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THE RELATIVE DISTRIBUTION OF PULMOCUTANEOUS BLOOD FLOW IN *RANA CATESBEIANA*: EFFECTS OF PULMONARY OR CUTANEOUS HYPOXIA

BY R. G. BOUTILIER*, M. L. GLASS† AND N. HEISLER

Abteilung Physiologie, Max-Planck-Institut fur experimentelle Medizin, Hermann-Rein-Strasse 3, D-3400 Göttingen, FRG

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SUMMARY

The distribution of pulmocutaneous heart output to lungs and skin was determined in non-anaesthetized, fully recovered bullfrogs (*Rana catesbeiana*) by application of the microsphere method in order to study the modulation of blood flow to different gas exchange sites in amphibians during environmental air and water hypoxia. The relative perfusion of various skin areas was found to be rather heterogeneously distributed with an over-proportionately high blood flow to the ventral body surface. This distribution of flow among different skin areas remained unaffected by any type of environmental hypoxia. The relative perfusion of lungs and skin, however, was significantly affected by the pattern of environmental oxygen partial pressure. The relative lung perfusion (≈80 % of pulmocutaneous flow in normoxic control conditions) was increased during water hypoxia, and reduced with lowered inspired P_{O_2}. This mechanism could be interpreted as a readjustment of blood flow towards the gas exchange site with higher oxygen partial pressure, but may also represent a mechanism to prevent oxygen loss from the body stores at gas exchange sites of low oxygen tension.

INTRODUCTION

A pulmocutaneous branchpoint in the anuran circulation has long been recognized as a potentially important locus for controlling blood flow to the lungs and skin (Couvreur, 1889; Arthaud & Butte, 1890). Increases in pulmonary blood flow occur during lung ventilation in *Xenopus* (Shelton, 1970, 1976), *Rana catesbeiana* (Johansen, Lenfant & Hanson, 1970) and *Bufo marinus* (West & Burggren, 1984), and are thought to be effected through vasodilation of the pulmonary vasculature. There is evidence that pulmocutaneous blood flow (Emilio & Shelton, 1972) and the distribution of flow between pulmonary and cutaneous arteries (West & Burggren,

*Present address: Department of Biology, Dalhousie University, Halifax, Nova Scotia, B3H 4J1, Canada.

†Present address: Department of Zoophysiology, University of Aarhus, DK-8000 Aarhus C, Denmark.

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1984) can be modulated by altering the composition of the intrapulmonary gas during a breath. Control mechanisms such as these would be most effective if they were responsive to changes in gas composition in both the intrapulmonary and aquatic environments. Using the microsphere entrapment method, we examined the relative distribution of pulmocutaneous blood flow in the bullfrog, *Rana catesbeiana*, to see if it could be modulated in response to pulmonary or cutaneous oxygen availability.

MATERIALS AND METHODS

Seven specimens of apparently healthy bullfrogs (body weight 250–450 g) were anaesthetized in buffered MS-222 solution ($1{\cdot}5\,g\,l^{-1}$, pH 7·5) and then intubated intratracheally for artificial ventilation throughout surgery. The left pulmocutaneous artery was exposed by a midventral incision and was transiently clamped off before a 4 mm length of PE 50 tubing was advanced downstream through a small cut in the vessel wall. A purse-string suture tightly closed the hole for the cannula entry by trapping a continuous piece of the outer wall of the vessel with fine polyamide thread. The operation caused virtually no blood loss and all incisions were carefully closed with sutures. After recovery from the operation, the frog was placed in a water-filled aquarium fitted with a screen slightly below the water surface to prevent access to the air except for a blowhole funnel large enough for the frog to introduce its head for air breathing. The blowhole funnel was flushed at a rate of $300\,ml\,min^{-1}$ with gases delivered by a gas mixing pump (Wösthoff, Bochum, FRG). The aquarium water was equilibrated with a different gas mixture from a separate gas mixing pump. Pressure changes at the tip of a cannula inserted below the water surface of the experimental chamber and connected to a Statham P23 Db pressure transducer were utilized to record breathing movements. The frogs were allowed to recover from surgery and get used to the experimental chamber for at least 48 h at a constant temperature of 20°C. The pulmocutaneous cannulae were flushed periodically with heparinized saline ($20\,i.u.\,heparin\,ml^{-1}$) in order to avoid blood clotting.

