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SHORT COMMUNICATION

CARDIAC OUTPUT IN CONSCIOUS TOADS (*BUFO MARINUS*)

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Cardiac output and peripheral resistance have not been measured in conscious anuran amphibians, although some data exist for pithed and anaesthetized frogs (Shelton and Jones, 1965*a*,*b*; Shelton, 1970; Tazawa *et al.* 1979). The periodic lung ventilation characteristic of conscious anurans could increase cardiac output reflexively or by changes in venous return, as in mammals. Furthermore, because the undivided ventricle places the pulmonary and systemic vascular beds effectively in parallel, changes in the ratio of systemic to pulmocutaneous vascular resistance have the potential to alter the distribution of cardiac output. Therefore, the objectives of this study were to measure cardiac output and peripheral resistance in undisturbed, conscious *Bufo marinus* and to determine the effect of increased ventilation, stimulated by CO_2, on pulmocutaneous blood flow and resistance.

We performed the experiments on 11 *B. marinus*, with a mean mass of 390±16 g, obtained from a commercial supplier in the USA. The toads were maintained on a 16 h:8 h L:D photoperiod at 22–23 ˚C, had free access to water and were force-fed a diet of canned dog food once a week. Surgery was not performed on the day the animals were fed. The experiments were performed at 23–24 ˚C.

Toads were anaesthetized by immersion in 0.25 % Tricaine (ethyl-*m*-aminobenzoate methanesulphonic acid, Sigma) adjusted to pH 7.0 by the addition of NaOH. A left axillary incision was made in order to approach the central cardiovascular area. The left common carotid artery, aorta and pulmocutaneous artery were carefully exposed. Clearing of tissue was kept to a minimum to preserve the innervation of the arteries. Miniature pulsed Doppler flow probes (Valpey-Fisher or Crystal Biotech, silastic) were implanted on the arteries (common carotid, 1.0 mm i.d.; aorta, 2.0–2.5 mm i.d.; pulmocutaneous artery, 2 mm i.d.). Carotid artery probes were cut down to accommodate the length of common carotid available for implantation. Probes were fixed in position with 5.0 gauge surgical silk, and the knot was anchored with cyanoacrylate (Zapagap,

Key words: cardiac output, carotid, aorta, pulmocutaneous arch, toad, *Bufo marinus.*

Pacer Technologies, CA). Leads were brought out through the axillary incision, which was closed, and secured dorsally to the skin. A right axillary incision was then made in order to implant a pulmocutaneous pressure cannula. The right cutaneous artery was occlusively cannulated with a 30 cm cannula (PE 50) and the tip of the cannula was advanced so that it lay flush to the wall of the pulmocutaneous artery. The right sciatic artery was occlusively cannulated with a 30 cm cannula (PE 50), advanced into the aorta, to record systemic arterial pressure and heart rate, and to take arterial blood samples for arterial blood gas and pH measurement. The cannulas were filled with heparinized saline (100 i.u. ml^{-1}; 0.65 % NaCl), coiled and attached to the dorsal surface of the toad with silk sutures until use. An air-filled cannula (PE 160) was introduced into the buccal cavity *via* the tympanum to monitor ventilation (West and Jones, 1975).

Signals from the miniature pulsed Doppler flow probes were conditioned using Valpey-Fisher PD-1 couplers. We recorded arterial and buccal cavity pressures with Beckman 4-327-0 pressure transducers and Beckman 9853A couplers. A blood gas analyzer (Instrumentation laboratories, Micro 13) was used to measure Pa_{O_2}, Pa_{CO_2} and pHa at 23–24 °C. Instantaneous left common carotid, aortic and pulmocutaneous artery blood flows, pulsatile pulmocutaneous and systemic arterial pressures and buccal cavity pressure were displayed on two four-channel Beckman R511A pen recorders writing on rectilinear coordinates. The recorders were coordinated by a common time signal displayed on one channel of each recorder. The flow signals and the pulmocutaneous pressure signal were simultaneously recorded for later analysis on a Hewlett Packard 3964A four-channel FM tape recorder.

