The Journal of Experimental Biology 204, 947–957 (2001)
Printed in Great Britain © The Company of Biologists Limited 2001
JEB3189

ADENOSINE INCREASES VENTILATION RATE, CARDIAC PERFORMANCE AND HAEMOLYMPH VELOCITY IN THE AMERICAN LOBSTER *HOMARUS AMERICANUS*

EVA STEGEN AND MANFRED K. GRIESHABER*

Institut für Zoophysiologie, Heinrich-Heine Universität, Universitätsstraße 1, D-40225 Düsseldorf, Germany

*Author for correspondence (e-mail: griesha@uni-duesseldorf.de)

Accepted 1 December 2000; published on WWW 12 February 2001

Summary

The effects of adenosine and adenine nucleotides on haemolymph velocity and on heart rate and scaphognathite frequency were investigated in the American lobster *Homarus americanus*. The infusion of 0.25–2.4 nmol g^{-1} min^{-1} adenosine produced steady-state concentrations of 2–3 μmol l^{-1} adenosine and approximately 80 μmol l^{-1} inosine in the haemolymph. No changes in haemolymph concentration of AMP, hypoxanthine, xanthine or IMP were observed.

Adenosine increases haemolymph velocity in the sternal artery from 55±29 to 204±53 mm s^{-1} and in the posterior aorta from 21±7 to 54±28 mm s^{-1} and reduces haemolymph velocity in the lateral arteries from 98±92 to 74±69 mm s^{-1}. Heart rate is increased from 69.3±7.4 to 81.2±6.2 beats min^{-1} and scaphognathite frequency from 86.9±29.0 to 147.1±35.0 beats min^{-1}. The effects of adenosine are rapidly reversed after the cessation of the infusion of this purine. The adenylates cause similar but lesser changes and the effects are protracted, probably because of low in vivo activities of nucleotidases.

Key words: adenosine, ventilation, heart performance, heamolymph velocity, lobster, *Homarus americanus.*

Introduction

The crustacean circulatory system is able to react to several ecological demands interfering with oxygen delivery such as environmental hypoxia (Butler et al., 1978; Wheatly and Taylor, 1981; McMahon, 1992; Reiber et al., 1992; Airries and McMahon, 1994), air exposure (Taylor and Wheatly, 1981; Airries and McMahon, 1996) or exercise (Herreid et al., 1983; Hamilton and Houlihan, 1992; Hokkanen and DeMont, 1992; Reiber, 1994; Reiber et al., 1997) with various physiological adaptations. An increase in gill ventilation, heart rate, stroke volume and haemolymph flow and a redistribution of the haemolymph to areas of enhanced oxygen demand may intensify the supply of oxygen to the tissues (Wheatly and Taylor, 1981; McMahon and Burnett, 1990; McMahon, 1995a).

Based on a deluge of work (McMahon and Wilkens, 1983), it was suggested (McMahon and Burnett, 1990) that the circulatory system of advanced decapod crustaceans might allow fine graded regulation of cardiac output and haemolymph flow as well as selective haemolymph distribution, and thereby augment oxygen supply to the tissues. To a certain extent, ventilatory and circulatory control mechanisms in advanced crustaceans may even be functionally equivalent to those of the vertebrate autonomic system (McMahon, 1995b). These statements and the availability of two non-invasive methods raised new interest in the efficiency of the crustacean cardiovascular physiology. In particular, the techniques of pulsed-Doppler-flowmetry and of photoplethysmography lend themselves well to the measurement of heart rate, haemolymph velocity (Reiber et al., 1992) and ventilation rate (Depledge, 1984).

Amongst cardiovascular adaptations, chronotropic and inotropic effects of the heart have been documented for many crustaceans. The influence of mediators such as proctolin, octopamine, dopamine, serotonine and crustacean cardioactive peptides on the circulatory system has been investigated (Wilkens et al., 1985; McGaw et al., 1994). These neurohormones, which are released from the pericardial organ, pass directly to the heart where they have been found to have both chronotropic and inotropic effects (Cooke and Sullivan, 1982). In addition to hormones, metabolites such as lactate or urate originating from muscle tissue or the hepatopancreas function as metabolic modulators, increasing the oxygen affinity of haemocyanin in many crustaceans (Truchot, 1980; Morris et al., 1985). These internal mediators may transfer to the cardio-ventilatory system information related to external abiotic factors such as lack of ambient oxygen or flight and fight responses.

Amongst the many internal factors known to act systemically in animals, adenosine is predominant since it can exert several important cardiovascular actions. In vertebrates especially, the adenosine-induced increase in coronary blood flow has been the subject of numerous investigations (Drury

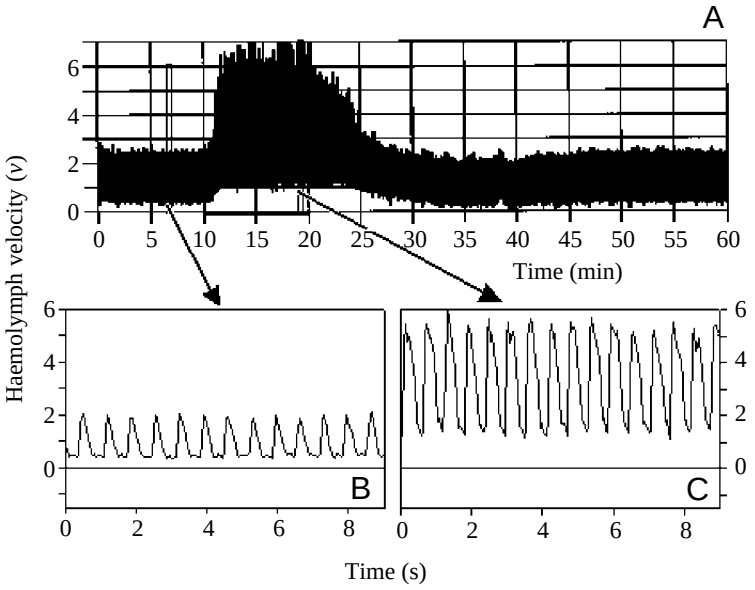

Fig. 1. Original tracing of the haemolymph velocity *v* in the sternal artery of the American lobster, *Homarus americanus*. (A) A tracing showing the haemolymph velocity (1 unit of velocity=110.66 mm s^{-1}) in a quiescent animal (1–10 min), during the infusion of 2.4 nmol g^{-1} body mass min^{-1} of adenosine (11–20 min) and during subsequent recovery lasting for 40 min. (B,C) Expanded velocity tracings during 9 s of the control and infusion phases.

and Szent-Györgyi, 1929; Gerlach et al., 1963; Böhm, 1987). Adenosine is produced by the breakdown of ATP and it accumulates within the tissues or is released into the blood (Zimmermann, 1992). It can therefore become effective during hypoxia or ischaemia, when the rate of energy consumption exceeds the rate of energy production. By analogy with experiments on vertebrates (Chiba and Himori, 1975; Böhm, 1987; Bennett and Drury, 1931), adenosine might also be expected to affect the circulatory and ventilatory system of some crustaceans. Therefore, we investigated adenosine- and adenine-nucleotide-induced changes in ventilation rate, cardiac performance and haemolymph velocity in the American lobster *Homarus americanus*.

