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CHRONOTROPIC AND INOTROPIC EFFECTS OF ADENOSINE AND AMP ON THE ISOLATED SYSTEMIC HEART OF *OCTOPUS VULGARIS*

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ABSTRACT: The effect of adenosine on the function of the heart in *Octopus vulgaris* was studied using an isolated heart preparation. Bolus injections of adenosine or AMP (adenosine precursor) induced both positive chronotropic and inotropic effects. The maximum inotropic effect preceded the maximum chronotropic effect. The impermeable adenosine analogue 2-chloroadenosine elicited a similar effect, while the adenosine uptake blocker dipyridamole did not affect the adenosine response. These results suggest that adenosine acted extracellularly. The concentration-response curves of adenosine and AMP were also determined, by evaluating the effects on ventricular and coronary function. Under these conditions, the potent chronotropic effect elicited by both substances apparently masked or compensated for the inotropic effect, owing to the negative force-frequency relationship known to occur in the octopus heart. The AMP displayed a lower threshold than adenosine, suggesting an higher affinity for the purinergic receptors involved or a strict association between 5'-nucleotidase and the adenosine receptor on the plasma membrane.

KEY WORDS: Adenosine, AMP, *Octopus vulgaris*, systemic heart, coronary circulation

INTRODUCTION

The purine nucleoside adenosine is an endogenous metabolic regulator, whose physiological actions include vasorelaxation and CNS depression (Berne, 1980; Daval *et al.*, 1991). On the mammalian heart, it has negative chronotropic (West *et al.*, 1987) and dromotropic (Clemo and Belardinelli, 1986) effects. It has also negative inotropic effects on the atrial muscle (Chiba and Himori, 1975; Bruckner *et al.*, 1985; Linden *et al.*, 1985) and is able to inhibit the positive inotropic response of the heart to catecholamines (Verhaeghe and Vanhoutte, 1977; Belardinelli *et al.*, 1982; Dobson, 1983).

A main role of adenosine in the regulation of myocardial blood flow has been proposed (Berne, 1980). Adenosine nucleotides and adenosine are powerful coronary vasodilators (Winbury *et al.*, 1953) causing coronary smooth muscle relaxation (Ramagopal *et al.*, 1989). Part of this effect is endothelium-dependent (Headrick and Berne, 1990; Olanrewaju *et al.*, 1995). Adenosine appears also to potentiate flow-induced vasodilation (Kuo and Chanallor, 1995). Adenosine formation in the heart has been related to the availability of oxygen relative to the demand for its use (Sparks and Bardenheuer, 1986; Olsson and Bunger, 1987), although it is now clear that the quantitative relationships between cardiac oxygen consumption, adenosine release and coronary flow rate can differ according to the kind of stimulus upon the heart (Olsson and Pearson, 1990).

Some of the effects of adenosine are strongly species-dependent, probably reflecting differences in the receptor density or degree to which these receptors are coupled to effector systems (Olsson and Pearson, 1990). Very few studies have been done on the role of adenosine in non-mammalian organisms (mainly chick and frog; see, e.g.,

Hartzel, 1979; Belardinelli *et al.*, 1982; Lazou and Beis, 1986). In invertebrates, information on the physiological role of adenosine and related compounds is relatively scant. Adenylate metabolizing enzymes have been reported, particularly in molluscs (Sciurba *et al.*, 1985; Lazou, 1989; Yoshida and Aikawa, 1993). Purine compounds appear to have variable cardiac action in mollusca, depending on the species (Knight *et al.*, 1992).

The present study was designed to evaluate the pharmacological effects of adenosine on the isolated and perfused heart of *Octopus vulgaris* (Cephalopoda: Octopoda). Cephalopods are a group of invertebrates with an efficient high pressure circulatory system. They have a complex nervous and endocrine cardiovascular regulation, in which, in analogy with the vertebrates, peptidergic mechanisms are involved together with the classical adrenergic and cholinergic mechanisms (Schipp, 1987; Agnisola *et al.*, 1989). The main active pump in the cephalopod circulation is the systemic heart, the highest complexity of which is found in the Octopoda (Kling and Schipp, 1987). A peculiar characteristic of the systemic heart of octopus is the existence of a coronary supply to the heart, the functional significance of which has been recently stressed (Houlihan *et al.*, 1987; Agnisola, 1990; Agnisola *et al.*, 1990; Agnisola and Houlihan, 1991; Agnisola and Houlihan, 1994). Using an isolated and perfused heart preparation, we have studied the effects on the heart rate and mechanical activity of the heart of bolus doses of adenosine alone and in presence of dipyridamole, a blocker of adenosine uptake, or 2-chloroadenosine, a stable adenosine analogue. The concentration-response curve in preparations chronically perfused with adenosine was also determined.