After surgery, animals were held in a translucent white plastic box. The floor of the box was kept moist with tap water. The buccal cannula was plugged to permit normal buccal ventilation during recovery. The animals were exposed to a low uniform noise level during both recovery and experimentation and were shielded from sight of the experimenters. To determine arterial blood flows, pressures and ventilatory activity, the flow leads and pressure cannulas were freed from the dorsal skin, and a length of flexible Tygon tubing was attached to the buccal catheter. Tubing and leads were led out of the box and connected to the appropriate transducers and signal conditioners.

Recordings of pressures and flows under undisturbed conditions were typically made for several hours on each post-operative day. Animals were allowed to settle for at least an hour before recording was started.

To record the responses to hypercapnia, the toads were placed in a Plexiglas box with a moist floor and a gas inlet and outlet. After a control period, during which the animals were allowed to settle, CO_2 was leaked into the box at a rate sufficient to provide 2–3 % CO_2 in inspired gas for approximately 1 min.

Flow velocities measured by the miniature pulsed Doppler flow probes were calibrated in terms of kHz Doppler shift during the course of the experiments, using internal calibration signals provided by the flow amplifiers. In order to calibrate the probes in terms of volume flow, toads were over-anaesthetized with Tricaine at the end of the experiments and partially exsanguinated *via* the sciatic artery cannula. The truncus arteriosus was exposed *via* a small mid-ventral incision. The carotid, aortic and pulmocutaneous divisions of the truncus were separately cannulated (PE 50) and the atria

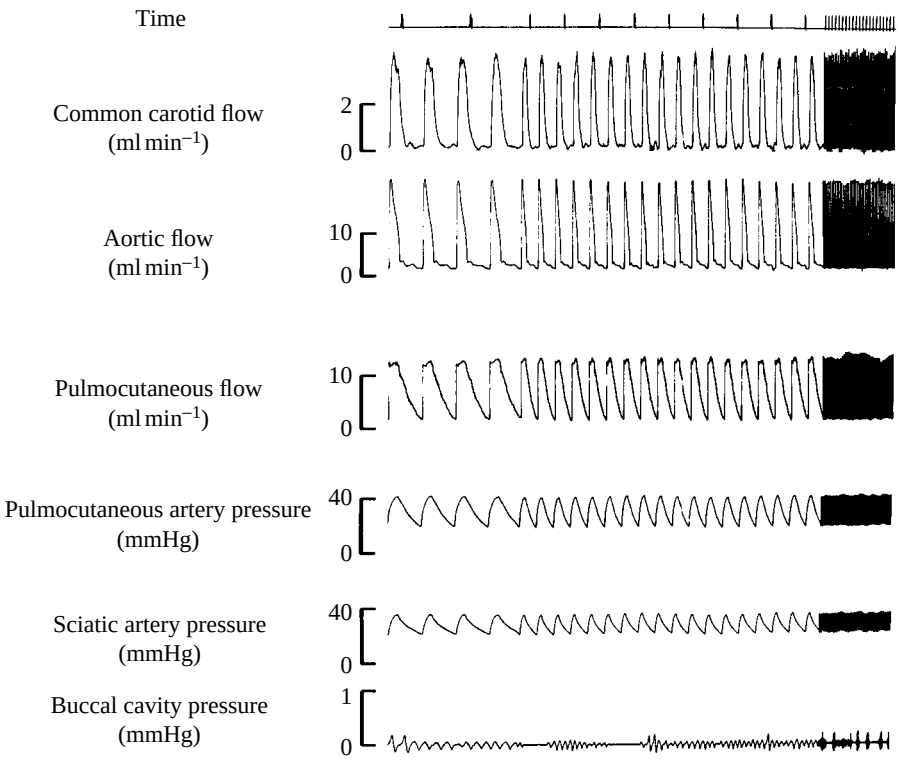

Fig. 1. Arterial pressures and instantaneous flows, and buccal cavity pressure, recorded in a conscious undisturbed toad in which low-amplitude lung ventilation cycles occurred infrequently. Traces, from the top: 5 s time marker; left common carotid blood flow; left aortic blood flow; left pulmocutaneous artery blood flow; pulmocutaneous arterial pressure; systemic pressure recorded *via* the sciatic artery; buccal pressure. Note that blood flow is relatively constant in all arches.

were opened. We took care not to disturb the positioning of the flow probes during this procedure. The relationship between Doppler shift (kHz) and the volume flow rate of heparinized toad blood was determined for the left common carotid, aorta and pulmocutaneous artery in turn. Blood was delivered to each vessel by a Harvard infusion pump at five appropriate flows and linear regressions were plotted for the relationship between Doppler shift (kHz) and volume flow (ml min^{-1}). Correlation coefficients (r) of the linear regressions ranged from 0.95 to 0.99. We used the regressions to calculate volume flows.