Materials and methods

Essentially following the method of Reiber et al. (Reiber et al., 1992), a directional pulsed-Doppler-flowmeter (545C-4 Bioengineering, University of Iowa, USA) and piezoelectric transducer crystals of 1 mm diameter (Crystal Biotech, Hopkinton, USA) were used to measure haemolymph velocity in the sternal artery, the posterior aorta and the lateral arteries of the American lobster *Homarus americanus*. The animals had a mean body mass of 500–1000 g. They were kept in aerated recirculating sea water at 15±2 °C. During the experiments, which were also carried out at this temperature, the animals were unrestrained in a 40 l aquarium. Animals were chilled on ice during surgical manipulation and they were allowed to recover for at least 7 h afterwards. The experiments were started when heart rate and haemolymph velocity achieved constant levels over at least 40 min. When the same animal was used for several purine infusions at different rates within one experimental set, the animal was allowed to recover for 2–7 h between treatments, while the catheter and the crystal remained implanted. The same experimental arrangement was used to measure heart frequency. Ventilation rate was monitored by means of photoplethysmography (Depledge, 1984).

Adenosine, dissolved in lobster saline (Zeis et al., 1992), was infused into the infrabranchial sinus via a catheter through the arthrodial membrane at the base of the fourth pereiopod by means of a syringe pump (Infors, Basel, Switzerland). All infusions, which lasted for 10 min, were carried out with an adenosine solution of 10 mmol l^{-1}, pH 8.0.

Haemolymph samples were drawn from the pericardial sinus, which was accessible through a perforation drilled into the carapace before the experiment. Blood samples were collected in ice-cold 0.6 mol l^{-1} perchloric acid. Immediately after mixing, the samples were centrifuged for 25 min at 20 000 ***g***. The supernatant was brought to pH 6.0 using 0.75 mol l^{-1} K_2HPO_4. The precipitate was removed, and purine derivatives were measured in the resulting supernatant according to Deussen et al. (Deussen et al., 1988) using high-performance liquid chromatography (HPLC). If the various peaks in the chromatogram could not accurately be assigned to a particular compound, the sample was treated with an appropriate enzyme of the purine catabolism to shift the peak in the chromatogram.

Values given in this study are means ± S.D. To compare control values with those obtained during and after the different interventions a Kruskal–Wallis ANOVA on ranks was performed on raw data. Multiple comparisons *versus* control values were carried out by Dunnett's test or Dunn's test. Differences were considered significant at $P<0.05$.

Results

Haemolymph velocity, heart rate and scaphognathite frequency

Haemolymph velocities were measured in the sternal artery, the posterior aorta and the lateral arteries before, during and after the application of various doses of adenosine. Fig. 1 shows

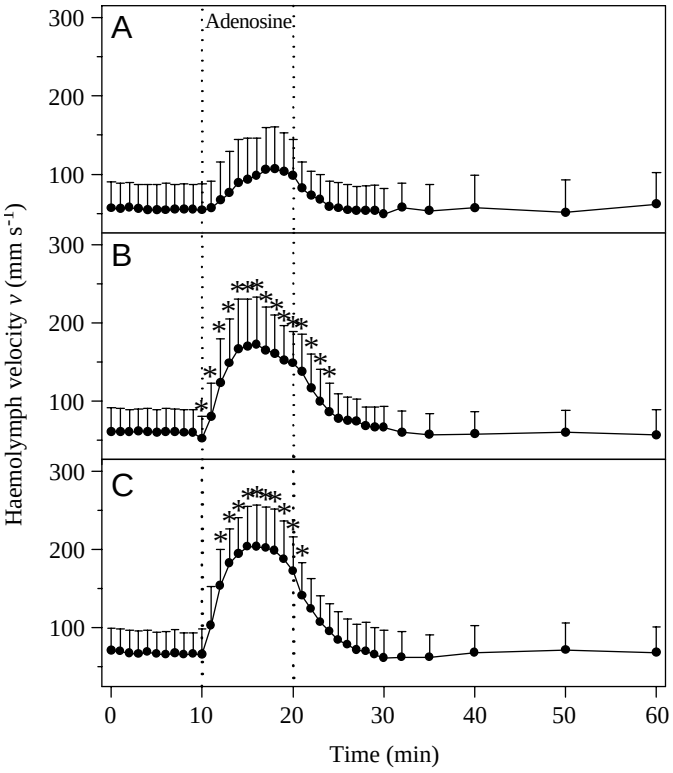

Fig. 2. Haemolymph velocity *v* in the sternal artery of the American lobster *Homarus americanus* in undisturbed animals (0–10 min), during the infusion of three different concentrations of adenosine (A) 0.25, (B) 1.2 and (C) 2.4 nmol g^{-1} body mass min^{-1} (10–20 min) and in recovering animals (20–40 min). Values are means ± S.D. (*N*=6, 10 and 9 in A–C, respectively). An asterisk denotes a value significantly different from the control value ($P \leq 0.05$).

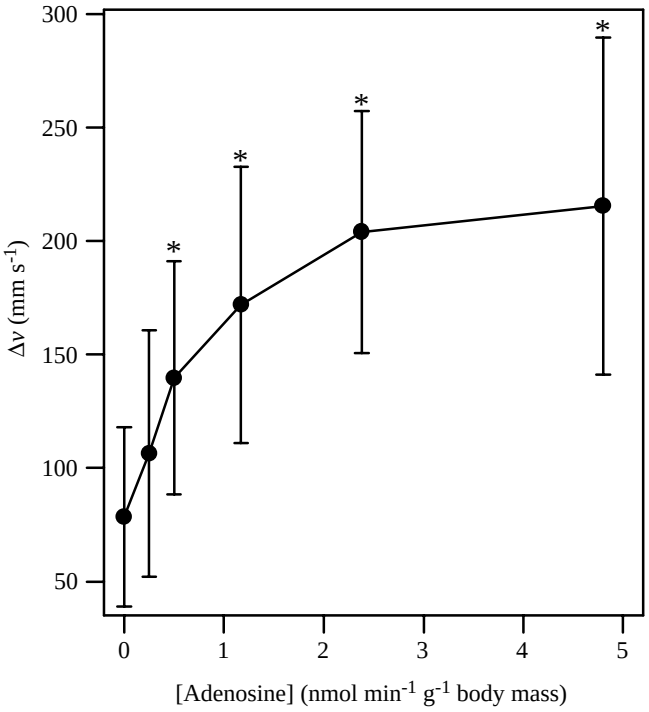

Fig. 3. Maximum increase of haemolymph velocities (Δv) in the sternal artery of the American lobster *Homarus americanus*, during the infusion of adenosine at 0, 0.25, 0.5, 1.2, 2.4 and 4.8 nmol g^{-1} body mass min^{-1}. Values are means ± S.D. (*N*=5, 6, 8, 10, 9 and 9, respectively). An asterisk denotes a value significantly different from the control value ($P \leq 0.05$).

an original tracing of haemolymph velocity in the sternal artery during an experiment which lasted for 60 min. After a 10 min control period, adenosine at 2.4 nmol g^{-1} body mass min^{-1} was infused over a period of 10 min. The animal then was allowed to recover, and the first 40 min of recovery are shown. Adenosine evoked an approximately threefold increase in haemolymph velocity during systole. Haemolyph flow did not return to zero in controls and it was increased during and following the infusion of adenosine.