In vertebrates, the main enzyme responsible of both intra- and extra-cellular adenosine formation is the 5'-nucleotidase (Sparks and Bardenheuer, 1986; Imai *et al.*, 1989). This enzyme is also present in the systemic heart of the *Octopus vulgaris* (Sciurba *et al.*, 1985). The study was then extended to the effects of adenosine 5'-monophosphate (AMP), as a putative extra-cellular source of adenosine in octopus.

MATERIALS AND METHODS

Animals:

The experiments were performed at the Zoological Station "A. Dohrn" of Naples, Italy. Specimens of both sexes of *Octopus vulgaris* captured in the Bay of Naples (April-May), were maintained in circulating sea water pools (20°C) and used within seven days of capture. The mean weight (± S.E.) of the animals was 0.780 ± 0.046 kg (N=42).

Systemic Heart Preparation:

The isolated systemic heart was prepared according to Agnisola and Houlihan (1991). The dissection was made at 4°C. Both the auricles and the dorsal aorta were cannulated and the gonadial aorta was ligatured at its base. A device was inserted through the abdominal aorta to record ventricular pressure. The heart was installed in the perfusion apparatus (Agnisola and Houlihan, 1991). Static input and output pressures were controlled by the height levels of respective reservoirs. The two auricles received aerated perfusion fluid (sea water + 0.05% glucose, pH 8.0) at the same controlled input pressure. The experiments were performed at room temperature (18-21 °C).

Chemicals:

Adenosine, adenosine 5'-monophosphate (AMP), dipyridamole and 2-chloroadenosine were purchased from Sigma Chem. Co. (St. Louis, MO, USA). Stock solutions of the above compounds (2 mg ml-1 or 10-3 M) were prepared, and dilutions were made in saline just before use. Other chemicals were at the highest grade available.

Experimental protocols:

I-Effects of bolus doses of adenosine, adenosine plus dipyridamole, 2-chloroadenosine and AMP on heart rate and mechanical activity of the isolated heart

This preliminary protocol allowed to evaluate the quality of the effects of adenosine and related compounds. The perfusate was not recirculating. The static input pressure and output pressure were set at 0.5 kPa and 2.5 kPa, respectively. A relatively high prelaod was necessary because in these experiments the heart performed slightly sub-physiologically, owing to the constrain represented by the clamp used for the mechanical activity recording (see below). After 10 min of perfusion, single doses (0.1-0.5 μmol) of adenosine, AMP or 2-chloroadenosine were injected, in a maximum volume of 0.4 ml, in one of the input ways, and the effect on the mechanical activity of the heart was recorded for 10-30 min. The effect of adenosine (0.5 μmol) plus dipyridamole (10 nmol) was also tested.

II- Concentration-response curves for adenosine and AMP

Each heart was chronically perfused with fixed drug concentrations and acted as its own control. The perfusate was not circulating. Two input reservoirs were used. The preload was adjusted to obtain a stroke volume of 0.8 ml g-1, a value which is within the physiological values for resting animals of the size range used (Wells and Smith, 1987). The diastolic output pressure was set at 2 kPa higher that the preload, according to the diastolic aortic pressure in resting animals (Wells and Smith, 1987). The tubes from the input reservoirs were connected to the input cannulae through three-way taps, so that 10 min after the onset of perfusion with the control perfusion fluid, it was possible to shift to a perfusate containing a fixed concentration of adenosine or AMP. Adenosine concentration ranged between 10-13 and 10-5 M, while AMP concentration ranged between 10-9 and 10-4 M. The effect on flows and ventricular pressure was recorded for at least 30 min.

Measurements and calculations:

Mechanical activity was evaluated with an isotonic transducer (Ugo Basile Biol. Res. Apparatus) connected to a Type 502A dual beam Oscilloscope (Tektronic Inc.) and permanently recorded on a 4 channel 3960 Recorder (Hewlett-Packard) for successive evaluations. A small clamp, connected to the isotonic lever of the transducer, was fixed to the heart close to the basis of the abdominal aorta.

Preload and afterload were defined as the mean values of input and output pressures respectively. They were obtained from ventricular pressure measurements made with an Elcomatic pressure transducer coupled to a Harvard Universal Oscillograph. The pressure signal was electronically differentiated to yield ventricular dP/dt. The dP/dtmax (= the maximum rate of pressure rise in the ventricle during isovolumic period) served as an index of contractility. Pressures are expressed in kPa.

