Central venous pressure and mean circulatory filling pressure in the dogfish *Squalus acanthias*: adrenergic control and role of the pericardium

Erik Sandblom,1,2 Michael Axelsson,1 and Anthony P. Farrell2

1Department of Zoology, Göteborg University, Gothenburg, Sweden; and 2Faculty of Agricultural Sciences and Department of Zoology, University of British Columbia, Vancouver, British Columbia, Canada

Submitted 26 April 2006; accepted in final form 22 June 2006


Central venous pressure and mean circulatory filling pressure in the dogfish *Squalus acanthias*: adrenergic control and role of the pericardium. *Am J Physiol Regul Integr Comp Physiol* 291: R1465–R1473, 2006. First published July 6, 2006; doi:10.1152/ajpregu.00282.2006.—Subambient central venous pressure (P_{ven}) and modulation of venous return through cardiac suction (vis a fronte) characterizes the venous circulation in sharks. Venous capacitance was estimated in the dogfish *Squalus acanthias* by measuring the mean circulatory filling pressure (MCFP) during transient occlusion of cardiac outflow. We tested the hypothesis that venous return and cardiac preload can be altered additionally through adrenergic changes of venous capacitance. The experiments involved the surgical opening of the pericardium to place a perivascular occluder around the conus arteriosus. Another control group was identically instrumented, but lacked the occluder, and was subjected to the same pharmacological protocol to evaluate how pericardiectomy affected cardiovascular status. Routine P_{ven} was negative (−0.08 ± 0.02 kPa) in control fish but positive (0.09 ± 0.01 kPa) in the pericardiectomized group. Injections of 5 μg/kg body mass (M_b) of epinephrine and phenylephrine (100 μg/kg M_b) increased P_{ven} and MCFP, whereas isoproterenol (1 μg/kg M_b) decreased both variables. Thus, constriction and relaxation of the venous vasculature were mediated through the respective stimulation of α- and β-adrenergic receptors. α-Adrenergic blockade with prazosin (1 mg/kg M_b) attenuated the responses to phenylephrine and decreased resting P_{ven} in pericardiectomized animals. Our results provide convincing evidence for adrenergic control of the venous vasculature in elasmobranchs, although the pericardium is clearly an important component in the modulation of venous function. Thus active changes in venous capacitance have previously been underestimated as an important means of modulating venous return and cardiac performance in this group.

elasmobranch; pulse pressure; venous capacitance

A CENTRAL AND FUNDAMENTAL MECHANISM in the maintenance of cardiovascular homeostasis in most vertebrate groups is active mobilization of venous blood reserves by means of neurohumorally mediated changes in venous capacitance (12, 17, 29, 31, 33–35, 37, 38, 40, 41, 46, 52). As demonstrated for mammals, small veins and venules are the primary blood volume reservoir in the circulation, containing ~70% of the total blood volume (31). Vascular capacitance is the relationship between contained blood volume and transmural pressure in the circulatory system, and a decrease in venous capacitance mobilizes blood volume. A common means to estimate venous capacitance changes in vivo is to measure the mean circulatory filling pressure (MCFP), which is the plateau venous pressure during a transient circulatory arrest (34). Because of the much higher elasticity (compliance) and volume in the venous compartment compared with the arterial compartment, changes in MCFP primarily reflects changes in the venous vasculature and/or, alternatively, in total blood volume (31, 34). MCFP has also been found to roughly equal blood pressure at the level of the venules and may thus provide an estimate of the upstream driving pressure for venous return to the heart (31, 34). Depending on the cardiac response, a decreased venous capacitance might shift blood toward the central venous compartment, which will be manifested as an increased cardiac filling pressure and possibly increased stroke volume (31, 34). Furthermore, during exercise, when cardiac output and venous return increase, the venous pressure difference must increase according to Pouisseille’s law. Consequently, an active increase in venous tone (which would be reflected as an increased MCFP) is likely to be an important mechanism that allows venular pressure to increase and prevents blood from pooling in the venous periphery as blood flow through the capillary beds increases.

In fish, our knowledge regarding the role and importance of the neurohumoral control of the venous circulation is still fragmentary. With few exceptions (e.g., Ref. 41), most studies to date have used rainbow trout, in which α-adrenergic control of the venous capacitance vasculature is well developed (38, 52). During exercise, MCFP increases in trout and European sea bass (*Dicentrarchus labrax*) (40, 41), and venous capacitance decreases during hypoxia in trout (37). These changes are largely mediated by increased α-adrenergic tone. It is unknown whether these mechanisms are present also in the ancient phylogenetic lineage occupied by elasmobranch fishes.