Analysis of the taped data was performed using a Compaq Deskpro 386s personal computer, running Lab tech Notebook software, and a Keithley-das series 500 A-D converter. Typically, two 10 min data segments were selected for analysis to provide mean values for flow and pressure. These periods were not selected randomly, but reflected times during which the toad was quiescent. In order that the measured cardiovascular variables should reflect conditions in undisturbed animals, we analyzed

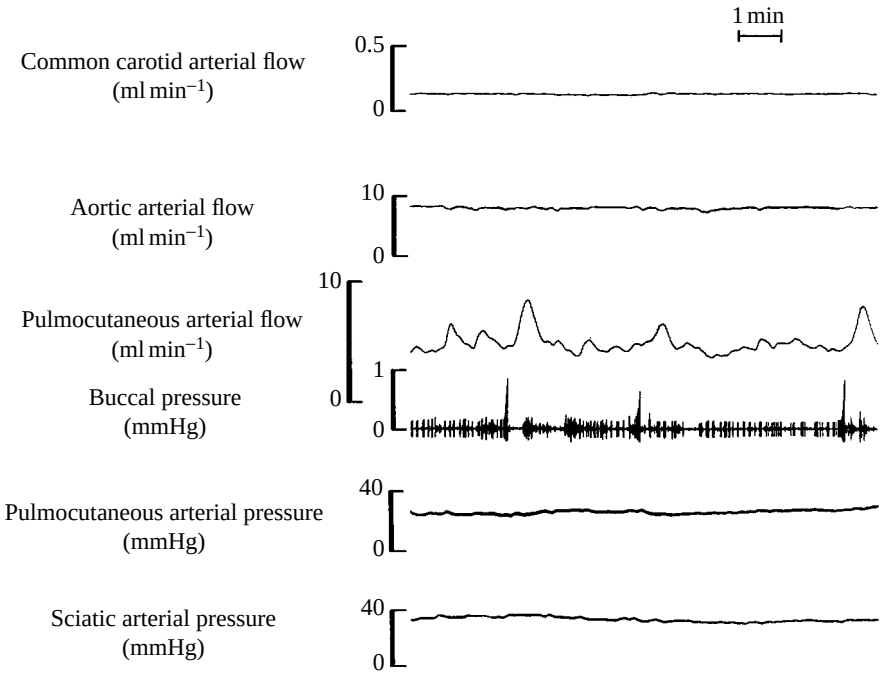

Fig. 2. Mean arterial pressures and flows recorded in a 390 g toad exhibiting sequences of lung inflation. Traces, from the top: left common carotid blood flow; left aortic blood flow; left pulmocutaneous artery blood flow; buccal pressure; pulmocutaneous arterial pressure; systemic pressure recorded *via* the sciatic artery. Pulmocutaneous artery flow increases as intrapulmonary pressure is elevated at the end of a sequence of lung inflation and peaks during the following non-ventilatory period, during which the lungs are held inflated. Ventilatory activity has no influence on common carotid or aortic blood flow.

data taken 3 days after surgery. The data trace not recorded on magnetic tape (usually the systemic arterial pressure trace) was digitized using a graphics tablet and Sigma Scan software (Jandel Scientific, Corte Madera, CA). Both sets of digitized data were stored and manipulated in a work sheet (Lotus 123, Lotus Development, Cambridge, MA). Blood flows were assumed to be equal in the left and right arterial arches, allowing cardiac output to be calculated. Pulmocutaneous flows have previously been determined to be bilaterally equivalent (Smits *et al.* 1986). To calculate peripheral resistance, systemic venous pressure was assumed to be 5 mmHg (0.67 kPa) and pulmonary venous pressure 4 mmHg (0.53 kPa) (Smits *et al.* 1986). To calculate total peripheral resistance, it was assumed that the carotid, aortic and pulmocutaneous resistances were arranged in parallel. They were therefore summed as their reciprocals. Mean values are given ± S.E.M.