Increasing the concentrations of adenosine increased haemolymph velocity (Fig. 2). During the first 10 min of the control period, velocity averaged 55±29 mm s^{-1}. The infusion of 0.25 nmol g^{-1} body mass min^{-1} of adenosine increased the haemolymph velocity to 106±54 mm s^{-1}, and adenosine concentrations of 1.2 and 2.4 nmol g^{-1} body mass min^{-1} resulted in haemolymph velocities of 172±61 and 204±53 mm s^{-1}, respectively. Higher concentrations of adenosine had no further effects; the haemolymph velocity levelled off between an infusion rate of 2.4 and 4.8 nmol g^{-1} body mass min^{-1} of adenosine (Fig. 3). From Fig. 2, it is also obvious that the haemolymph velocity had already slightly decreased before the end of the infusion of adenosine and dropped to control levels within 10 min after cessation of the treatment.

The Doppler signals resulting from the haemolymph velocity in the posterior aorta were different from those in the sternal artery. As can be seen from the original tracings (Fig. 4), the haemolymph flow in this artery reverses to some extent with every heart beat. The retrograde velocity of the haemolymph was highly variable during control and experimental periods. The net increase of the haemolymph velocity was at most only twofold and thus smaller in this vessel than in the sternal artery. Under control conditions, the average haemolymph velocity was 21±7.0 mm s^{-1} and this gradually increased to 28±27, 39±20 and 54±28 mm s^{-1} in the presence of adenosine at 0.25, 1.2 and 2.4 nmol g^{-1} body mass min^{-1}, respectively. Cessation of the adenosine infusion again caused a rapid decrease of the purine effect and control levels were reached after 10 min (Fig. 5).

The haemolymph velocity in the lateral arteries was very variable among specimens even under control conditions (Fig. 6). Only if the animals were left undisturbed for several hours could stable haemolymph velocities of 98±82 mm s^{-1} be estimated. In contrast to the results from the haemolymph velocities in the sternal artery and the posterior aorta, the value of this variable was reduced by 25 % in the lateral arteries when different amounts of adenosine were infused, but the reduction was not significant. Again, haemolymph velocities within the range of control values were achieved within minutes after the infusion was stopped.

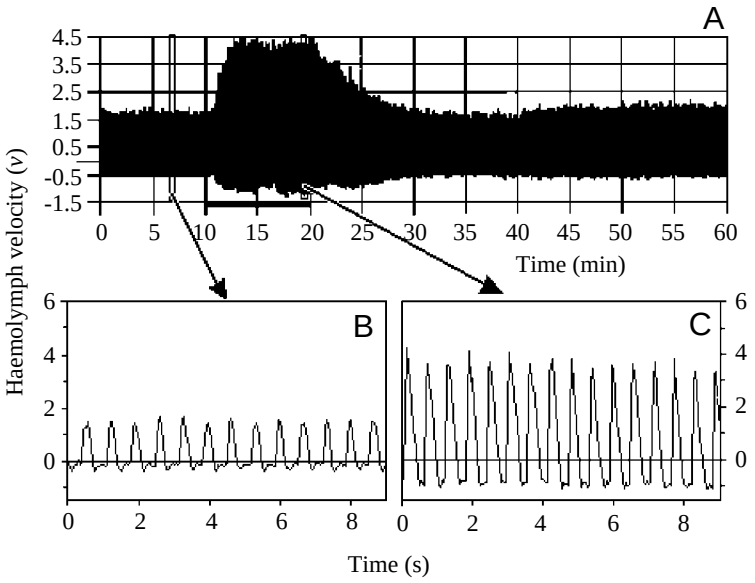

Fig. 4. Original tracing of the haemolymph velocity *v* in the posterior aorta of the American lobster, *Homarus americanus*. (A) A tracing showing the haemolymph velocity (1 unit of velocity=110.66 mm s^{-1}) in a quiescent animal (1–10 min), during the infusion of 2.4 nmol g^{-1} body mass min^{-1} of adenosine (10–20 min) and during subsequent recovery lasting for 40 min. (B,C) Expanded velocity tracings during 9 s of the control and infusion phases.

Besides its effects on haemolymph velocities, adenosine also affects heart rate and scaphognathite frequency. Single contractions of the heart cause a pulsatile haemolymph flow. Thus, the frequency can be resolved from the Doppler signal and quantified in the presence of different adenosine dosages (Fig. 7). Quiescent specimens showed an average heart rate of 69.3±7.4 beats min^{-1}. The frequency was significantly increased by a factor of 1.09, 1.14 and 1.17 in the presence 0.25, 1.2 and 2.4 nmol g^{-1} body mass min^{-1} of adenosine, respectively. Higher doses of adenosine often caused a transient bradycardia and even sometimes cardiac arrest which switched over to a tachycardia (Figs 7, 8C). The positive adenosine-dependent, chronotropic effect disappeared within approximately 10 min after the infusion of adenosine was terminated. The beat frequency of the scaphognatite was significantly elevated in the presence of 4.8 nmol g^{-1} body mass min^{-1} of adenosine and increased from 86.9±29.0 to 147.1±35.0 beats min^{-1} (Fig. 8C).

Several publications report a release of adenine compounds from endothelial cells (Deussen et al., 1993) or from isolated vessel preparations from the rabbit aorta (Sedaa et al., 1990), causing many different responses (Burnstock, 1996). It is conceivable that not only adenosine, but also ATP, ADP and AMP similarly influence respiration and circulation in Crustacea. Fig. 8A shows the haemolymph velocities in the sternal artery resulting from a 10 min infusion of 4.8 nmol g^{-1} body mass min^{-1} each of adenosine, AMP, ADP and ATP. The effect of adenosine was most pronounced, significantly increasing haemolymph velocity from 86.8±29.2 to 147.1±35.0 mm s^{-1}. When adenylates at the same concentration were infused, this effect was reduced as the number of phosphate groups increased (AMP>ADP>ATP). Recovery following infusion was protracted and, in the case of ATP, haemolymph velocities remained significantly elevated 40 min after cessation of the nucleotide application.