Historically, the control of venous function in elasmobranchs has been dominated by the notion that cardiac filling is mediated by suction of a heart enclosed in a rigid pericardial cavity (i.e., vis a fronte) (1–3, 14, 16, 20, 42, 44, 47) and subambient central venous pressures (P_{ven}) (7, 10, 21, 45, 47). Evidence for active venous regulation in elasmobranchs is conflicting. For example, Satchell (43) suggested a passive role for the venous vasculature in elasmobranchs, based on the apparently strong dependence on vis a fronte filling and the observation that large veins in sharks have a low density of smooth muscle cells that virtually lack innervation. He hypothesized that the potential redistribution of venous blood volume must be mediated by passive changes in upstream arteriolar resistance rather than by active changes from venoconstriction. Additionally, elasmobranchs appear to tolerate gravitational stress very poorly compared with teleosts (27). On the other hand, we provide convincing evidence for adrenergic control of the venous vasculature in elasmobranchs, although the pericardium is clearly an important component in the modulation of venous function. Thus active changes in venous capacitance have previously been underestimated as an important means of modulating venous return and cardiac performance in this group.
hand, \( P_{\text{ven}} \) in elasmobranchs does increase in response to an injection of epinephrine (10) and during exercise (24). Furthermore, pericardial pressure is elevated during exercise, which is indicative of an increased filling pressure (23), and the perfused dogfish heart responds well to changes in cardiac filling pressure in accordance with the Frank-Starling mechanism (14, 16). Thus, despite the apparently strong dependence on vis a \( \text{via} \) fronte for cardiac filling, peripheral venoconstriction may also be important in modulating venous return and cardiac filling in this group.

Measurements of MCFP in the dogfish could resolve these conflicting results by providing an effective method to examine the possibility for active venous capacitance changes in changing venous return and cardiac filling pressure. As far as we are aware, no such measurements have been published for any cartilaginous fish. Therefore, the present study on the dogfish was undertaken with two primary objectives in mind. First, we were interested in providing an effective method to examine this group.

MATERIALS AND METHODS

Animals

Pacific spiny dogfish (\( Squalus acanthias \)) of either sex in the size range of 904–1,780 g were caught using long lines with baited barbless hooks. Animals were held outdoors in a fiberglass tank (2,000 liters) supplied with through-flowing seawater of ambient temperature (10°C). The fish were not fed while in captivity (<2 wk).

All experiments were approved by the University of British Columbia Animal Care Committee in accordance with the Canadian Council for Animal Care.

Surgery and Experimental Setup

Sharks were individually anesthetized in seawater containing ~100 mg/l of ethyl 3-amino benzoate methanesulfonate salt (MS-222) and placed in a V-shaped trough, where they were continuously supplied with recirculating (~10°C) seawater containing MS-222 (~50 mg/l). To obtain dorsal aortic blood pressure (\( P_{\text{a}} \)), the dorsal aorta was cannulated percutaneously from the tail with a polyethylene (PE)-50 catheter using a 16-gauge needle as a trochar. The catheter was advanced retrograde ~20 cm along the length of the vessel and secured to the skin with a silk suture (19). A dab of cyanoacrylate glue on the tissue sealed the cannulation site. \( P_{\text{ven}} \) was measured in the ductus of Cuvier, which was cannulated with a PE-50 catheter fashioned with the tip pulled slightly to obtain a snug fit around the tip of the guide and an extra side hole made 0.5 cm from the tip to optimize patency. To position this catheter, the fish was positioned ventral side up, and an ~1.5-cm ventral incision was made to gain access to the most posterior branchial cavity. The catheter was introduced blind into the vessel through the posterior wall of the branchial cavity using a sharpened steel wire guide. The catheter was secured with a single silk suture to the wall of the branchial cavity and with additional sutures to the skin. The incision was closed with interrupted silk sutures and cyanoacrylate glue. Postmortem examination of several specimens revealed that the venous catheter was located in the ductus of Cuvier just before the entry into the sinus venosus. Both catheters were filled with heparinized (100 IU/ml) dogfish saline (1.3% NaCl) and sealed with pins. The ventral aorta was exposed with a midline ventral incision, and a Transonic transit-time blood-flow probe (25 or 45) was positioned around the ventral aorta posterior to the first two anterior pairs of branchial arteries. The placement of the flow probe meant that the cardiac output going to the gill arches posterior to this position was not measured, and so only partial values of cardiac output (\( Q_{\text{arch}} \)), stroke volume (\( S_{\text{V volunteers}} \)), systemic resistance (\( R_{\text{sys}} \)), and venous resistance (\( R_{\text{ven}} \)) were reported. Previously, Taylor et al. (49), using the Fick equation, estimated blood flow to the anterior two pairs of gill arches as 37% of the total cardiac output, a percentage that was unchanged during hypoxia.

In most of our experiments where MCFP was measured, a cuff-type vascular occluder was placed around the conus arteriosus near the ventral aorta. This method has been used previously in teleosts and has been described elsewhere (38, 41). However, due to the anatomic arrangement in dogfish, a 0.5-cm incision had to be made in the rostral wall of the pericardium to gain access to the conus arteriosus. The pericardium was subsequently closed with uninterrupted 4-0 silk sutures. The entire midline incision was closed with interrupted silk sutures and cyanoacrylate glue, and the catheters and leads were additionally secured to the skin with silk sutures.

Numerous experiments were attempted in which a Fogarty embolectomy catheter was introduced into the conus arteriosus via an afferent branchial artery so that cardiac outflow could be occluded without cutting the pericardium. This type of catheter has previously been successfully used to cannulate and occlude the dorsal aorta in rainbow trout (38). Although successful balloon placement allowed for the complete stoppage of cardiac outflow (Fig. 1A), accurate placement of the Fogarty catheter proved to be extremely difficult due to the conal valves. Typically, the tip of the catheter would lodge between the conal wall and the first conal valve, precluding further retrograde advancement of the catheter from the ventral aorta into the conus arteriosus. Inflation of the balloon in this position resulted in only partial stoppage of cardiac output (Fig. 1B). Given the unusually low success rate of this surgical approach, it was abandoned in favor of the pericardioectomy as described above, but this necessitated an evaluation of pericardioectomy on venous function.