We obtained complete sets of simultaneous flow and pressure data from six animals, and partial data sets from an additional five. Systemic arterial gases and pH in instrumented animals were $P\mathrm{a}_{O_2}$=63.0±3.5 mmHg (8.4 kPa), $P\mathrm{a}_{CO_2}$=15.2±0.7 mmHg (2.0 kPa), pHa=7.83±0.01. These values are similar to those previously measured in

Table 1. *Mean values for cardiovascular variables in resting* Bufo marinus

Variable	Mean ± S.E.M.	*N*
Blood flows		
Common carotid blood flow (ml min^{-1} kg^{-1})	3.3±0.82	9
Aortic blood flow (ml min^{-1} kg^{-1})	26.8±2.87	9
Pulmocutaneous blood flow (ml min^{-1} kg^{-1})	31.5±6.18	8
Cardiac output (ml min^{-1} kg^{-1})	57.2±6.46	6
Blood pressures		
Mean systemic arterial pressure (mmHg)	23.7±1.18	8
Mean pulmocutaneous pressure (mmHg)	23.3±1.91	8
Vascular resistances		
Common carotid resistance (mmHg ml^{-1} min kg^{-1})	15.6±6.28	7
Aortic resistance (mmHg ml^{-1} min kg^{-1})	0.89±0.12	6
Pulmocutaneous resistance (mmHg ml^{-1} min kg^{-1})	0.91±0.29	5
Total peripheral resistance (mmHg ml^{-1} min kg^{-1})	0.45±0.13	4
Heart rate and stroke flows		
Heart rate (beats min^{-1})	29.8±2.53	11
Common carotid stroke flow (ml $beat^{-1}$ kg^{-1})	0.13±0.04	9
Aortic stroke flow (ml $beat^{-1}$ kg^{-1})	1.01±0.16	9
Pulmocutaneous stroke flow (ml $beat^{-1}$ kg^{-1})	1.11±0.17	8

Blood flows, stroke flows and vascular resistances were calculated as bilateral and adjusted for body mass.

All flows are reported as calculated bilateral flow. Carotid, aortic and pulmocutaneous flows and stroke flows reported represent the means for all experiments in which flow was measured for that arch. Flows measured from any arch were not statistically different in animals from which complete and incomplete sets of data were obtained. Cardiac output, vessel resistances and total peripheral resistance represent the means for preparations in which the appropriate variables were measured simultaneously. In order to calculate resistance, systemic venous pressure was assumed to be 5 mmHg and pulmonary venous pressure was assumed to be 4 mmHg (Smits *et al*. 1986). Data were taken from the same post-surgical day for each animal.

undisturbed *Bufo marinus* (West *et al.* 1987). This suggests that the presence of implanted flow probes had little effect on ventilation or arousal state.

In those undisturbed animals in which low-amplitude lung ventilation cycles occurred infrequently and lung inflation cycles were not prominent, mean flow remained constant in all three arterial arches (Fig. 1). However, in animals that exhibited lung inflation cycles, pulmocutaneous artery flow started rising during the inflation sequence and often peaked during the following non-ventilatory period. Fig. 2 illustrates results from an animal in which this phenomenon was pronounced.

Table 1 contains mean values for carotid, aortic and pulmocutaneous blood flow, stroke flow and peripheral resistance. Cardiac output was calculated at 57.2 ml min^{-1} kg^{-1}. Carotid flow represented 8.0±2.9 %, aortic 44.9±5.3 % and pulmocutaneous 48.4±5.9 % of the cardiac output in six animals in which all three variables were measured simultaneously.

