submerged ducks (Fig. 1*A*) was 24.1 ml·min^{-1}·100 g^{-1}·Torr $Pa_{CO_2}{}^{-1}$ ($r^2 = 0.798$, $P < 0.01$). There was also a positive correlation between whole brain CBF and MABP in submerged ducks ($r = 0.734$; Fig. 1*D*), but a multiple regression analysis of CBF vs. Pa_{CO_2} and MABP reduced r^2 to 0.665, and the partial regression coefficients for Pa_{CO_2} and MABP were not statistically significant ($P > 0.05$). Multiple regression with Pa_{CO_2}, Pa_{O_2}, and arterial pH as predictors was not performed because of strong colinearity between these variables. Thus it was not possible to separate the effects of these variables on CBF.

Seventeen brain nuclei could be reliably identified (Table 3). These nuclei were visually distinguishable because they received a higher blood flow than surrounding areas in control and submerged groups. The proportional difference in blood flow (submerged/control) in most of these nuclei (Table 3) was similar to the whole brain average (Table 2), ranging from 1.41 in the ectostriatum to 2.41 in the locus ceruleus. In terms of absolute difference in blood flow (ΔrCBF), the smallest (168 ml·min^{-1}·100 g^{-1}) occurred in the ectostriatum and the largest (322 ml·min^{-1}·100 g^{-1}) was in the nucleus ruber (Table 3).

Table 1. *Physiological variables in 2 groups of Pekin ducks*

	Control (n = 7)	Submerged	
		Presubmergence (n = 6)	Asphyxia (n = 6)
HR, beats/min	192 ± 10	177 ± 14	49 ± 13*
MABP, mmHg	140 ± 11	139 ± 3	135 ± 11
Pa_{O_2}, Torr	96.3 ± 1.9	94.6 ± 2.1	51.7 ± 1.5*
Minimum Pa_{O_2}, Torr			37 ± 3
Pa_{CO_2}, Torr	35.8 ± 1.3	33.4 ± 1.2	51.1 ± 2.2*
pH_a	7.48 ± 0.01	7.50 ± 0.01	7.36 ± 0.02*
Time submerged, min			4.61 ± 0.4
Body temp, °C	41.3 ± 0.2		40.9 ± 0.3

Values are means ± SE; n, no. of animals. HR, heart rate; MABP, mean arterial blood pressure; Pa_{O_2}, arterial P_{O_2}; Pa_{CO_2}, arterial P_{CO_2}; pH_a, arterial pH. *Significantly different from control ($P < 0.05$, independent-samples t test) and from presubmergence ($P < 0.05$, paired-sample t test).

Table 2. *CBF of whole brain and major brain regions*

	Control (n = 7)	Submerged (n = 6)	Submerged/ Control
Whole brain	158 ± 14	320 ± 61*	2.03
Cervical spinal cord	103 ± 6	233 ± 54*	2.26
Medulla oblongata	139 ± 15	309 ± 71*	2.22
Pons	144 ± 14	330 ± 60*	2.29
Cerebellum	142 ± 13	299 ± 59*	2.11
Mesencephalon	144 ± 16	319 ± 72*	2.22
Diencephalon	146 ± 14	329 ± 63*	2.26
Telencephalon	169 ± 17	293 ± 59*	1.73

Values are means ± SE, expressed as ml·min^{-1}·100 g wet tissue^{-1}; n, no. of animals. CBF, cerebral blood flow. *Significantly different from control ($P < 0.05$, independent-sample t test of log-transformed data).