To investigate the effect of the surgical pericardioectomy, a second group of animals was instrumented with the flow probe and catheters as described above but without an occluder. Hence, in this control group, the pericardium remained intact, but MCFP could not be measured. After the surgery, fish were transferred to opaque holding tanks supplied with through-flowing seawater at 10–11°C and left to recover for 24 h before the experiment commenced.

Drug Injection Protocol

To investigate the adrenergic control of venous capacitance, 100 \( \mu \text{g/kg body mass (} M_\text{b} \text{)} \) of phenylephrine was injected into the dorsal aorta to stimulate \( \alpha \)-adrenoceptors and isoproterenol (1 \( \mu \text{g/kg } M_\text{b} \)) to stimulate \( \beta \)-adrenergic receptors. Epinephrine bitartrate (5 \( \mu \text{g/kg } M_\text{b} \)) was used as a combined \( \alpha \)- and \( \beta \)-adrenoceptor agonist. The doses were chosen because, in preliminary experiments, they produced clear and consistent venous responses that wore off within 30–50 min. Assuming a blood volume of 6% for dogfish (28) and that the entire dose stays within the intravascular compartment, the injected epinephrine dose would result in a final circulating concentration of ~250 nmol/l. The injections of epinephrine and phenylephrine (same dosages as above) were repeated 1.5–2 h after the \( \alpha \)-adrenergic blockade with prazosin (1 mg/kg \( M_\text{b} \)). All pharmacological substances were purchased from Sigma (St. Louis, MO).

In the pericardioectomized group and in two fish with Fogarty catheters where MCFP was measured, baseline cardiovascular variables were recorded after a bolus of saline (0.5 ml/kg \( M_\text{b} \)) administered via the arterial catheter. After 10 min, when any potential effect of the saline injection and the ventral aortic occlusion had worn off, a bolus (0.5 ml/kg \( M_\text{b} \)) of any of the adrenergic drugs was administered in a randomized manner followed by 0.4 ml of saline to clear the dead space in the catheter. Each injection was separated by at least 30 min or until cardiovascular variables had returned to baseline levels.
Because MCFP cannot be measured continuously, all drug responses reported in this study were always measured 2 min after the injection of epinephrine and phenylephrine and 5 min after the injection of isoproterenol, because initial experiments showed that the peak responses to the respective drugs roughly occurred within these time intervals.

In the control group, agonist and antagonist injections were performed in the same manner as described for the pericardioectomized group. However, because MCFP was not measured in this series, saline was not injected during the control period as we assumed that the small volume loading effect the injection might have per se was negligible for the interpretation of the drug effects on the dynamic pressure and flow responses.

Data Acquisition and Calculations

Blood pressures were measured using disposable pressure transducers (model DPT-6100, Medizintechnik, Kirchseeon, Germany) calibrated several times daily against static water columns. The signals generated by the transducers were amplified using a 4ChAmp amplifier (Somedic, Hörby, Sweden). The flow probe around the ventral aorta was connected to a Transonic flowmeter (model T206) to obtain partial ventral aortic blood flow. Blood flow data were subsequently normalized to body weight. Data were digitally stored for subsequent analysis using a Power Lab unit (ADInstruments) connected to a portable computer.

Heart rate (fH) was calculated from pulsatile pressure or flow records. MCFP was taken as Pven during the last 5–10 s of a 20-s occlusion. This time interval was chosen because Pven reached a stable plateau within this period. In trout, shorter occlusion periods generally have to be applied when measuring MCFP because pronounced barostatic reflexes are activated after 8–12 s of ventral aortic occlusion (38, 51). No compensatory cardiovascular reflexes were observed during occlusion in dogfish (S. acanthias) (see Fig. 1 and the DISCUSSION). The pressure gradient driving venous return (ΔPven) was calculated as MCFP − Pven. SVpart was calculated as Qpart/fH. Rsys part was calculated as (Pda − Pven)/Qpart, and Rven part was calculated as (MCFP − Pven)/Qpart (41).

Statistical Analysis

In the pericardioectomized group, 20-s mean values immediately before each MCFP maneuver were compared. In the control group with an intact pericardium, where MCFP was not measured, the baseline value was compared with a 20-s mean value after an identical time interval (i.e., 2 min after the injections of epinephrine and phenylephrine and 5 min after the injection of isoproterenol). All reported values are means ± SE (13). A Wilcoxon matched-pairs signed-ranks test (two tailed) was used to compare cardiovascular variables before and after drug injection, and a Mann-Whitney U-test was used to compare baseline values between groups. A significance level of 5% was applied for all statistical comparisons.

