POSSIBLE MECHANISMS OF CONTROL OF VASCULAR RESISTANCE IN THE LOBSTER HOMARUS AMERICANUS

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Summary

In Homarus americanus, the resistance to fluid flow through each of the arteries leaving the heart, including the complete hemocoelic return pathways, can be controlled. Each of the five arterial types (anterior median, paired anterior lateral, paired hepatic, sternal and dorsal abdominal) exhibits a unique spectrum of responses to a battery of neurotransmitters (acetylcholine, glutamic acid, y-aminobutyric acid) and neurohormones (dopamine, octopamine, 5-hydroxytryptamine, crustacean cardioactive peptide, FLRFamide-related peptides F1 and F2, and proctolin). Acetylcholine causes increases in resistance in all arteries except the anterior median artery; in the dorsal abdominal artery, this increase is antagonized by y-aminobutyric acid. All neurohormones that are effective in a particular artery cause increases in resistance to flow. The sites of action of these compounds in the dorsal abdominal artery are valves located at major branch points; the sites of control in the other arteries are not known. It is concluded that the control of arterial resistance is a mechanism which the animal can exploit to produce different flow patterns among the various arteries.

Key words: vascular resistance, lobster, Homarus americanus, neurohormone, neurotransmitter, artery, arterial valve, acetylcholine, glutamic acid, y-aminobutyric acid (GABA), dopamine, octopamine, serotonin, 5-hydroxytryptamine, crustacean cardioactive peptide, F1, F2, proctolin.

Introduction

The requirement for a large animal to be able to regulate the distribution of blood to different organ systems to meet varying perfusion demands is axiomatic. In mammals, all systemic blood leaves the left ventricle via a single aorta. Thereafter, the distribution of blood throughout different vascular beds is primarily under the active control of the resistance of individual segments (Berne and Levy, 1992). Flow rate in an artery will be inversely proportional to resistance, and resistance is increased or decreased by contraction or relaxation of the smooth muscle layers in the walls of the arteries and arterioles. In crustaceans, the flow rate through individual arteries also varies over time, and the distribution pattern among the various arteries is known to change, often in predictable ways, in response to changing conditions such as hypoxia and exposure to neurohormones (Reiber et al. 1992; Airriess and McMahon, 1994, 1966; McGaw et al. 1994a,b). Compared with mammals, the cardiovascular system is quite differently constructed and organized, and the mechanism controlling distribution will be different as well. The heart pumps blood into seven arteries (McLaughlin, 1983), but these arteries are generally assumed to lack muscle layers (Martin and Hose, 1995). By what mechanism can the distribution of blood be controlled? At the heart, the entrance into each artery, except the dorsal abdominal artery, is guarded by a bicuspid muscular valve. These valves passively prevent the reflux of blood during diastole, but they can also actively control outflow. The valve muscles are variously innervated (Alexandrowicz, 1932) by excitatory and inhibitory neurons (lobsters, Kuramoto et al. 1992; isopods, Kihara et al. 1985; Fujiwara-Tsukamoto et al. 1992), and some of these valves respond to neurohormones (Kuramoto and Ebara, 1984; Kuramoto et al. 1992, 1995). Excitation will cause the valve muscles to contract and impede flow, while inhibition will facilitate flow. Thus, at the level of the cardioarterial valves, crustaceans possess the means to regulate the distribution of blood to individual arteries.

It is generally assumed that the arterial supply-side and hemocoel sinus return-side of the crustacean hemolymph distribution system make up a passive system that lacks vasomotor components that would allow fine control (Farrell, 1991; Martin and Hose, 1995). Indeed, the walls of all arteries except the dorsal abdominal artery are devoid of muscle layers. They are constructed of epithelial and connective tissue cells and are richly invested with fibrillar layers (Martin and Hose, 1995; Wilkens et al. 1997). In contrast, blocks of striated muscle occur in the lateral walls of the dorsal abdominal artery.

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Materials and methods

American lobsters (Homarus americanus Milne Edwards), ranging from 400 to 600 g, were purchased from a commercial supplier and maintained in artificial sea water at 12 °C. The dissections and observations were made under a stereomicroscope. All dissections and tissue manipulations were performed in aerated Cole’s (1941) saline, adjusted to pH 7.6.