Adenine compounds caused similar response patterns in heart and ventilation rates. Again a large increase in beat frequency was found following adenosine application. The

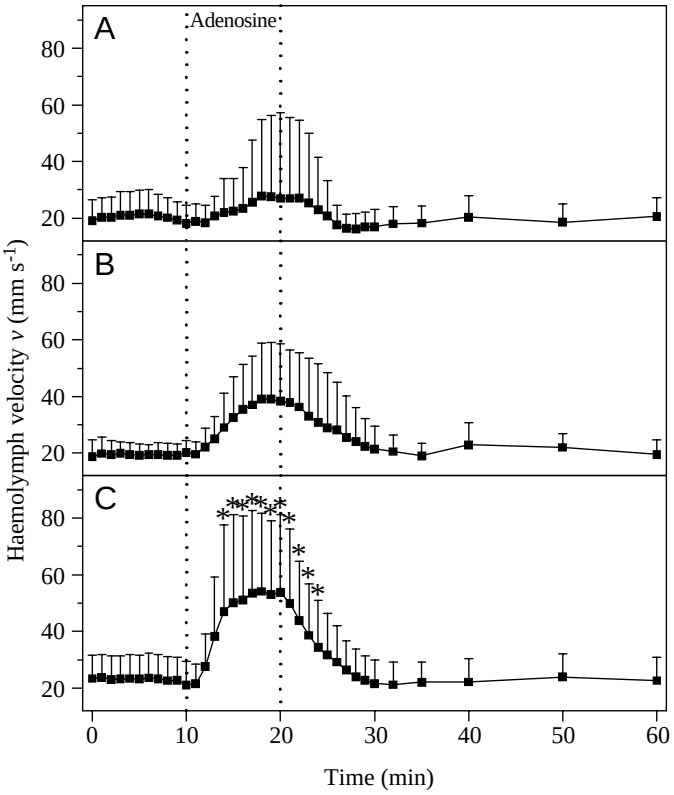

Fig. 5. Haemolymph velocities *v* in the posterior aorta of the American lobster *Homarus americanus* in undisturbed animals (0–10 min), during the infusion of three different concentrations of adenosine (A) 0.25, (B) 1.2 and (C) 2.4 nmol g^{-1} body mass min^{-1} (10–20 min) and in recovering animals (20–40 min). Values are means ± S.D. (*N*=8, 6 and 10 in A–C, respectively). An asterisk denotes a value significantly different from the control value ($P \leq 0.05$).

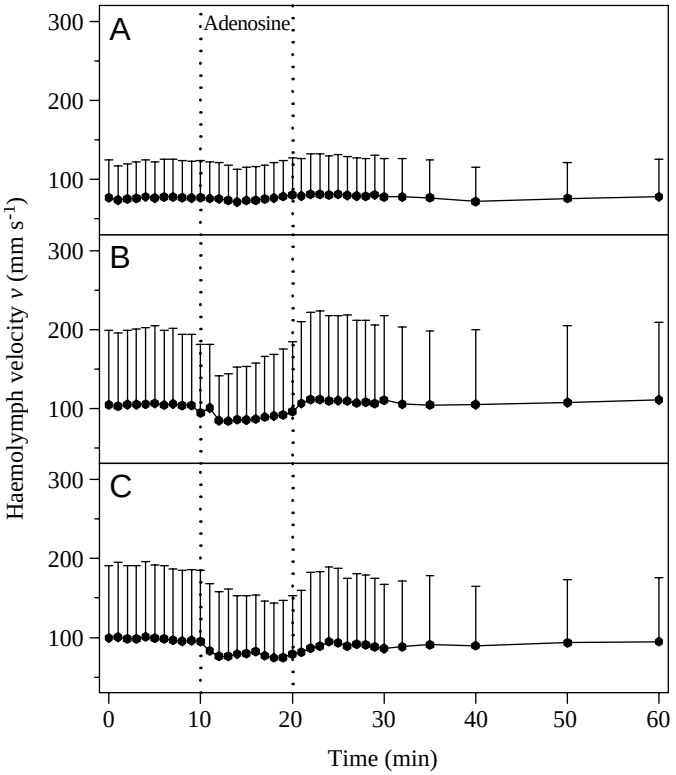

Fig. 6. Haemolymph velocities (v) in the lateral arteries of the American lobster *Homarus americanus* in undisturbed animals (0–10 min), during the infusion of three different concentrations of adenosine (A) 0.25, (B) 1.2 and (C) 2.4 nmol g^{-1} body mass min^{-1} (10–20 min) and in recovering animals (20–40 min). The low velocity values during the infusion period are not significantly different from the control values. Values are means ± S.D. (N=6, 7 and 10 in A–C, respectively).

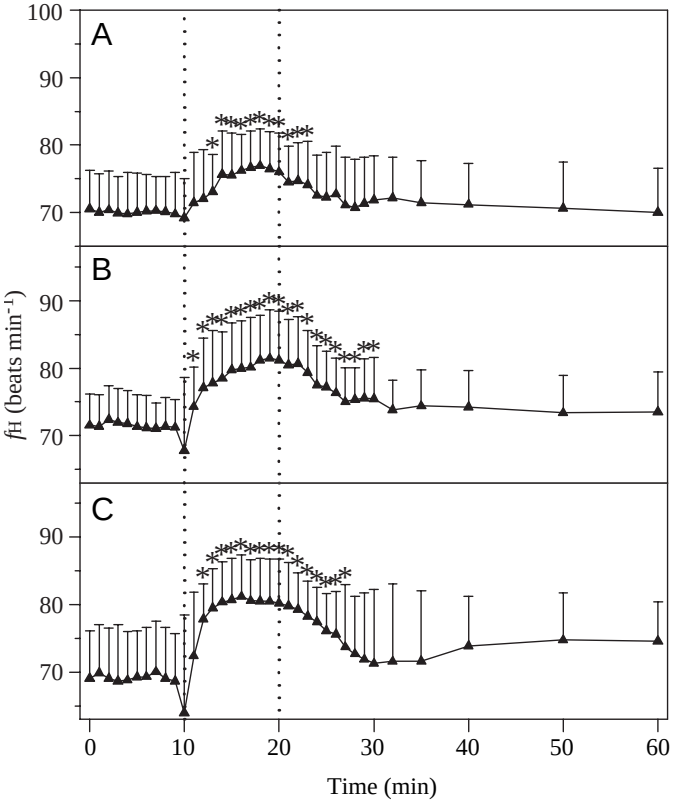

Fig. 7. Heart rate f_H of the American lobster *Homarus americanus* in undisturbed animals (0–10 min), during the infusion of three different concentrations of adenosine (A) 0.25, (B) 1.2 and (C) 2.4 nmol g^{-1} body mass min^{-1} (10–20 min) and in recovering animals (20–40 min). Values are means ± S.D. (N=13, 15 and 19 in A–C, respectively). An asterisk denotes a value significantly different from the control value (P≤0.05).

infusion of the adenylates initially caused a transient bradycardia and a reduction in the beat frequency of the scaphognathite. With higher numbers of phosphate groups, the elevation of heart and ventilation rates was less pronounced during the infusion period, but remained high or even increased in the case of ATP during recovery (Fig. 8B,C).