RESULTS

Ventral Aortic Occlusion

Examples of effects of occlusions are presented in Fig. 1. Successful placement of a Fogarty catheter in the conus arteriosus allowed for complete occlusion of cardiac outflow, as judged from the nonpulsatile Pda (Fig. 1A). In contrast, unsuccessful placement of the Fogarty catheter resulted in only partial occlusion (Fig. 1B). Inflation of the perivascular occluder around the conus arteriosus also produced an immediate
rise in $P_{\text{ven}}$ and a decline in $P_{\text{da}}$ (Fig. 1, C and D). Occasionally, the heart did not resume beating immediately after the occlusion was released (Fig. 1D).

**General Effects of Pericardioectomy and $\alpha$-Adrenoceptor Blockade**

In Fig. 2, baseline cardiovascular variables are compared for fish with and without an open pericardium. The only statistically significant difference in baseline cardiovascular status as a result of pericardioectomy was an elevated $P_{\text{ven}}$ ($0.09 \pm 0.01$ vs. $-0.08 \pm 0.02$ kPa) and a significantly reduced venous pulse pressure ($P_{\text{ven pulse}}$).

$\alpha$-Adrenoceptor blockade had somewhat more depressant effects in the pericardioectomized group because $P_{\text{ven}}$, $f_H$, and $R_{\text{sys part}}$ were not reduced by prazosin in the control group, whereas only $f_H$ and $R_{\text{ven part}}$ were unaffected in the pericardioectomized group. All other variables decreased significantly after prazosin. These results suggest that pericardioectomy may increase the general $\alpha$-adrenergic tone on the circulation. After prazosin, $P_{\text{ven}}$ and $P_{\text{ven pulse}}$ remained significantly different in the control group compared with the pericardioectomized group. Both $P_{\text{ven pulse}}$ and dorsal aortic pulse pressure ($P_{\text{da pulse}}$) decreased significantly after $\alpha$-adrenoceptor blockade in both groups.

**Effects of Adrenergic Agonists**

**Cardiovascular responses in pericardioectomized fish.** The venous responses to adrenergic agonist injection are shown in Fig. 3. Intravenous injections of epinephrine and phenylephrine both significantly increased $P_{\text{ven}}$ and MCFP, whereas isoproterenol significantly decreased the same variables. Phenylephrine, but not epinephrine, also reduced $\Delta P_{\text{ven}}$ significantly. Prazosin treatment completely abolished the venous effects of epinephrine and significantly attenuated the increases in $P_{\text{ven}}$ and MCFP in response to phenylephrine. These results show that both $\alpha$- and $\beta$-adrenergic mechanisms control the venous circulation in dogfish.

Epinephrine also increased $P_{\text{da}}$, $P_{\text{da pulse}}$, and $R_{\text{sys part}}$, whereas phenylephrine decreased $f_H$ and increased $P_{\text{da pulse}}$ without affecting $P_{\text{da}}$ and $R_{\text{sys part}}$ (Table 1). Isoproterenol decreased $P_{\text{da}}$, $P_{\text{da pulse}}$, and $R_{\text{sys part}}$. Prazosin significantly attenuated the increases in $R_{\text{sys part}}$ and $P_{\text{da}}$ in response to
epinephrine, whereas phenylephrine resulted in a small but significant increase in \(P_{\text{da}}\) and a significantly smaller decrease in \(f_{\text{H}}\). \(P_{\text{da}}\) pulse still increased in response to epinephrine and phenylephrine, but these responses were significantly smaller compared with the responses in untreated sharks (Table 1).

Cardiovascular responses in control fish with intact pericardium. Table 2 shows the responses to agonist injection in fish with an intact pericardium. The qualitative responses were largely similar compared with the pericardioectomized group. Both epinephrine and phenylephrine increased \(P_{\text{da}}\), \(P_{\text{ven}}\), \(P_{\text{da}}\) pulse, and \(R_{\text{sys part}}\), and epinephrine also resulted in tachycardia. Isoproterenol decreased \(P_{\text{da}}\), \(P_{\text{ven}}\), and \(P_{\text{da}}\) pulse, but, in contrast to the situation in pericardioectomized fish, \(P_{\text{ven}}\) and \(P_{\text{da}}\) pulse were unchanged. Instead, \(f_{\text{H}}\) increased, whereas \(SV_{\text{part}}\) decreased.

Prazosin significantly attenuated both venous and arterial pressor effects of epinephrine, but it only attenuated the change in \(P_{\text{ven}}\) in response to phenylephrine, while the increases in \(R_{\text{sys part}}\), \(P_{\text{da}}\) pulse, and \(P_{\text{da}}\) pulse were unchanged. Again, epinephrine increased \(f_{\text{H}}\) resulting in a rise in \(Q_{\text{part}}\), while phenylephrine significantly reduced heart rate after prazosin (Table 2).