DISCUSSION

This study has utilized quantitative autoradiography to make the first measurements of CBF in a number of discrete nuclei and regions of the avian brain. The results of the study refute the hypothesis that there is an oxygen-conserving redistribution of blood flow within the brain during submergence asphyxia. There were no regional decreases in CBF, as would be required to significantly extend underwater survival time through reduction of perfused (i.e., aerobic) tissue mass. The results confirm the generally held assumption that the entire brain is continually perfused during submergence (3, 21). Increased CBF during involuntary submergence functions to prolong underwater survival time by ensur-

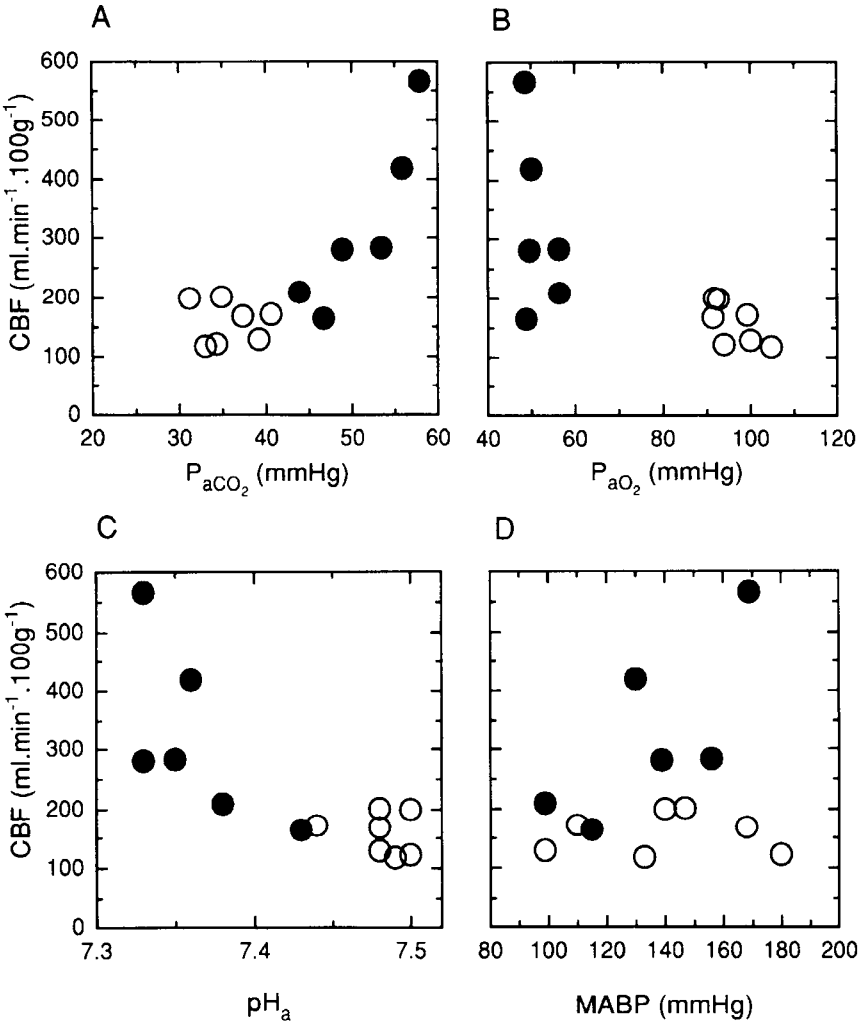

Fig. 1. Relationships between average whole brain blood flow and several physiological variables in Pekin duck. Symbols represent mean value for each animal: ○, control group; ●, submerged group. CBF, cerebral blood flow; MABP, mean arterial blood pressure; Pa_{O_2}, partial pressure of O_2 in arterial blood; Pa_{CO_2}, partial pressure of CO_2 in arterial blood.

ing adequate oxygen delivery to the brain until nearly all oxygen reserves are consumed (6).

These results in the duck differ from previous studies in submerged seals in which heterogeneous changes in CBF were observed. Zapol et al. (36) found a 1.5-fold increase in medullary blood flow and no change in blood flows in the cerebral cortex, cerebellum, thalamus, and hypothalamus in submerged Weddell seals. In contrast, Blix et al. (3) found no change in blood flow in the pons/medulla, a 1.6-fold increase in the cerebellum, and an ~2-fold increase in both the cortical and thalamic/hypothalamic regions in submerged spotted seals and gray seals. In the submerged Pekin ducks of the present study, average CBF of most major brain regions was ~2.25 times control values, although slightly smaller increases were observed in the cerebellum and telencephalon. Large differences in body mass, blood gas tensions, pH, and probably also arterial oxygen content prevent useful comparison of cerebrovascular responses to submergence asphyxia between ducks and seals and also between the two seal studies.