From these experiments it is obvious that the adenosine-dependent upregulation of some systemic variables was readily abolished within 10 min of the cessation of the treatment, indicating a rapid catabolisation of this purine or its rephosphorylation to AMP. Therefore, the steady state concentrations of AMP and adenosine and of some of its degradation products were measured in the haemolymph (Fig. 9). In quiescent, non-infused animals 4±4 nmol l^{-1} adenosine (detection limit 2 nmol l^{-1}) can be measured in the haemolymph. The infusion of 2.4 nmol g^{-1} body mass min^{-1} adenosine over a period of 10 min resulted in a significant increase in the steady-state concentration of this purine in the haemolymph to 3.1±3.9 μmol l^{-1}, but its concentration fell to 1.6±1.6 μmol l^{-1} before the infusion was terminated. Even though the standard deviations were enormous, the increase in adenosine concentration was significant during the infusion period. Within 2 min of ending the application of adenosine, the concentration in the haemolymph dropped to 0.05±0.05 μmol l^{-1}, a value not significantly different from the control value. Inosine, the first metabolite arising from adenosine degradation, showed a near linear concentration increase from 1.1±1.4 to 81.5±33.7 μmol l^{-1} during 10 min of adenosine infusion. During recovery, the inosine concentration decreased logarithmically, reaching 18.5±9.1 μmol l^{-1} after 30 min of recovery. Hypoxanthine and xanthine levels tended to increase slightly, but individual variability is too pronounced to obtain significant results. IMP concentrations were always below the detection limit of 1 μmol l^{-1}.

An infusion of an adenosine solution of 0.03 and 0.24 μl min^{-1} g^{-1} body mass could elicit a response from baroreceptors known to occur in some Crustacea (Burggren et al., 1990). Lobster Ringer's solution was therefore infused at a flow rate of 0.24 μl min^{-1} g^{-1} body mass for 10 min. This was followed by an adenosine application for 10 min at the same flow rate of 2.4 nmol g^{-1} body mass min^{-1}. Fig. 10 shows that the infusion of lobster saline at this flow had no effect on haemolymph velocity in any of the three vessels investigated or on heart rate. Subsequent infusion of adenosine (Fig. 10A)

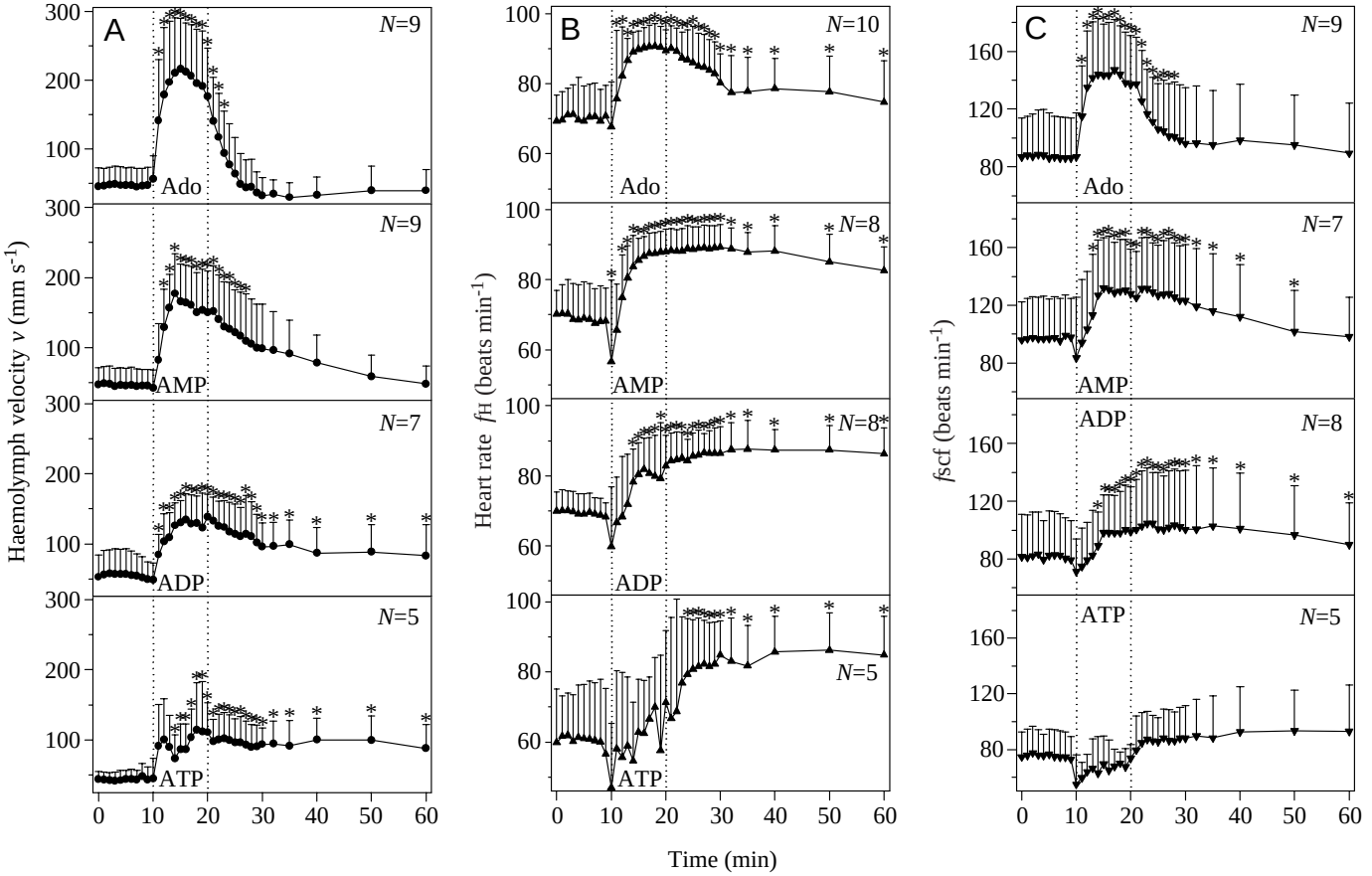

Fig. 8. Haemolymph velocities (v) in the sternal artery (A), heart rate (f_H; B) and scaphognathite frequencies ($f$scf; C) of the American lobster, *Homarus americanus*, in undisturbed animals (0–10 min), during the infusion of adenosine (Ado), AMP, ADP and ATP at a rate of 4.8 nmol g^{-1} body mass min^{-1} each (10–20 min) and during recovery (20–40 min). Values are means ± S.D. (N is given in the respective graphs). An asterisk denotes a value significantly different from the control value ($P \leq 0.05$).

showed the same effect on haemolymph velocity and on heart frequency as documented previously (Figs 2, 5–7).

Since adenosine is reported to be rapidly metabolized to inosine in human and dog plasma (Möser et al., 1989) and in lobster haemolymph (Fig. 9), this purine could also have a potentiating effect on haemolymph velocities in the blood vessels and on heart rate. To check this possibility, 2.4 nmol g^{-1} body mass min^{-1} inosine was infused followed by 2.4 nmol g^{-1} body mass min^{-1} adenosine. Inosine increased neither haemolymph velocity in the arteries investigated nor heart rate, but adenosine still had the same effects (Fig. 10B).