The CO_2 stimulus increased both the frequency and amplitude of lung ventilation cycles in undisturbed conscious toads (Fig. 3). Resulting systemic arterial blood gas

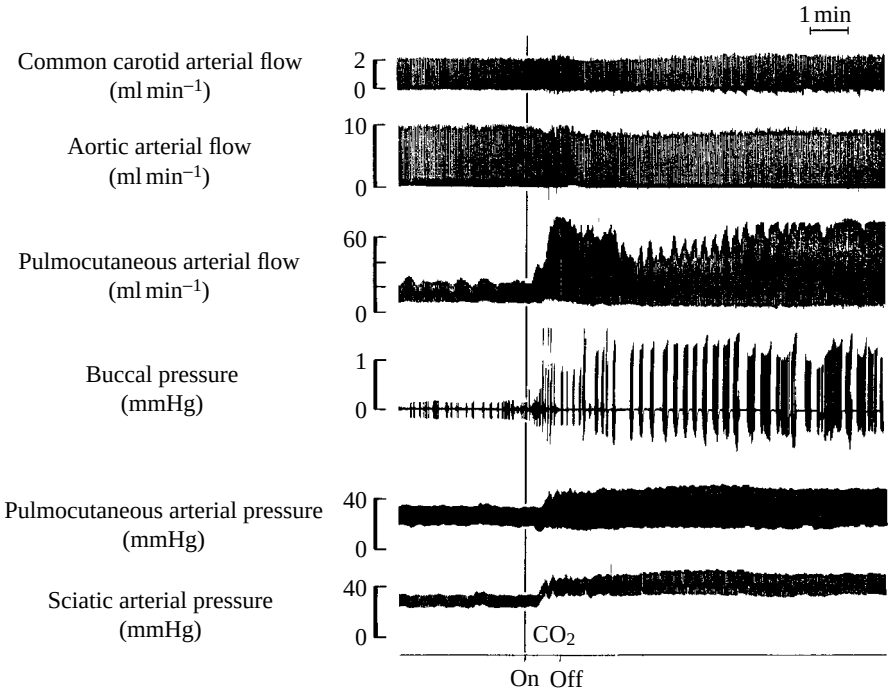

Fig. 3. Cardiovascular and respiratory responses to an increase in hypercapnic ventilatory drive in a 380 g conscious toad. Traces, from the top: left common carotid blood flow; left aortic blood flow; left pulmocutaneous artery blood flow; buccal pressure; pulmocutaneous arterial pressure; systemic pressure recorded *via* the sciatic artery. 10 % CO_2 was introduced into the experimental chamber at the event marker.

pressures and pH were Pa_{O_2}=66.8±2.4 mmHg (8.9 kPa), Pa_{CO_2}=16.1±1.0 mmHg (2.2 kPa), pHa=7.69±0.04 (*N*=3). Systolic pressure increased in both the systemic and the pulmocutaneous circulations. Diastolic pressure in the systemic, but not the pulmocutaneous, circulation also increased. Instantaneous and mean pulmocutaneous blood flow increased, but carotid and aortic flows remained constant or fell slightly. Fig. 4 illustrates the averaged pulmocutaneous blood flow and pressure responses for five animals.

There are no comparable measurements of cardiac output and its distribution in conscious anurans. However, Table 2 provides a comparison with estimates based on information found in the literature. In pithed bullfrogs, *Rana catesbeiana,* the systemic arterial vessels received 44 %, and the pulmocutaneous system 56 %, of total flow (Tazawa *et al.* 1979). There is no adrenergic vasoconstrictor tone in resting *Bufo marinus (*Wahlquist and Campbell, 1988), but vagally mediated constrictor tone is present in the extrinsic pulmonary artery (Luckhardt and Carlson, 1921). Therefore, pithing might have been responsible for pulmocutaneous flow representing a larger proportion of cardiac output in bullfrogs.

Arterial blood flow was constant in all three arches in conscious toads showing only