The radioactive microspheres used during experimentation (diameter $25 \pm 2{\cdot}5\,\mu m$, New England Nuclear) were matched in size to the larger diameter of the bullfrog erythrocytes, and were labelled with ^{141}Ce (cerium), ^{51}Cr (chromium) and ^{46}Sc (scandium) for three different determinations of blood flow distribution. Immediately before injection, the microspheres were resuspended by sonication (up to 5 min) in 10% dextran solution with 0·05% Tween 80 added to prevent aggregation. The suspension was occasionally checked by light microscopy for microsphere aggregation and disintegration. A sample of 0·5 ml microsphere suspension (containing between 3×10^5 and 5×10^5 spheres) was slowly infused into the pulmocutaneous vessel over a period of 30 s. The microsphere infusion was immediately followed with an additional infusion of microsphere-free dextran solution (0·5 ml in 30 s) to ensure that all microspheres resident in the cannula had been flushed into the circulation. Recirculation of microspheres not trapped in the vascular beds during the first tissue passage was checked in experiments on open

chest preparations of bullfrogs cannulated with systemic venous, left auricle and systemic artery catheters. Microspheres were injected into the systemic arterial blood streambed, and blood was simultaneously withdrawn from the return sites of circulation (for details of the procedure see Heisler, Neumann & Maloiy, 1983). These preliminary experiments indicated that more than 96 % of 25 μm diameter microspheres were trapped during their first pass through the systemic capillary beds.

Each animal received three successive infusions of differently labelled microspheres corresponding to three different environmental conditions of oxygenation: (1) control conditions (air $P_{O_2} \approx 155$ Torr, water $P_{O_2} \approx 140$ Torr); (2) aquatic hypoxia ($P_{O_2} \approx 40$ Torr) combined with air normoxia (inspired $P_{O_2} \approx 155$ Torr); (3) aquatic normoxia ($P_{O_2} \approx 155$ Torr) combined with lung gas hypoxia (inspired air $P_{O_2} \approx 40$ Torr). All injections were conducted after a 10-min period of voluntary submergence and were spaced approximately 2·5 h apart during which time the animals were exposed to a different combination of environmental oxygen partial pressures. 15 min after the final injection the animals were killed by an overdose of anaesthetic, and the distribution of injected microspheres between lungs and skin was assessed by differential determination of ^{141}Ce, ^{51}Cr and ^{46}Sc radioactivity by multichannel gamma scintillation counting (Model 5986, Packard Instruments, Inc.) in combination with on-line spectrum analysis (DEC PDP 11) on the basis of standard spectral analysis performed before each batch of tissue samples (see Heisler *et al.* 1983). The relative amount of microspheres spilled over into the systemic circulation was determined from the activity in the homogenized remainder of the animal.

The relative flow distribution (RFD) of pulmocutaneous blood to the skin (S) and left lung (L) was assessed as:

$$RFD_S = \frac{A_S}{A_L + A_S},$$

or:

$$RFD_L = \frac{A_L}{A_L + A_S},$$

where A is the activity of any one of the three labels (injections were always made into the pulmocutaneous artery feeding the left lung).

The specific regional skin perfusion (RSP) was calculated as the ratio of the specific activity of a selected skin region (activity/tissue weight) to the average specific tissue activity (activity/tissue weight of entire skin).

Significance of differences was tested by application of Student's t-test ($P < 0{\cdot}05$).

RESULTS AND DISCUSSION

The microspheres administered into the pulmocutaneous artery were mainly distributed between lungs and skin, with the larger proportion of total lung and skin flow directed to the lungs (80·1 ± 5·3 %, $\bar{x} \pm$ S.E.). In seven animals only 16–33 % of the infused activity was found to be spilled over into the systemic circulation. Total

body microsphere activity has not been reported in earlier studies on amphibians, so that these data cannot be compared to literature reports. Presumably, anatomical shunts can account for most of the observed distribution to body tissues other than the lungs and skin, retrograde flow and capillary passage of microspheres being factors of presumably less importance. Checks of microsphere recirculation in both pulmonary and systemic circulation of *Varanus exanthematicus* suggest that the capillary diameters are not greatly different between the two vascular beds, at least in this lower vertebrate species. The effect of microspheres being spilled over into the systemic circulation on the results is negligible: only a small proportion is supplied by the systemic circulation to the relatively small skin tissue fraction. Any direct reflux from the pulmocutaneous injection site will end up mostly in the systemic tissues, any further passage of these spheres through systemic tissues will be redistributed to lungs and skin in the same way as the originally injected batch.