Discussion

Adenosine-dependent regulation of haemolymph flow

The haemolymph flow of many crustaceans is mainly channelled to the tissues through seven arteries, which exit from the ventricle. With the exception of the posterior aorta, each vessel has at its origin a muscular, innervated semilunar valve, which can be stimulated either electrically by the central nervous system (Kihara et al., 1985) or by a variety of aminergic and peptidergic neurohormones (Kuramoto and Ebara, 1984; Kuramoto and Ebara, 1989; Wilkens and Kuramoto, 1998). Several of these compounds have been identified in the pericardial organs of decapods, from where they can be released into the haemolymph and transported to their site of action (Alexandrowicz, 1953). Their chrono- and inotropic effects on the heart of decapod crustaceans have been studied in detail (Beltz and Kravitz, 1986). Furthermore, the circulatory and ventilatory sytems are tightly regulated by these compounds and allow appropriate physiological responses by an animal to compensate for natural perturbations (McMahon, 1995a; McMahon, 1995b; McMahon, 1999). These compounds alone, however, cannot explain the range of physiological reactions involved in environmental adaptation.

The actions of purine nucleotides and nucleosides on invertebrate organs and tissues have been known for many years (Burnstock, 1996). In the olfactory organ of lobsters and other decapod crustaceans a population of purinergic receptors exists that can be excited by adenine nucleotides (Carr et al., 1986). There are no recent investigations of the influence of adenylates and adenosine on circulation and respiration in crustaceans, therefore the effects of adenosine and adenylates were assessed on respiration and circulation of the American lobster *Homarus americanus*.

The infusion of adenosine gradually increased the

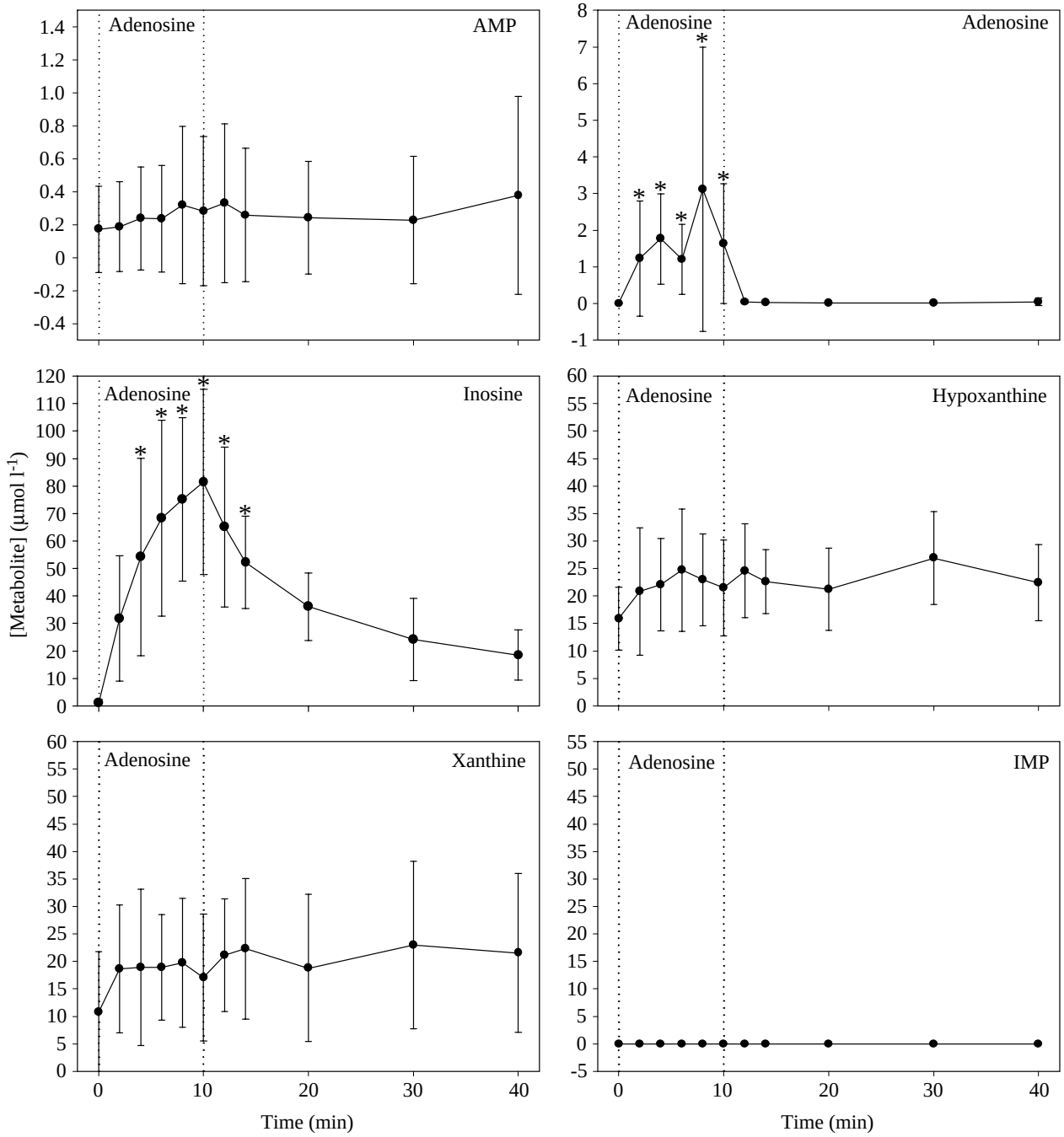

Fig. 9. Steady-state concentrations (µmol l^{-1}) of various metabolites in the haemolymph of the American lobster *Homarus americanus* during a period (0–10 min) of infusion of 2.4 nmol g^{-1} body mass min^{-1} of adenosine and the following recovery (10–40 min). Values are means ± S.D. (*N*=8). An asterisk denotes a value significantly different from the control value ($P \leq 0.05$).

haemolymph velocity approximately threefold in the sternal artery. This effect could be saturated at an infusion rate of between 2.4 and 4.8 nmol g^{-1} body mass min^{-1} of adenosine (Figs 2, 3), which led to a steady-state concentration of approximately 2 µmol l^{-1} adenosine in the haemolymph (Fig. 9). Thus, low concentrations of this purine were sufficient to exert a maximal response which, however, was sustained for longer periods. During the infusion period of adenosine a reduction in the haemolymph velocity occurred that could have several causes. The diameter of the vessel might have increased or the resistance to flow decreased, either of which would result in a reduction of velocity. There might even be CNS-receptors activated by adenosine, in which case the CNS drive to the heart might show adaptation. So far, none of these possibilities have been investigated.