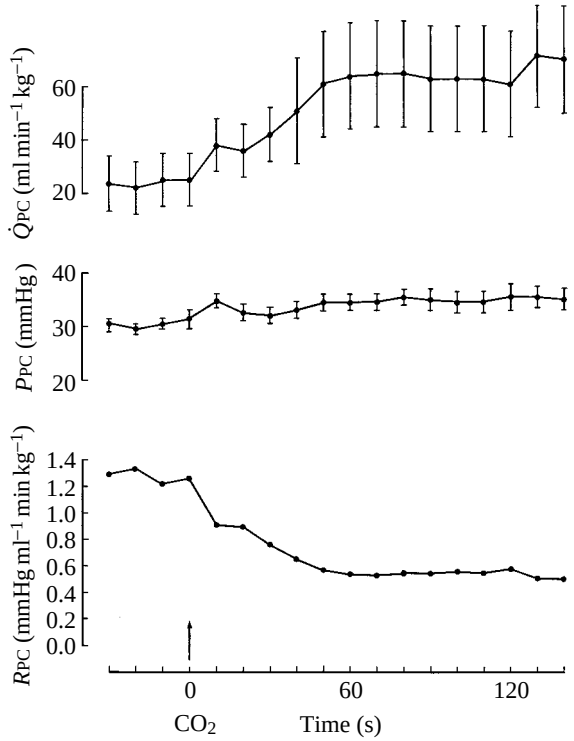

Fig. 4. Changes in pulmocutaneous artery flow ($\dot{Q}_{PC}$), blood pressure (P_{PC}) and calculated resistance (R_{PC}) in response to an increase in hypercapnic ventilatory drive in conscious toads. N=5. The vertical bars represent the standard error of the mean values. Pulmocutaneous flow is reported as bilateral flow.

Table 2. *Cardiac output comparisons*

Species	*N*	Mass (g)	Cardiac output (ml min^{-1} kg^{-1})	Conditions	Authors
Bufo marinus	6	360	57	Conscious	Present study
Rana catesbeiana†		269	76	Pithed	Tazawa *et al*. (1979)
*R. pipiens**	1	21	114	Anaesthetized	Shelton and Jones (1965*a*)
*R. temporaria**	1	35	63	Anaesthetized	Shelton and Jones (1965*a*)
*R. pipiens**	1	20–30	237–355	Anaesthetized	Shelton and Jones (1965*b*)
*Xenopus laevis**	1	104	66	Anaesthetized	Shelton (1970)

*Calculated from figures.

†Statistical composite, nine groups of 6–14 animals.

Shelton (1970). Tricaine anaesthesia, electromagnetic flow probes on aorta and pulmocutaneous artery.

Shelton and Jones (1965*a*). Tricaine anaesthesia, cardiometer.

Shelton and Jones (1965*b*). Tricaine anaesthesia, ciné film of ventricle.

Tazawa *et al*. (1979). Pithed frogs, Fick principle.

intermittent lung ventilation (Fig. 1). However, when several lung inflation cycles occurred spontaneously (Fig. 2) or in response to CO_2 (Fig. 3), pulmocutaneous artery flow alone increased. Evidently, the increase in pulmocutaneous flow on ventilation does not simply represent a 'steal' of cardiac output from the systemic arches through the undivided ventricle, because there was no marked decrease in carotid or aortic flow (Figs 2, 3). This suggests that lung inflation results in a fall in pulmocutaneous vascular resistance that closely matches a ventilation-related increase in cardiac output. A similar phenomenon occurred in anaesthetized *Xenopus laevis* (Shelton, 1970), in which large increases in pulmocutaneous flow followed lung ventilation, but aortic flow stayed constant.

The decrease in pulmocutaneous resistance in the face of increased pulmocutaneous blood flow may have both passive (change in transmural pressure) and active (neurally mediated) components (Milnor, 1989). In urethanized toads, peripheral resistance in a single pulmocutaneous arch fell from 2.1 to 1.5 mmHg ml^{-1} min when flow increased from 4.5 to 9 ml min^{-1}, the range of flow illustrated in Fig. 2 (calculated from Van Vliet and West, 1986). Presumably this is caused by recruitment and distention of pulmonary capillaries when pulmonary flow, or arterial pressure, increases. These flows in anaesthetized animals were achieved at pulmocutaneous pressures of only 9.5–13.5 mmHg, compared with 24.2 mmHg in the present study. This suggests that the pulmocutaneous outflow tract was relatively distended in anaesthetized toads, with little smooth muscle tone. In contrast, pulmonary vascular resistance in conscious toads is almost certainly neurally regulated. A vagally innervated muscular sphincter is located within the extrinsic pulmonary artery of anurans (de Saint-Aubain and Wingstrand, 1979). Emilio and Shelton (1972) suggested that pulmonary stretch receptors may be the afferent limb of a reflex pathway that regulates pulmonary vascular resistance by this means.

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