The perfusion of the skin originating from the pulmocutaneous artery is rather inhomogeneously distributed, as indicated by the specific regional skin perfusion (RSP, Table 1). The skin of the ventral body surface (Fig. 1) is overproportionately perfused as compared to the more-or-less average blood flow to the skin of the dorsal body surface and the foreleg, whereas the RSP of buccal and hindleg skin is only about one-quarter or half of that for skin on the ventral and dorsal body surface, respectively. These data are in contrast with those of Moalli, Meyers, Jackson & Millard (1980), who found that the dorsal regions of the bullfrog received a greater than average blood supply from the cutaneous artery. However, in contrast to our experiments on intact, unrestrained, unanaesthetized and fully recovered animals their study was performed on open chest preparations of anaesthetized frogs with portions of the circulation ligated in order to facilitate the discrete labelling of the pulmocutaneous and systemic arterial sources.

The preferential perfusion of the ventral body surface with blood from the pulmocutaneous branch favours utilization of this area as a site for gas exchange or ionic regulation. However, there is no selective redistribution of blood flow between specific skin regions (Table 1) during changes in the oxygenation of environmental water and inspired air, whereas the relative flow distribution (RFD) between skin and lungs is significantly affected ($P < 0{\cdot}05$). This indicates that although the total skin blood flow may be subject to changes, the regional distribution of cutaneous artery blood flow to various skins areas is fixed. These data suggest that blood supply to specific skin areas may be optimal even under conditions of largely changed environmental conditions, or more likely, is less controlled.

Under all conditions of oxygenation, the lungs rather than the skin received the greatest proportion of pulmocutaneous blood flow during a voluntary dive ($80{\cdot}1 \pm 5{\cdot}3\%$, $\bar{x} \pm$ S.E., Fig. 2). Similar distribution of pulmocutaneous blood flow has been reported in *Bufo marinus*, in response to mechanical and chemical stimulation of the lungs during breathing and breath-holding periods (West & Burggren, 1984). Compared to normoxic conditions, aquatic hypoxia in the bullfrog led to a reduction in the proportion of pulmocutaneous blood flowing to the skin,

favouring increased relative perfusion of the lung. Conversely, relative blood flow to the skin increased during the breathing of hypoxic gas mixtures (Fig. 2).

The functional significance of the ability to modulate blood flow to the lung and skin may seem simple; the blood is directed towards that site of gas exchange where most oxygen is available. A full interpretation may, however, be more complex. The most important aspect of the response to hypoxic water may not be that the relative flow to the lungs is increased; rather, a reduction of cutaneous blood flow could be directed towards conservation of the body oxygen stores (Shelton, 1985), since oxygen gained by air breathing may be lost transcutaneously to hypoxic water (Johansen, 1968; Feder & Burggren, 1985). Equally, the ability to redirect pulmocutaneous blood flow away from the lungs and to the skin during conditions of low pulmonary P_{O_2} (Fig. 2) may serve to alleviate hypoxic conditions during prolonged

Table 1. *Specific regional skin perfusion (RSP, see text for calculation) of pulmocutaneous circulation of bullfrogs exposed to various conditions of environmental oxygenation*

Series/Conditions	Specific regional skin perfusion				
	Ventral	Dorsal	Buccal	Hindleg	Foreleg
(1) Control: water and lung normoxia	2·63 ± 0·51	0·98 ± 0·23	0·47 ± 0·09	0·47 ± 0·09	0·87 ± 0·32
(2) Aquatic hypoxia, lung normoxia	1·97 ± 0·42	1·01 ± 0·22	0·44 ± 0·11	0·55 ± 0·16	0·94 ± 0·30
(3) Aquatic normoxia, lung hypoxia	1·94 ± 0·41	1·12 ± 0·28	0·52 ± 0·13	0·64 ± 0·19	0·90 ± 0·26

Scheme of skin regions shown in Fig. 1.
RSP values are means ± S.E. of seven experiments.
Values of RSP greater than 1 indicate that blood flow to that region is above average and *vice versa*.