During and after cessation of the infusion, adenosine was rapidly metabolized by adenosine deaminase, resulting in an inosine accumulation in the haemolymph, whereas adenosine

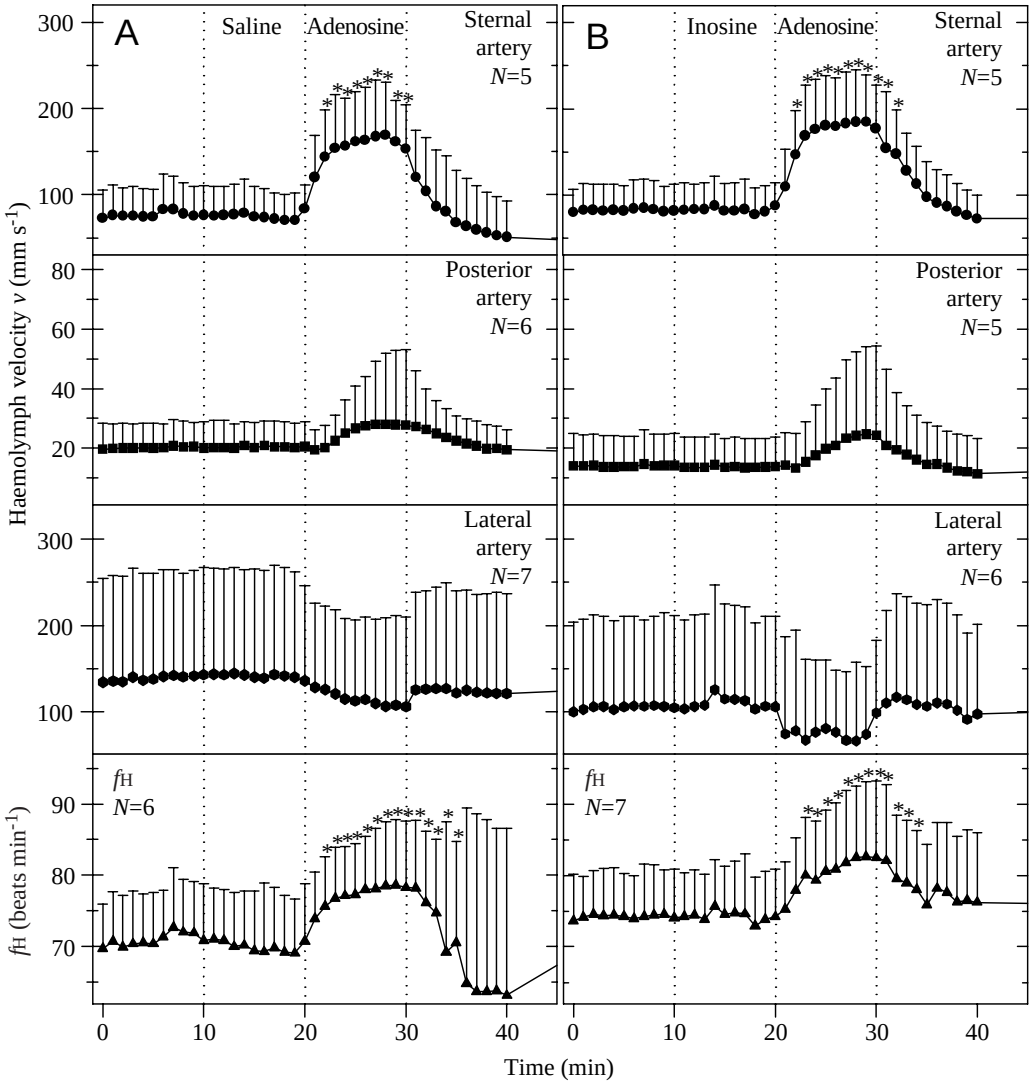

Fig. 10. Changes in heart rate (f_H) and haemolymph velocities (v) in three different vessels of the American lobster *Homarus americanus* following the infusion of either saline (A) or inosine (B) followed by an infusion of adenosine. Values are means ± S.D. (N is given in the respective graphs). An asterisk denotes a value significantly different from the control value ($P \leq 0.05$).

could barely be detected 2 min after the infusion had been terminated (Fig. 9). Its impact on the circulation is immediate (Fig. 8) and as pronounced as in other mediators such as proctolin, crustacean cardioactive peptide (McGaw et al., 1994) or 5-hydroxytryptamine (Wilkens et al., 1985). Responses to adenosine are of short duration because of a rapid enzymatic degradation by adenosine deaminase (Arch and Newsholme, 1978). Probably adenosine is geared to mediate rapid, systemic responses to environmental stress such as flight and fight reactions.

Haemolymph velocity (v, in mm s^{-1}) can be related to haemolymph flow (Q, in ml min^{-1}) if the diameter (d) of the vessel is known. Reiber et al. (Reiber et al., 1997) measured the diameter of several blood vessels in the American lobster. The mean diameter (d) of the sternal artery was 1.8 mm (1.9 mm during systole and 1.7 mm during diastole) in quiescent animals. Using the relation $Q=v(d/2)^2B$ (Chauveau et al., 1985), the haemolymph flow in the sternal artery was estimated to increase from 5.6±2.9 to 21.5±7.5 ml min^{-1} kg^{-1} body mass when 2.4 nmol g^{-1} body mass min^{-1} of adenosine were infused in *Homarus americanus*. This value for control animals is considerably lower than that given by Reiber et al., who measured 38.9±4.1 ml min^{-1} in quiescent American lobster (Reiber et al., 1997), which probably approximates to 59.8±6.3 ml min^{-1} kg^{-1} body mass in a specimen weighing 650 g. Whether this tenfold discrepancy is due to methodological differences, to false measurements, to the treatment of the animals (free roaming *versus* tethered) or to an inaccurate calculation of the data has not been resolved yet.

The posterior aorta also showed an adenosine-dependent increase in haemolymph velocity by a factor of 2.6, which is less pronounced than in the sternal artery. This amounts to an increase in haemolymph flow from 2.3±0.8 ml min^{-1} kg^{-1} body mass in quiescent animals to 4.4±1.0 ml min^{-1} kg^{-1} body mass during adenosine infusion, using a mean diameter of 1.86 mm for the posterior aorta (Reiber et al., 1997). In contrast to the other vessels investigated, a retrograde flow already occurred in the posterior aorta under resting conditions (Fig. 4B) and was enhanced following adenosine infusion (Fig. 4C). This retrograde flow could be explained by the histological peculiarities of this

vessel. All crustacean arteries have elastic layers adjacent to the lumen and should thus function as windkessels to dampen the pulsatile flow produced by the pumping heart (Wilkens et al., 1997). The posterior aorta is the only vessel to contain striated muscles in the lateral walls, and these muscles are expected to counteract the transmural pressure generated by the heart during systole. Furthermore the posterior aorta has no cardioarterial valves at its origin; in this vessel the resistance is adjusted by segmental valves at the origin of the six paired segmental arteries (Wilkens, 1997). If the posterior aorta, which is extended during systole, returns to its diastolic diameter, haemolymph should not only be driven through the six segmental branches, but also a certain portion of haemolymph must be pumped back towards the venticle since no valves are present to prevent this flow. The haemolymph in the segmental branches flows perpendicularly to the haemolymph flow direction in the posterior aorta. Because of that disadvantageous angle the transducer can only detect flow in both directions of the main vessel. This is a weakness in the applied method in the case of a macruran, because additional haemolymph, which is driven to the tissues by means of actively contracting muscular parts of the vessel, cannot be quantified. Nevertheless, the Doppler method gives a good estimate of velocity, and the 2.6-fold increase of haemolymph velocity in the vessel supplying the gut, midgut gland, pleopods, uropod and tail muscles in the presence of 2.4 nmol g^{-1} body mass min^{-1} of adenosine represents an augmented oxygen supply to the tail muscle, as for example during enhanced activity. If the vascular muscles and the valves indeed work as suggested above, this 2.6-fold increase should even be higher.