The first step in the preparation of an animal for arterial perfusion was to replace the hemolymph with ice-chilled saline as detailed in Wilkens et al. (1997). Briefly, the carapace over the heart of an ice-chilled animal was removed and the heart and pericardial sinus were perfused with cold saline for 20–30 min until all traces of cloudy hemolymph were washed out. Following exsanguination, the lobsters were placed in a bath thermostatted to 12 °C. The carapace opening was enlarged until the proximal segment of each of the dorsal arteries, the anterior median artery (AMA), anterior lateral arteries (ALAs) and the dorsal abdominal artery (DAA), was visible. More extensive surgery was required to gain access to the deep hepatic arteries (HAs) and sternal artery (SA). The circulatory path from the beginning of each artery back to the area of the heart was intact. Arteries were cannulated, one-by-one, and perfused with saline chilled to 12 °C. The supply source to the perfusion pump could be switched between saline or saline containing the desired test compound. Except for the tests shown in Fig. 7, all measurements were made at a perfusion rate of 2 ml min⁻¹.

Arterial resistance (kPa s ml⁻¹) to flow was calculated using the hydraulic resistance equation (Berne and Levy, 1992) where the pressure drop from the perfusion cannula to the pericardial sinus (kPa) is divided by flow rate (ml min⁻¹) produced by a peristaltic pump:

\[ \text{resistance} = \frac{P_{1} - P_{o}}{\text{flow rate}}, \quad (1) \]

where \( P_{1} \) is the perfusion pressure measured from a T-junction side-arm placed close to the cannula tip and \( P_{o} \) is the hydrostatic pressure in the pericardial sinus adjacent to the heart.

All compounds were tested at a range of concentrations, and the results presented here are those obtained at concentrations that produced the maximum effect. Full concentration–response relationships were not determined. Test compounds were the neurotransmitters acetylcholine (ACh), glutamic acid and γ-aminobutyric acid (GABA); the aminergic neurohormones dopamine, octopamine and 5-hydroxytryptamine (5-HT) (Cooke and Sullivan; 1982, Sigma Chemical Co., St Louis, MO, USA) and the peptidergic neurohormones crustacean cardioactive peptide (CCAP) (Strangier et al. 1987; Peninsula Laboratories, Inc., Belmont, CA, USA), proctolin (Sullivan, 1979; Sigma Chemical Co.) and the FLRFamide-related peptides F1 and F2 (Kobierski et al. 1987; gift from I. Orchard, University of Toronto).

Results

The effects of adding a neurotransmitter (ACh) or each of three neurohormones (proctolin, F1 and F2) on the back pressure to perfusion in a DAA are shown in Fig. 1. Important points to note in these traces, which are taken from a single artery, are the rates of onset and recovery for each compound. The duration of responses to a 30 s exposure to the FLRFamide-related peptides was 20–60 min. A summary of the time course of the effects of these compounds is shown in Table 1.

The sensitivity to neurotransmitters and neurohormones varied among the five arteries tested. To allow comparisons, the data plotted in Fig. 2 are presented as percentage change in resistance from the control value. The AMA is relatively insensitive to all of the compounds perfused except glutamic acid. Note the reduced ordinate range of this graph. For the ALAs, HAs and SA, the profiles of sensitivities were similar to each other, but different from that of the AMA. In these
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Contr ol of v ascular r esistance in lobster s arteries, the peptides F1 and F2 produced the most dramatic increases in resistance. Proctolin (0.01 mol l⁻¹) was as effective as 5-HT (1 mol l⁻¹), but the other aminergic neurohormones and neurotransmitters were only moderately effective. For the DAA, all compounds except glutamic acid caused the resistance to increase by twofold or more.

There was a clear dichotomy between the effects of the two excitatory neurotransmitters tested. Glutamic acid increased the resistance of the AMA, ALAs, SA and DAA by about 30% but had virtually no effect on the HAs. Acetylcholine increased the resistance of the DAA by more than 150% and of the ALAs and SA by approximately 30%; it had a minimal effect on the resistance of any artery, but for the DAA it antagonized the effects of acetylcholine (Fig. 3) and proctolin (data not shown).

Combinations of GABA and other vasoactive compounds were not tested on any of the other arteries.