In the two fish instrumented with Fogarty catheters, routine \(P_{\text{ven}}\) was \(-0.09\) kPa, and MCFP was \(0.12\) kPa (data

---

**Table 1. Cardiovascular responses to agonist injection in the dogfish Squalus acanthias with perivascular occluder**

<table>
<thead>
<tr>
<th></th>
<th>(P_{\text{da}}) kPa</th>
<th>(P_{\text{ven}}) kPa</th>
<th>(P_{\text{da}}) pulse kPa</th>
<th>MCFP, kPa</th>
<th>(Q_{\text{ven}}) ml/min (1) kg (M_b)</th>
<th>(f_{\text{H}}), beats/ min</th>
<th>(SV_{\text{part}}) ml/kg (M_b)</th>
<th>(R_{\text{sys part}}) kPa/ml (1) kg (M_b)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Untreated</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>(2.2) \pm 0.1</td>
<td>(0.9) \pm 0.01</td>
<td>(0.7) \pm 0.1</td>
<td>(0.17) \pm 0.01</td>
<td>(9.2) \pm 1.0</td>
<td>(20.5) \pm 1.0</td>
<td>(0.47) \pm 0.6</td>
<td>(0.25) \pm 0.04</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>(+0.9) \pm 0.1*</td>
<td>(+0.04) \pm 0.01*</td>
<td>(+0.3) \pm 0.0*</td>
<td>(+0.03) \pm 0.01*</td>
<td>(-0.9) \pm 0.7</td>
<td>(+0.9) \pm 0.6</td>
<td>(-0.06) \pm 0.05</td>
<td>(+0.16) \pm 0.05†</td>
</tr>
<tr>
<td>Baseline</td>
<td>(+0.2) \pm 0.2</td>
<td>(0.09) \pm 0.01</td>
<td>(0.7) \pm 0.1</td>
<td>(0.17) \pm 0.01</td>
<td>(9.3) \pm 0.9</td>
<td>(21.0) \pm 1.0</td>
<td>(0.45) \pm 0.03</td>
<td>(0.24) \pm 0.03</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>(+0.1) \pm 0.1</td>
<td>(+0.04) \pm 0.01*</td>
<td>(+0.2) \pm 0.0*</td>
<td>(+0.03) \pm 0.01*</td>
<td>(-0.4) \pm 0.5</td>
<td>(-2.8) \pm 0.4*</td>
<td>(+0.04) \pm 0.03</td>
<td>(+0.03) \pm 0.02†</td>
</tr>
<tr>
<td>Baseline</td>
<td>(+0.3) \pm 0.2</td>
<td>(0.10) \pm 0.01</td>
<td>(0.8) \pm 0.1</td>
<td>(0.18) \pm 0.01</td>
<td>(9.2) \pm 0.9</td>
<td>(20.1) \pm 1.1</td>
<td>(0.48) \pm 0.06</td>
<td>(0.25) \pm 0.03</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>(-0.2) \pm 0.0</td>
<td>(-0.02) \pm 0.01*</td>
<td>(-0.1) \pm 0.0*</td>
<td>(-0.01) \pm 0.01*</td>
<td>(+0.2) \pm 0.5</td>
<td>(+0.9) \pm 0.5</td>
<td>(-0.02) \pm 0.02</td>
<td>(-0.03) \pm 0.01*†</td>
</tr>
<tr>
<td><strong>Prazosin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>(1.3) \pm 0.1</td>
<td>(0.07) \pm 0.01</td>
<td>(0.4) \pm 0.1</td>
<td>(0.13) \pm 0.01</td>
<td>(6.8) \pm 1.0</td>
<td>(19.9) \pm 1.3</td>
<td>(0.36) \pm 0.06</td>
<td>(0.19) \pm 0.02</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>(+0.4) \pm 0.1†</td>
<td>(+0.01) \pm 0.00†</td>
<td>(+0.1) \pm 0.00†</td>
<td>(+0.00) \pm 0.00</td>
<td>(+0.1) \pm 0.2</td>
<td>(+0.1) \pm 0.5</td>
<td>(-0.01) \pm 0.01</td>
<td>(+0.05) \pm 0.01†</td>
</tr>
<tr>
<td>Baseline</td>
<td>(+1.3) \pm 0.1</td>
<td>(0.06) \pm 0.01</td>
<td>(0.4) \pm 0.1</td>
<td>(0.12) \pm 0.00</td>
<td>(6.8) \pm 0.8</td>
<td>(20.7) \pm 1.4</td>
<td>(0.34) \pm 0.05</td>
<td>(0.18) \pm 0.02</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>(+0.1) \pm 0.0</td>
<td>(+0.01) \pm 0.00†</td>
<td>(+0.1) \pm 0.00†</td>
<td>(+0.01) \pm 0.00†</td>
<td>(+0.1) \pm 0.3</td>
<td>(-1.3) \pm 0.5†</td>
<td>(+0.02) \pm 0.02</td>
<td>(+0.01) \pm 0.01</td>
</tr>
</tbody>
</table>

Values are means \(\pm\) SE; \(n = 9–11\) untreated dogfish and 9–11 dogfish treated with 1 mg/kg body mass \((M_b)\) prazosin. Shown are changes from baseline after epinephrine \((5 \mu g/kg M_b)\), phenylephrine \((100 \mu g/kg M_b)\), and isoproterenol \((1 \mu g/kg M_b)\) in untreated and prazosin-treated dogfish. \(P_{\text{da}}\), dorsal aortic blood pressure; \(P_{\text{ven}}\), central venous blood pressure; \(P_{\text{da}}\) pulse, dorsal aortic pulse pressure; MCFP, mean circulatory filling pressure; \(Q_{\text{ven}}\), partial cardiac output; \(f_{\text{H}}\), heart rate; \(SV_{\text{part}}\), partial stroke volume; \(R_{\text{sys part}}\), partial systemic resistance. *Significant effect of agonist injection; †significant difference from the corresponding value in untreated fish \((P < 0.05)\).
CONTROL OF VENOUS FUNCTION IN DOGFISH