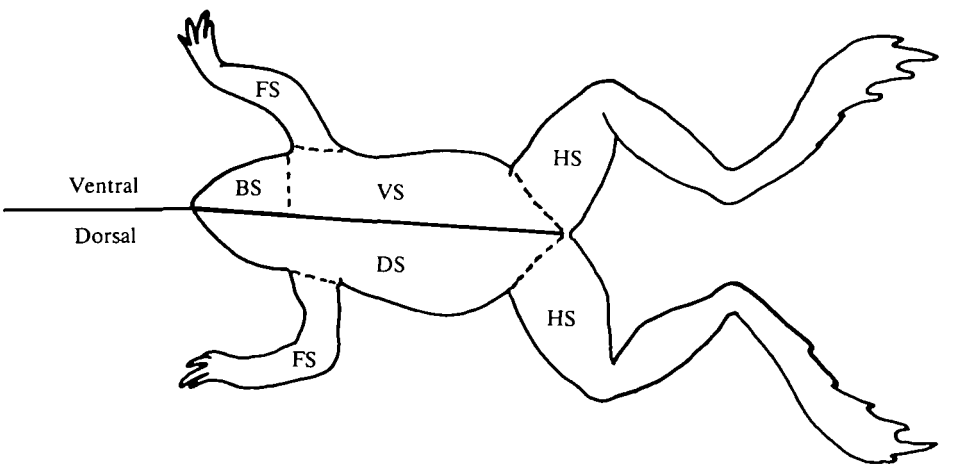

Fig. 1. Regions of the dorsal and ventral skin sampled for microsphere activity. DS, dorsal skin; VS, ventral skin; BS, buccal skin; HS, hindleg skin; FS, foreleg skin.

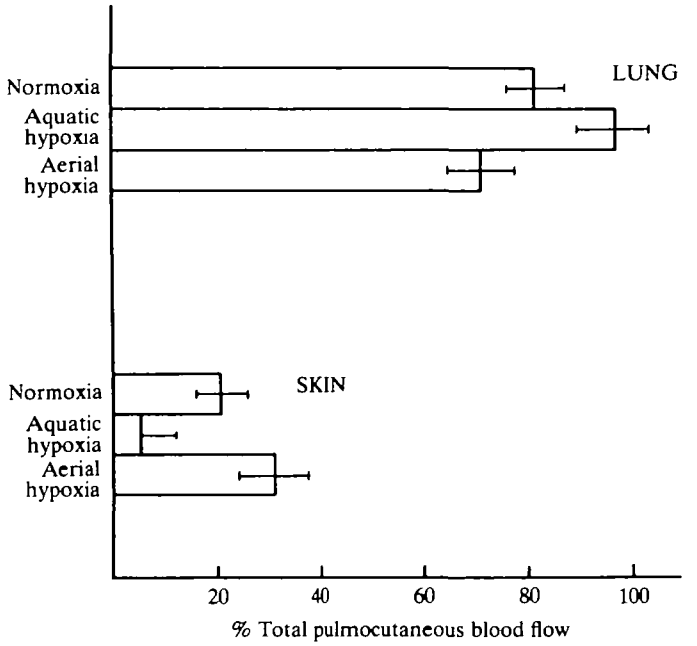

Fig. 2. Histogram showing the relative flow of pulmocutaneous blood in bullfrogs subjected to normoxia ($P_{O_2} \approx 155$ Torr), aquatic hypoxia ($P_{O_2} \approx 40$ Torr) delivered only at the skin, and aerial hypoxia ($P_{O_2} \approx 40$ Torr) delivered only at the lungs. Radioactive microspheres were injected 10 min into a voluntary dive. Data are means (±S.E.) of seven experiments at 20°C. Significant changes throughout ($P < 0{\cdot}05$, Student's paired t-test).

dives. Indeed, there is evidence that cutaneous oxygen uptake increases as the body oxygen stores decline during extended dives in *Xenopus laevis* (Emilio & Shelton, 1972; Boutilier & Shelton, 1986). Evidently, changes such as these can be produced not only as a consequence of the increase in the P_{O_2} gradient across the skin (Shelton & Boutilier, 1982), but also as the number of cutaneous capillaries open to blood flow (Poczopko, 1957, 1959; Burggren & Moalli, 1984) alters as the dive progresses. The latter are difficult to assess in intact animals (cf. Moalli *et al.* 1980), owing to blood flow contributions from both cutaneous and systemic arterial sources, directing respectively, partly deoxygenated and oxygenated blood to the same respiratory exchanger.

The basis for controlling blood flow at the pulmocutaneous branchpoint may well reside with muscular sphincters which have been located in both the pulmonary and cutaneous arteries of anuran amphibians. Vagal stimulation and acetylcholine exert a strong pressor influence on the sphincter located in the distal segment of the pulmonary artery in *Rana temporaria* (de Saint-Aubain & Wingstrand, 1979), whereas the sphincter located in the proximal segment of the cutaneous branch of the pulmocutaneous vessel in *Bufo marinus* receives a rich supply of adrenergic constrictory fibres (Smith, 1976). Direct relationships between chemical stimuli, their origin and the vasoactive nature of the pulmonary or cutaneous sphincters have yet to be established. However, reciprocal innervation of this sort in *Rana*

catesbeiana would provide an obvious vehicle for selectively altering the resistance, and therefore the blood flow, to the two vascular networks.

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