The sites of action of active compounds in the DAA were investigated by measuring the responses when the outflow was variously restricted. For these determinations, the dorsal carapace and superficial flexor muscles were removed along the full length of the abdomen to expose the DAA and the proximal portions of the segmental lateral arteries. The DAA and segmental lateral vessels of a single abdominal segment were viewed through the stereomicroscope as the vasoactive compounds were delivered. The anatomical arrangements of these arteries are shown in Fig. 4. Fig. 5 shows the changes in resistance to fluid flow in response to three vasoactive compounds when the flow was restricted either to the segmental lateral arteries of segments one to five (left four bars) or to the two large bifurcating modified segmental

Fig. 2. Relative change in resistance to flow of each of the five arterial types in response to a variety of neurotransmitters and neurohormones. The actual magnitude of the change can be estimated by multiplying the percentage change shown here by the resistance at the same flow rate, 2 ml min⁻¹, from Fig. 2 of Wilkens et al. (1997). AMA, anterior median artery; ALA, anterior lateral artery; HA, hepatic artery; SA, sternal artery; DAA, dorsal abdominal artery; ACh, 10⁻³ mol l⁻¹ acetylcholine; Glu, 10⁻³ mol l⁻¹ glutamic acid; 5-HT, 10⁻⁶ mol l⁻¹ 5-hydroxytryptamine; DA, 10⁻⁶ mol l⁻¹ dopamine; CCAP, 10⁻⁶ mol l⁻¹ crustacean cardioactive peptide; PR, 10⁻⁸ mol l⁻¹ proctolin; F1, F2, 10⁻⁸ mol l⁻¹ peptides F1 and F2. Values are means ± S.E.M. (N=4–10).

Table 1. Time course of action of a neurotransmitter and three peptide neurohormones on the dorsal abdominal artery

<table>
<thead>
<tr>
<th>Compound</th>
<th>N</th>
<th>Time to peak (s)</th>
<th>Half-relaxation time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholine (1 mmol l⁻¹)</td>
<td>5</td>
<td>55±4</td>
<td>83±15</td>
</tr>
<tr>
<td>Proctolin (1 µmol l⁻¹)</td>
<td>6</td>
<td>50±6</td>
<td>145±44</td>
</tr>
<tr>
<td>F1 (1 µmol l⁻¹)</td>
<td>6</td>
<td>91±19</td>
<td>1291±1026</td>
</tr>
<tr>
<td>F2 (1 µmol l⁻¹)</td>
<td>3</td>
<td>135±35</td>
<td>3771±1763</td>
</tr>
</tbody>
</table>

Test compounds were perfused for 30 s followed by a return to saline perfusion. Values are mean ± S.E.M.

Fig. 3. Record showing the increase in perfusion back pressure to the dorsal abdominal artery when exposed to acetylcholine (ACh) (1 mmol l⁻¹) and the antagonistic effect of γ-aminobutyric acid (GABA) (1 mmol l⁻¹), which was applied 30 s after the onset of the second challenge by ACh. The anticipated time course of the response to the second ACh application is drawn as a dashed line.
arteries in segment five that supply the tail fan (right four bars). In response to a vasoactive compound, the resistance to perfusion increased, the diameter of the DAA increased and that of the segmental lateral vessels decreased. It was apparent that the valve muscles had contracted and reduced the volume of fluid flowing into the lateral vessels. The walls of the segmental lateral vessels do not contain muscle layers (not illustrated).

The bicuspid valves located at the origins of the segmental lateral vessels from the DAA fold outwards with anterograde hemolymph flow and will resist back flow, thus acting as passive rectifiers of flow. Histological sections show that the upstream face of each valve leaflet contains a layer of striated muscle (Fig. 6A,B). The fibers of these muscles are oriented parallel to the valve edges. In addition to the pair of segmental lateral arteries, a large number of small-diameter vessels arise from the ventral wall of the DAA and from the two terminal bifurcating branches and travel to the wall of the intestine (Fig. 4). The origins of these intestinal vessels are not guarded by valves, as can be seen in Fig. 6C, where an extension of a mass of clotted hemolymph is seen exiting from the right artery.

The effects of neurotransmitters and hormones on flow resistance are dependent on flow rate. As shown in Fig. 7, the resistance to flow in the DAA decreased non-linearly as flow rate increased. The resistance to perfusion was increased when the perfusate contained ACh; however, the difference between these control and ACh relationships decreased with increasing flow rate. The curves in Fig. 7 fall within the 95% confidence intervals of a fourth-order regression equation.