DISCUSSION

Resting \( P_{\text{ven}} \) and Effect of Pericardectomy

\( P_{\text{ven}} \) in elasmobranchs is subambient. Johansen and Hanson (21) reported a pressure of 0 to \(-0.1 \) kPa in the posterior cardinal sinus in free-swimming \( S. \text{acanthias} \) and a pressure of around \(-0.15 \) kPa in the sinus venosus in one restrained specimen. Similarly, Capra and Satchell (10) reported a pressure of \(-0.4 \) to 0 kPa in the lateral abdominal vein. In another dogfish species, \( Scyliorhinus \text{canicula} \), Short et al. (45) reported a pressure of \(-0.45 \) kPa in the sinus venosus. The routine subambient \( P_{\text{ven}} \) values recorded here (\(-0.08 \) to 0.02 to \(-0.07 \) \pm 0.01 kPa; Table 2 and Fig. 2) on dogfish with an intact pericardium are consistent with these previous values.

Pericardectomy clearly resets \( P_{\text{ven}} \) because \( P_{\text{ven}} \) was always positive in fish with a vascular occluder (0.09 \pm 0.01 to 0.10 \pm 0.01 kPa; Table 1). This was associated with a significantly lower \( P_{\text{ven}} \) pulse (Figs. 2 and 4), which suggests that the effects of vis a fronte filling may be manifest in \( P_{\text{ven}} \) measured in the ductus of Cuvier. In situ perfused hearts of dogfish and rainbow trout can pump routine cardiac outputs with subambient filling pressures (15, 16). When the pericardium is cut, however, there is a right shift of the Starling curve of the heart as it switches from vis a fronte to vis a tergo filling (15, 16). This may be the case here too for dogfish in vivo after pericardiectomy. The increase in \( P_{\text{ven}} \) after pericardiectomy is probably both an effect of blood pooling in the central venous compartment as cardiac suction force is impaired but presumably also a result of an increased adrenergic venous tone (see below). Nevertheless, these findings are important for future experiments on venous function in sharks, because they reveal that different surgical methods can produce significantly different venous values.

The only exception of negative \( P_{\text{ven}} \) in sharks that we are aware of is for \( Triakis \text{semifasciata} \), where cardiac sinus pressure was positive, ranging from 0.20 \pm 0.05 to 0.26 \pm 0.05 kPa, and, during exercise, it rose even higher (24). These values, which are even higher than those we report here for the pericardiectomized dogfish, point to either an important species difference among elasmobranchs or that the surgical method used in the intact group probably overestimate MCFP in the dogfish (Fig. 2). This value is similar to previously reported values for rainbow trout (\(-0.17 \) kPa; Refs. 37 and 38) but somewhat lower than those for the European sea bass (\( D. \text{labrax} \), \(-0.27 \) kPa; Ref. 41). It is likely, however, that the intact pericardium in the pericardiectomized group might have resulted in peripheral venoconstriction, which is believed to occur in trout as a means of augmenting venous return and cardiac filling pressure when cardiac suction force is impaired after pericardiectomy (15, 16, 37, 51). Thus, the values obtained in the pericardiectomized group probably overestimate MCFP in the dogfish slightly. In fact, in the two fish instrumented with Fogarty catheters, where the pericardium was left intact, MCFP was 0.12 kPa.

The MCFP values above should be related to the \( P_{\text{ven}} \) Values for these animals because the difference between these two pressures is a strong determinant of the rate of venous return (31). Routine \( P_{\text{ven}} \) is around 0.06 kPa in rainbow trout (37, 39) and 0.11 kPa in sea bass (41). Thus, it seems that \( P_{\text{ven}} \) is reflected in MCFP, such that animals with a low \( P_{\text{ven}} \) also have a low MCFP.

The fast vascular baroreflex responses typically observed during ventral aortic occlusion in trout (38, 51) and mammals (32) were absent in the dogfish as judged by the stable occlusion values of \( P_{\text{da}} \) and \( P_{\text{ven}} \) (Fig. 1). While there is evidence for the cardiac limb of the baroreflex in sharks (5, 22, 25, 50), these results indicate that nervous baroreflex control of the systemic vasculature of the dogfish is relatively limited or absent. This line of argument is supported by the apparently sparse nervous control of the vasculature in elasmobranchs (9, 18, 26, 30, 43). Instead, sharks seem to rely on another, somewhat slower baroreflex mechanism, where