The results reported here also contrast with studies of CBF in fetal and neonatal mammals, where heterogeneous changes in rCBF, including local decreases in perfusion, were observed during asphyxia (13, 19, 20, 29, 34). Unfortunately many of the latter studies were confounded by changes in blood pressure during asphyxia, whereas Pekin ducks retained good control over arterial blood pressure during submergence. Massik et al. (27) observed that hypoxia and hypercapnia caused additive increases in whole brain CBF at normal MABP in anesthetized newborn lambs, but differences in rCBF were not investigated. CBF was positively correlated with MABP in submerged ducks but not in the control group, suggesting that autoregulation is lost during submergence in ducks. Unfortunately strong correlations between MABP and blood gas tensions and pH prevented a direct analysis of this hypothesis. The utility of comparisons of cerebrovascular responses between submerged ducks (or seals) and asphyxiated neonates is reduced by the fact that neonates were usually subjected to much more severe asphyxic insults and often showed signs of cardiovascular instability during CBF measurements, whereas ducks and seals invoked controlled oxygen-conserving cardiovascular adjustments during submergence.

The results of the present study also differ quantitatively from previous studies of avian CBF. Average CBF of normoxic-normocapnic whole brain and single brain regions has been reported to range from 43 to >100

Table 3. *rCBF in specific nuclei of control and submerged Pekin duck brain*

	Control ($n = 7$)	Submerged ($n = 6$)	Δ	Submerged/ Control
Cerebellar nuclei	282 ± 40	526 ± 91*	244	1.87
Complexus olivaris caudalis	251 ± 37	520 ± 105*	269	2.07
Ectostriatum	412 ± 64	580 ± 95	168	1.41
Hippocampus	238 ± 22	456 ± 84*	218	1.92
Locus ceruleus	170 ± 14	409 ± 91*	239	2.41
Neostriatum	320 ± 47	570 ± 97*	250	1.78
Nuclei gracilis and cuneatus	285 ± 42	550 ± 90*	265	1.93
Nuclei habenularis	203 ± 29	447 ± 76*	244	2.20
Nuclei hypothalami	267 ± 55	449 ± 92	182	1.68
Nuclei raphes	214 ± 26	427 ± 85*	213	2.00
Nucleus mesencephalicus lateralis	350 ± 47	586 ± 101*	236	1.67
Nucleus motorius dorsalis nervus vagi	231 ± 39	445 ± 75*	214	1.93
Nucleus ovoidalis	283 ± 27	566 ± 82*	283	2.00
Nucleus rotundus	327 ± 42	557 ± 87*	230	1.70
Nucleus ruber	231 ± 31	553 ± 116*	322	2.39
Nucleus sensorius principalis nervi trigemini	250 ± 29	541 ± 90*	291	2.16
Nucleus tractus solitarii	224 ± 36	454 ± 79*	230	2.03

Values are means ± SE in ml·min^{-1}·100 g wet tissue^{-1}; n, no. of animals. rCBF, regional CBF; Δ, submerged − control. *Significantly different from control ($P < 0.05$, independent-sample t test of log-transformed data).

ml·min^{-1}·100 g^{-1} in ducks, geese, and chickens (1, 8–10, 16, 21, 35). In the control group of the present study, CBF (158 ± 14 ml·min^{-1}·100 g^{-1}) was higher than previously reported. There are several possible causes of the elevated CBF in the present study, but no firm conclusions can be drawn in this regard from the available data. One study, reporting low CBF (~50 ml·min^{-1}·100 g^{-1}) used geese anesthetized with pentobarbital sodium (1), which is known to depress CBF in mammals (30) and may have had the same effect in geese. Two other studies, reporting CBF at ~71 (16) and 99 ml·min^{-1}·100 g^{-1} (9), used ducks and geese that had been allowed 2 h of recovery from halothane anesthesia. With one exception (21), studies involving awake ducks and geese reported CBF values ≥100 ml·min^{-1}·100 g^{-1} (8, 10, 31, 35). Thus there is circumstantial evidence that the use of anesthetics may have decreased CBF in some studies.