In the accompanying study, the elasticity of the proximal portions of each of the five types of artery was measured (Wilkens et al. 1997). To make these measurements, one end of a length of artery was cannulated with a polyethylene tube leading to a pressure transducer and the other to a tube coming from a saline-filled supply bottle which could be raised and lowered. No changes in compliance were detected in any of the arteries when they were filled with ordinary saline or saline containing 5-HT (1 \(\mu\)mol l\(^{-1}\)) or proctolin (0.01 \(\mu\)mol l\(^{-1}\)) (data not illustrated).

**Discussion**

In the absence of vascular muscles, there are three ways in which resistance to flow through a crustacean artery could occur. First, facultative changes in blood flow through the gills occur during the transition from water to air, possibly as the gills collapse and the branchial resistance increases (Taylor and Greenaway, 1984), and facultative changes in the resistance to flow through the DAA occur during changes in abdominal position from extended to flexed (Wilkens et al. 1997). Second, the cardioarterial valves, located at the origin of each artery from the heart, can regulate entry of blood into that artery. The muscles of these valves are under both nervous and neurohormonal control (see Introduction for references). Excitation will cause the valve muscles to contract and impede flow, while inhibition will facilitate flow. The third
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The control of vascular resistance in lobsters, as illustrated by the data presented here, is the active control of arterial resistance. For this study, each artery except the DAA was treated as if it were a black box with respect to the effects of compounds on the resistance to fluid flow. The data are presented with the assumption that changes in resistance arise in the arteries themselves, although it is recognized that the total path of fluid flow includes the return blood-flow pathways through lacunae and sinuses. The validity of this assumption is confirmed for the DAA, as will be discussed below; however, in an abstract, Taylor et al. (1995) indicated that some cardioactive hormones can modulate resistance to perfusion in crab gills in vitro. The magnitudes of these changes in gill resistance are not known, but this control point may be important to the general peripheral resistance. Gill resistance changes would presumably not contribute to the selective distribution of blood through different arteries since blood from all arteries returns to the heart via the gills.

The spectrum of sensitivities to the cardioactive substances tested differs for each artery. The AMA is sensitive to glutamic acid, but not to ACh or any of the neurohormones. A logical target for glutamic acid may be the muscles attached to the cor frontale, an auxiliary pump located behind the brain (Steinacher, 1979). Note that the range of AMA responses in Fig. 2 is six times lower than for the other arteries. The ALAs, HAs and SA, with a few exceptions, exhibit similar spectra and magnitudes of sensitivities to each other; however, the ALAs are the only arteries of these three to respond to CCAP, while the FLRFamide-related peptides F1 and F2 produced the greatest increases in resistance. The resistance to flow in the DAA is dramatically increased by all compounds except glutamic acid.

The absence of resistance changes in the AMA during exposure to all cardioactive substances except glutamic acid has interesting implications. In crustaceans, this artery supplies the brain, the eyestalks and the antennules. This artery is supplied by an auxiliary pump, the cor frontale (Steinacher, 1979; McLaughlin, 1983). Even though the resistance to flow in this artery is high (Wilkens et al. 1997), it is important that it is maintained at all times. The flow rate in the AMAs of lobsters and crayfish is relatively high (Reiber et al. 1992; Reiber, 1994), while in crabs it is quite low in normoxic conditions and elevated during hypoxia (Airriess and McMahon, 1994, 1996). Increased resistance in response to glutamic acid may be an experimental artifact since it may have induced tonic contraction of the muscles that control the cor frontale; normally, these muscles are probably under phasic nervous control. In vertebrates, and in particular diving birds and mammals, the coronary supply to the brain is conserved at all times, including during diving bradycardia, while other peripheral vascular beds display a wide variety of use-related changes in blood flow (Stephenson and Butler, 1987; Farrell, 1991). By being exempt from those potentially circulating...
compounds that affect the other arteries, the AMA is thus one more in a long list of examples where the blood flow to the brain is preserved under a variety of conditions.

The rest of the discussion will focus on the abdominal system since detailed information is not yet available for the other arteries. In contrast to all other arteries leaving the heart, the dorsal flap valve at the back of the heart leading into the bulbus arteriosus and thence to the DAA is not muscular and is apparently not controlled (Kuramoto et al. 1992). Thus, the control of peripheral resistance is the only way in which the distribution to and from this artery can be regulated. The primary, if not the only, sites of action of effective compounds are the muscular bicuspid valves located at the origin of each of the segmental lateral vessels including the bifurcating pair in segment five. The uropods are the movable appendages of segment six (Ruppert and Barnes, 1994), and the two bifurcating arteries at the posterior boundary of abdominal segment five are the segmental arteries of segment six whose point of origin has moved slightly forward into segment five, but which have followed the uropods to their current position. This homology explains the observation that the drug sensitivity of the valves to these two vessels is the same as for the more anterior segmental lateral vessels of segments one to five. Information about the sites of action of these compounds is only available for the DAA; however, the time course over which resistance changes occur in the other arteries is similar to that in the abdominal artery. This leads to the suggestion that valves may exist in the other arteries and that they may be the sites of transmitter and hormone action.