<table>
<thead>
<tr>
<th></th>
<th>( P_{\text{da}} ), kPa</th>
<th>( P_{\text{ven}} ), kPa</th>
<th>( P_{\text{da}} ) pulse, kPa</th>
<th>( Q_{\text{ven}} ), mL/min·kg(^{-1})</th>
<th>( f_{\text{hr}} ), beats/ min</th>
<th>( SV_{\text{part}} ), mL/kg</th>
<th>( R_{\text{sys part}} ), kPa·min(^{-1})·kg(^{-1})</th>
<th>( M_{\text{b}} ), g/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>2.4 \pm 0.2</td>
<td>-0.08 \pm 0.02</td>
<td>0.7 \pm 0.1</td>
<td>11.0 \pm 1.3</td>
<td>23.4 \pm 1.3</td>
<td>0.49 \pm 0.06</td>
<td>0.25 \pm 0.03</td>
<td></td>
</tr>
<tr>
<td>Epinephrine</td>
<td>+1.0 \pm 0.1*</td>
<td>+0.14 \pm 0.2*</td>
<td>+0.3 \pm 0.1*</td>
<td>-0.1 \pm 0.6</td>
<td>+2.5 \pm 0.4*</td>
<td>-0.05 \pm 0.03</td>
<td>+0.09 \pm 0.02*</td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>2.3 \pm 0.1</td>
<td>-0.07 \pm 0.01</td>
<td>0.7 \pm 0.1</td>
<td>11.2 \pm 1.2</td>
<td>23.7 \pm 1.2</td>
<td>0.49 \pm 0.06</td>
<td>0.23 \pm 0.03</td>
<td></td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>+0.5 \pm 0.1*</td>
<td>+0.12 \pm 0.2*</td>
<td>+0.2 \pm 0.0*</td>
<td>-0.8 \pm 0.5</td>
<td>-0.5 \pm 0.3</td>
<td>-0.03 \pm 0.03</td>
<td>+0.06 \pm 0.01*</td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>2.3 \pm 0.2</td>
<td>-0.08 \pm 0.02</td>
<td>0.7 \pm 0.1</td>
<td>11.4 \pm 1.3</td>
<td>23.9 \pm 1.2</td>
<td>0.48 \pm 0.05</td>
<td>0.23 \pm 0.03</td>
<td></td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>-0.4 \pm 0.1*</td>
<td>-0.11 \pm 0.2*</td>
<td>-0.3 \pm 0.0*</td>
<td>-0.8 \pm 0.5</td>
<td>+1.9 \pm 0.2*</td>
<td>-0.07 \pm 0.02*</td>
<td>-0.01 \pm 0.1</td>
<td></td>
</tr>
<tr>
<td>Prazosin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1.7 \pm 0.2</td>
<td>-0.08 \pm 0.02</td>
<td>0.4 \pm 0.1</td>
<td>9.0 \pm 0.9</td>
<td>23.7 \pm 0.9</td>
<td>0.38 \pm 0.03</td>
<td>0.21 \pm 0.03</td>
<td></td>
</tr>
<tr>
<td>Epinephrine</td>
<td>+0.6 \pm 0.1*†</td>
<td>+0.05 \pm 0.1†</td>
<td>+0.2 \pm 0.0†</td>
<td>+0.7 \pm 0.2†</td>
<td>+1.3 \pm 0.2†</td>
<td>+0.01 \pm 0.01</td>
<td>+0.05 \pm 0.01†</td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1.6 \pm 0.2</td>
<td>-0.09 \pm 0.02</td>
<td>0.4 \pm 0.1</td>
<td>8.7 \pm 0.8</td>
<td>24.5 \pm 0.7</td>
<td>0.37 \pm 0.04</td>
<td>0.21 \pm 0.03</td>
<td></td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>+0.3 \pm 0.1*</td>
<td>+0.05 \pm 0.1†</td>
<td>+0.2 \pm 0.0*</td>
<td>-0.1 \pm 0.3</td>
<td>-1.7 \pm 0.2†</td>
<td>+0.02 \pm 0.01†</td>
<td>+0.04 \pm 0.01*</td>
<td></td>
</tr>
</tbody>
</table>

Values are means \( \pm \) SE; \( n = 8 \) 9 untreated dogfish and 8 9 prazosin (1 mg/kg \( M_{\text{b}} \))-treated dogfish. Shown are changes from baseline after epinephrine (5 \( \mu g/kg \) \( M_{\text{b}} \)), phenylephrine (100 \( \mu g/kg \) \( M_{\text{b}} \)), and isoproterenol (1 \( \mu g/kg \) \( M_{\text{b}} \)) in untreated and prazosin-treated dogfish. *Significant effect of agonist injection; †significant difference from the corresponding value in untreated fish (\( P < 0.05 \)).
catecholamines and angiotensins are released into the bloodstream in response to arterial hypotension (6, 11).

Occasionally, transient cardiac arrest was observed after conal occlusion, but the heart always resumed beating (Fig. 1). We have previously observed similar responses in the Antarctic fish *Pagothenia borchgrevinki* after mechanical ventral aortic occlusion (M. Axelsson, unpublished observations) but never in the trout or sea bass. The mechanism behind this cardiac response remains unknown.

**Vascular Responses to Adrenergic Agonists and Antagonists**

Overall, the cardiovascular responses to adrenergic agonist injections were qualitatively similar in the two groups, but the changes were more pronounced in fish with an intact pericardium (Tables 1 and 2). This may have been related to a higher routine adrenergic tone after pericardioectomy, as noted above. For example, only in the group with an intact pericardium did both Pda and Rsyst part increase in response to α-adrenoceptor stimulation (Tables 1 and 2). Although routine Rsyst part was not significantly different in the two groups, routine vascular α-adrenergic tone was presumably higher in the pericardioctomized fish because both Pven and Rsyst part fell significantly after prazosin treatment in this group (Fig. 2). Thus, these findings suggest that adrenergic venous mechanisms were clearly still functioning after pericardioectomy but that the available scope for altering vascular tone was attenuated.