In awake animals, the levels of stress experienced by the birds may have affected CBF (4). However, experimental protocols varied widely between studies, and comparisons of stress levels are subjective. As is the case in mammals (4), there is no obvious correlation between the level of restraint and CBF in birds (avian studies have used containment within a small space and physical or pharmacological immobilization). Nevertheless it seems likely that involuntary head immersion represents an added stress that could augment CBF. Plasma catecholamines are substantially elevated in forcibly submerged ducks (25) and may be involved in mediating a stress-related hyperperfusion by their action on β-receptors (4). The blood gas changes that occur during submergence stimulate the carotid body chemoreceptors (22). These receptors might cause cerebral vasodilation indirectly by activating the defense-arousal system (26) as well as by a direct reflex effect on the cerebral vasculature (28), although the latter effect, if present, is likely to be small (32).

This was the first study of avian CBF in which hypercapnia and hypoxia occurred simultaneously, and this may account for the higher apparent CBF-Pa_{CO_2} sensitivity than in previous reports. In the present study, Pa_{O_2} decreased to ~40–50 Torr and Pa_{CO_2} increased to >50 Torr (Table 1). According to the data of Grubb et al. (14, 16), hypoxia and hypercapnia at these levels would increase CBF by 1.5–2.5 times and ~2 times, respectively. Because average CBF approximately doubled in this experiment, our results do not support those of Jones et al. (21), who concluded that the effects of hypoxia and hypercapnia are additive during submergence asphyxia. Absolute CBF during submergence asphyxia was similar in this and the study by Jones and Purves (22), but presubmergence (control) CBF differed widely between studies. The reasons for this discrepancy are unclear.

Few studies have addressed the question as to which regions of the central nervous system (CNS) are involved in the integration and modulation of systemic cardiovascular responses during submergence. Exploratory studies using electrical stimulation within the brain stem of the duck (11, 12) and elephant seal (33) revealed various sites within the mesencephalon and hypothalamus that were potentially involved in controlling the "diving response." However, this work was not pursued. Recent observations of the variability of cardiovascular responses to diving under different circumstances (31) have renewed speculation about the likely role of suprabulbar regions of the CNS in modulating cardiovascular diving responses.

Given the high spatial resolution of the technique, quantitative autoradiography could potentially be used for functional mapping of the avian brain. This relies on the assumption that rCBF is proportional to regional metabolic rate, so that changes in rCBF infer local changes in neural activity (18). However, in the present study there were no obviously "activated" (i.e., hyperperfused) or "deactivated" (i.e., hypoperfused) nuclei in submerged ducks. In general, the elevation of rCBF in identified nuclei was similar in magnitude to that of the entire CNS.

None of the areas described by Feigl and Folkow (11) and Folkow and Rubinstein (12) were apparent in the autoradiographs. The largest proportional increase in blood flow occurred in the locus ceruleus. This nucleus is known to be involved in cardiovascular control in mammals, including a role in the development of conditioned bradycardia (17). The largest absolute increase in blood flow occurred in the nucleus ruber located in the ventromedial region of the midbrain. To our knowledge, the nucleus ruber has not been implicated in cardiovascular control and may be worthy of further investigation in this context.

We found rCBF to be highly variable between individuals (Tables 2 and 3), despite use of Pa_{O_2} as an objective

criterion for determining the timing of IPIA injection in submerged ducks, which compromises the usefulness of the technique for functional mapping of the brain. Further work is necessary to quantify the potential effects of stress or arousal in awake submerged birds and the confounding effects of global changes in blood gas tensions and pH. Other factors such as increased blood catecholamine concentrations, inputs from peripheral sensory receptors, especially trigeminal and vagal afferents, and a possible influence of cerebrovascular sympathetic innervation must be investigated in birds before rCBF can be confidently used to indicate neural activity.

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