Insight into a possible regulation of blood flow among the branches of a single artery is offered by the observations on the DAA. Two types of vessel branch from this artery. The relatively large, paired segmental lateral vessels supply blood to the abdominal musculature and to the swimmerets. From the ventral wall of the DAA, numerous small-diameter vessels supply the walls of the intestine. Valves are not present at the origin of the small intestinal vessels. Thus, when the segmental arterial valve muscles are relaxed, the majority of blood will be delivered to the abdominal muscles, swimmerets, telson and uropods. However, when these valves contract, in response to excitatory transmitters and neurohormones, more blood will be forced into the digestive system. A. J. Mercier (reported in Wilkens, 1995) found that the circulating level of FLRFamide-related peptides in crayfish was increased following the ingestion of a meal. However, when these valves contract, in response to excitatory transmitters and neurohormones, more blood will be forced into the digestive system. A. J. Mercier (reported in Wilkens, 1995) found that the circulating level of FLRFamide-related peptides in crayfish was increased following the ingestion of a meal. Thus, these hormones, by causing the arterial valves to contract, will cause a redistribution of blood to the gut, at least in the abdominal regions, and thereby facilitate food absorption. Circulating hormones will activate all valves simultaneously and maximize blood flow to the gut.

Independent control of individual valves would further refine blood distribution in the DAA. Each of the segmental lateral vessel valves receives excitatory and inhibitory innervation (Wilkens and Davidson, 1995). The very large increases in abdominal arterial resistance in response to ACh and its antagonism by GABA support the suggestion that these may be the transmitters of the valve neurons. The neural control of valve muscles is an effective means of regulating blood flow in individual arteries (Fuj iwara-Tsukamoto et al. 1992; Kuramoto et al. 1995). If further research shows that the cardioarterial valve muscles and the control points in arteries themselves exhibit some form of intrinsic or neurohormonally induced tone, the activation of inhibitory valve nerves would provide a mechanism to increase perfusion of specific tissues. A general increase in inhibitory input to the arteries would also reduce the overall resistance against which the heart must pump and could be the long-sought-after mechanism by which changes in stroke volume are effected.

Muscular valves are not unexpected, since Alexandrowicz (1932) described innervation of the valves; however, to the author’s knowledge, this report presents the first histological images of the muscular arrangement of the arterial valve. The fibers are located on the upstream faces of the valves and arranged longitudinally in parallel with the lips of the valve. The valve flaps fold away from the orthodromic direction of blood flow to prevent back flow, and contraction of these fibers will stiffen the valve and reduce forward flow. The flow-rate-dependent effects of hormones in the DAA indicate that the contractions of the valve muscles can be partially overcome at higher flow rates, but even a small reduction in the valve aperture can have a profound effect on flow, as predicted by Poiseuille’s equations where flow is proportional to the fourth power of the radius of a vessel.

In the lobster, the capacitance-type vessels will have a resistance related primarily to their diameter and flow rate (Wilkens et al. 1997). As flow rate increases and the vessel diameter increases, the resistance to flow decreases as the reciprocal of the fourth power of the radius. Vessels without muscle layers will not be able to alter their passive elasticity, and the compliance of the vessel walls is not altered during exposure to low concentrations of 5-HT or proctolin. In the conditions of the present determinations, there should have been no nervous or neurohormonal input to any of the arteries; however, in intact animals, the valves in the DAA, and possibly elsewhere, could be partially contracted, and active relaxation by inhibitory nervous input would allow fine control of blood distribution. The role of the striated muscle in the lateral walls of the DAA is not known.

In summary, the data presented here clearly show that the mechanisms for the regulation of blood flow among the different arterial systems include the control of resistance of these pathways, in addition to the previously documented control exerted by the cardioarterial valves. Clearly, the resistance of individual arterial systems can be actively controlled, and this may be one of the processes responsible for different patterns of circulatory distribution and for changes in stroke volume observed in intact animals.

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References


GOVER, M. N. (1982). Hormones and


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