The hypothesis that the vascular muscles and the valves of the posterior aorta work as suggested is corroborated by a peculiarity of the Doppler traces of the sternal artery, which was also described (Reiber, 1994; Reiber et al., 1997) for macrurans. Under resting conditions, the diastolic velocity in the sternal artery did not return to 0 mm s^{-1} (Fig. 1). Similar to the diastolic retrograde flow in the posterior aorta (Fig. 4B,C), the residual diastolic flow in the sternal artery was enhanced in the presence of adenosine (Fig. 1B,C). It is therefore possible that the haemolymph that was pumped out of the posterior aorta during diastole by the contracting vascular muscles was not only represented by the diastolic retrograde flow (Fig. 4) but also by the diastolic residual flow in the sternal artery (Fig. 1), because it was driven through the bulbus arteriosus and further through the opened cardioarterial valves of the sternal artery.

The lateral arteries, which have control flow rates of 1.9±1.7 ml min^{-1} kg^{-1} body mass and transport only 1 % of the cardiac output (McMahon, 1995a), show opposite effects. In some specimens a slight increase of the haemolyph velocity resulted from adenosine infusion, but in most experiments a slower flow occurred (Fig. 6), which amounted to 1.5±0.9 ml min^{-1} kg^{-1} body mass, assuming a mean diameter of 0.8 mm (Reiber et al., 1997). This decrease in the adenosine response may result from shunting between arterial systems, as proposed by McMahon (McMahon, 1995b) for this vessel in the presence of 5-hydroxytryptamine.

Adenosine- and adenine-nucleotide-dependent increases in circulation and ventilation

Heart rate in a quiescent American lobster averaged 70.2±7.0 beats min^{-1} and increased significantly to 82± 7.0 beats min^{-1} when 2.4 nmol g^{-1} body mass min^{-1} of adenosine were infused (Fig. 7). Prior to the frequency increase, a conspicuous drop in heart rate occurred. Heart rates were then elevated during the later period of infusion and returned to control levels between 5 to 8 min. Except for the lateral arteries, this increase in beat frequency contributes to an increase in haemolymph flow through the vessels. In addition, the increase in scaphognathite frequency (Fig. 8C) enhances water flow through the gill chamber, providing more oxygen to the haemolymph.

It is unknown (1) to what extent adenosine itself is released to the extracellular space, (2) whether adenosine nucleotides are extracellularly converted to adenosine by ectoenzymes, and (3) whether the proportion of released adenyl compounds depends on the site of production (Rubio et al., 1973). In addition, throughout the animal kingdom different classes of purinoceptors have been reported that are excited by ATP, ADP and AMP (Burnstock, 1996). It is therefore conceivable that the nucleotides evoke reactions similar to that of adenosine in crustaceans by binding either to the same or to different receptors as adenosine, or by extracellular degradation of nucleotides to adenosine. Fig. 8A demonstrates the effects of adenosine and its nucleotides on haemolymph velocity in the sternal artery. All four purines elicited a significant increase in haemolymph velocity. The effect was graded, with adenosine showing the greatest and ATP the smallest effect. Although a direct stimulation of purinoceptors by adenylates cannot be excluded, it is assumed that ectonucleases dephosphorylate these purines to adenosine, which is not only indicated by the phosphorylation-dependent gradation of the effects, but also by the delay of recovery. The latter effect is probably due to the action of phosphatases and the 5′-nucleotidase, which provide a long-lasting supply of adenosine because their *in vivo* activities are much lower than the high *in vivo* activity of the adenosine deaminase (Arch and Newsholme, 1978).

The heart of *Cancer magister* is also known to react with an increase in rate after a bolus of 5-hydroxytryptamine, which is released during aggressive encounters that are usually coincident with enhanced activity (McMahon, 1995b; Kravitz, 1988). It is tempting to speculate that 5-hydroxytryptamine and adenosine act in a concerted manner, one mediator being released from the neurohaemal organ, the other from muscle tissue.

Applications of adenyl compounds evoked similar graded effects on heart (Fig. 8B) and ventilation (Fig. 8C) rates. With increasing phosphorylation of the adenylate the respective maxima of both systemic variables were protracted; even at the end of recovery, high heart and scaphognathite rates were observed, which again indicates that adenine nucleotides serve as an adenosine pool and that the nucleoside is protected from rapid deamination by means of the phosphate groups. These conspicuous similarities between adenosine- and adenine-

nucleotide-dependent reactions of the circulatory and those of the respiratory system strongly suggest that both systems do not perform in concert by chance, but are under the common control of adenosine exciting the central pacemaker (Wilkens et al., 1974).

Control experiments using the infusion of lobster Ringer and inosine

Instead of applying adenosine as a bolus, we infused this purine to obtain a steady rate into the haemolymph over a 10 min period. This procedure could also lead to an increase in haemolymph flow through the activation of baroreceptors or simply by irritating the animal. In addition, we also have found that adenosine is rapidly metabolized to inosine, which accumulates in the haemolymph to steady-state concentrations approximately 25-fold higher than those of adenosine (Fig. 9). Thus, inosine instead of adenosine could elicit an increase in haemolymph velocity; however, none of the adenosine-dependent effects occurred when lobster Ringer's solution or inosine was infused (Fig. 10A,B). Following the infusion of inosine the subsequent infusion of adenosine changed cardiac variables in the usual way. These experiments again prove that adenosine potently mediates systemic functions in lobster. Since inosine is also metabolized to urate, which is a transient end product in the purine catabolic pathway, a systemic influence of this latter compound can also be excluded.

Adenosine clearly evokes an increase of heart rate and it changes haemolymph velocity in the different arteries in *Homarus americanus*, which is probably due in part to an enhanced cardiac performance and to heamolymph redistribution. The simultaneous elevation of scaphognathite frequency even further augments the provision of oxygen. Effects on heart rate and haemolymph distribution can also be obtained by the application of 5-hydroxytryptamine and dopamine (Airriess, 1994; cited in McMahon, 1995) as well as by the peptidergic mediators proctolin, F1 and F2 (McGaw et al., 1994; Wilkens and Kuramoto, 1998). In contrast to these compounds, adenosine is mainly produced in muscle tissue and thus can be referred to as a metabolic regulator of systemic functions in lobster. It remains to be determined during which physiological reactions adenosine is produced.

We are very grateful to Brian McMahon and Carl Reiber for their initial help with the Doppler method and the coconut and for valuable discussions. Thanks are also due to Christopher Bridges and Axel Schubert for introducing us to the method of photoplethysmography. We thank the Deutsche Forschungsgemeinschaft and Fonds der Chemischen Industrie for continuous support.

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