Phenylephrine and epinephrine typically increased Pven and MCFP (Tables 1 and 2), responses that were blocked or attenuated by prazosin. Thus, α-adrenergic constriction is an important means of regulating capacitance vessel tone in the dogfish. Because the venous responses to epinephrine injection mimicked those to phenylephrine, it is clear that, with the doses used in the present experiments, the α-adrenergic response to this naturally occurring catecholamine dominates over the β-adrenergic effects. This is consistent with previous studies (8, 10, 19, 26) on the arterial circulation of dogfish, where α-adrenoceptor stimulation increased blood pressure.

Capra and Satchell (10) reported that β-adrenergic stimulation reduced central and caudal venous pressures in dogfish. The present study revealed that β-adrenergic stimulation relaxed the venous vasculature, as judged by the reduced MCFP after isoproterenol (Fig. 3). This indicates that the drop in Pven is at least in part mediated through venous relaxation. Similar to the situation in trout, however, the possibility that tachycardia could passively decrease Pven remains (4), especially because isoproterenol increased fH in the group with an intact pericardium (Table 2). In contrast, in mammals, the capacitance vasculature is normally not affected by β-adrenergic stimulation (36, 48), but our findings of β-adrenergic venodilation in dogfish agree well with those recently reported for the rattlesnake (46).

The changes in Pda pulse in response to adrenergic agonists were similar in the two groups (Tables 1 and 2). These changes probably reflected changes in arterial compliance and resistance and/or cardiac contractility. These arguments are supported by the drop in routine Pda pulse after prazosin (Fig. 2). The changes in Pven pulse, however, differed markedly between the two groups. Routine Pven was much more pulsatile in the control fish without the occluder and remained so even after prazosin, indicating that this difference was due to the severely impaired cardiac suction force in the pericardioctomized group (Figs. 2 and 4). The agonist injections in the control group primarily affected diastolic pressure, such that the pulse pressure decreased in response to epinephrine and phenylephrine and increased in response to isoproterenol. However, after pericardioectomy, only small changes in mean Pven were observed in response to the agonists (Fig. 4).

**Cardiac Responses to Adrenergic Agonists and Antagonists**

From perfused heart preparations, it is well recognized that the elasmobranch heart responds in accordance with the Frank-Starling mechanism, i.e., an increased cardiac filling pressure increases cardiac output via increased stroke volume and cardiac contraction force (14, 16). Although all agonist injections

![Fig. 4. Representative original recordings of Pven in the dogfish *S. acanthias* showing the effects of injections of adrenergic agonists, including epinephrine (5 μg/kg M9), phenylephrine (100 μg/kg M9), and isoproterenol (1 μg/kg M9), in pericardioctomized fish instrumented with a perivascular occluder (A) and in control fish without an occluder and with an intact pericardium (B). Dashed vertical lines denote the start of drug administration. Control fish with an intact pericardium displayed larger routine Pven pulse and larger changes in response to drugs compared with pericardioctomized fish.](http://ajpregu.physiology.org/doi/10.1210/pa-r1471)
significantly increased or decreased cardiac filling pressure ($P_{\text{ven}}$), neither $SV_{\text{part}}$ nor $Q_{\text{part}}$ changed. This is probably because $R_{\text{sys}}$ and $f_H$ were also affected by the injections. For example, the lack of increase in stroke volume after epinephrine could be explained by a combined effect of an increased afterload ($R_{\text{sys}}$ part) in both groups and possibly also by an increased $f_H$ in the group with an intact pericardium (Tables 1 and 2). Other investigators, using similar drug injection protocols in intact animals, have also reported large changes in filling pressure with no changes in stroke volume or cardiac output (40, 52). Under natural conditions such as exercise, however, when cardiac filling pressure and stroke volume are likely to increase from adrenergic stimulation, the increase in afterload is significantly less because a metabolic vasodilation counters the adrenergic constriction of the resistance vasculature. The only significant change in $SV_{\text{part}}$ that was found in the present study was the drop after isoproterenol in the control group, but because $f_H$ increased in conjunction with the drop in filling pressure, it is impossible to conclude whether the reduced stroke volume was induced by tachycardia, reduced filling pressure, or both.

Conclusions

This study presents the first conclusive evidence that elasmobranchs have the capability to alter venous capacitance via adrenergic control mechanisms. Given the apparently sparse innervation of the circulation in sharks, these changes are presumably mediated through circulating catecholamines. How these mechanisms are utilized during natural cardiovascular challenges, such as exercise and hypoxia, will require future experiments similar to those recently conducted for teleosts (37, 40, 41). Nevertheless, from the above experiments, it is possible to conclude that the machinery necessary to regulate venous return and cardiac preload, by means of active changes in venous capacitance, is present in the ancient phylogenetic lineage occupied by sharks.

ACKNOWLEDGMENTS

The authors thank Christopher Wilson for excellent assistance during the experimental phase of this study.

GRANTS

Financial support was received from the Swedish Research Council (to M. Axelson), the National Sciences and Engineering Research Council of Canada (to A. P. Farrell), and the Helge Axelsson Foundation (to E. Sandblom).

REFERENCES