The heart rate was the intrinsic rhythm of the heart and was calculated from mechanical or pressure records. Aortic and coronary outputs were determined volumetrically as described by Houlihan *et al.* (1987). Stroke volume and coronary volume (=volume of pefusate passing through the coronary system during each contraction cycle, Agnisola and Houlihan, 1991) were determined from the following relationships: stroke volume (ml g-1) = aortic output (ml min-1 g-1) / heart rate (beats min-1); coronary volume (ml g-1) = coronary output (ml min-1 g-1) / heart rate (ml min-1).

The coronary resistance (GPa s m-3, G=109) was determined as = mean ventricular pressure (kPa) x 60/ coronary output (ml min 1) (Agnisola and Houlihan, 1991). Mean ventricular pressure was calculated by integration of pressure records.

Stroke work was calculated as (mJ g-1): (afterload - preload) (kPa) ´ (stroke volume + coronary volume) (ml)/weight of the ventricle (g). The power output (mW g-1) of the heart was calculated as: (afterload - preload) (kPa) ´ cardiac output (ml min 1)/(60 ´ weight of the ventricle), where cardiac output was equal to aortic output plus coronary flow.

Test to control ratios were used to evaluate the effects of adenosine and AMP in the concentration-response curve determinations. The standard errors for the percent variations were calculated from the errors of the baseline and test values of the corresponding parameters. Student's t test was used to compare means (5% level of confidence). In figures, data are reported as percent change.

RESULTS

Qualitative effects of Adenosine and related compounds:

Figure 1 reports the effects of the highest bolus dose used (0.5 µmol) of adenosine, AMP or 2-chloroadenosine on mechanical activity and heart rate of the octopus isolated and perfused systemic heart. These experiments gave preliminary information on the response of the heart to adenosine and related compounds. Adenosine and AMP displayed similar effects, which were dose-dependent (data not shown). The minimal dose which elicited a response was 0.2 mmol. The data reported in figure 1 indicate that the inotropic and chronotropic effects induced by the two substances following a bolus treatment have different time-dependency. Just after the injection a positive inotropic effect was observed (Fig. 1A), followed by a negative inotropic effect. The latter was coincident with a maximum in the chronotropic effect, as indicated in figure 1b, were the peack to peack intervals as a function of time from the bolus injection is reported: a maximum chronotropic effect after 1 min was observed. Both chronotropic and inotropic effects disappeared within 4 min, and the control performance was recovered. Interestingly, the blocker of adenosine uptake, dipyridamole (up to 6´10-9 mol), did not interfere with the action of adenosine (Fig. 1A).

The effects of 2-chloroadenosine (up to 0.5 mmol) were similar to those of adenosine. However, the positive chronotropic effect was lasting for a much longer time (more than 20 min) (Fig. 1B).

Concentration-response curves of Adenosine and Amp.:

Table 1 reports the mean values of control performance parameters of the perfused isolated systemic heart preparations used for the determination of the concentration-

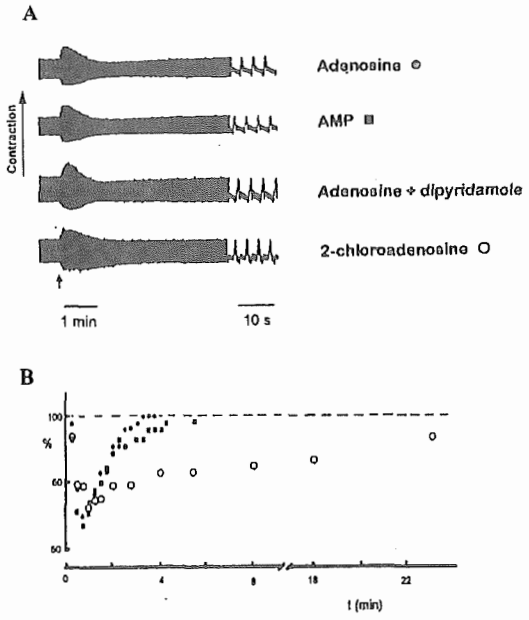

Fig. 1. A) Effect of of adenosine (0.6 μmol), AMP (0.5 μmol) and 2-chloroadenosine (0.5 μmol) on the mechanograms of the isolated and perfused systemic heart of *Octopus vulgaris*. The effect of dipyridamole (10 nmol) on the adenosine action is also reported. B) heart period of the isolated and perfused octopus heart as a function of time after bolus injection of adenosine (●), AMP (■), or 2-chloroadenosine (○). The data have been derived from the records reported in (A). Because the inverse relationship between heart period and heart rate, the observed reductions indicate a positive chronotropic effect.

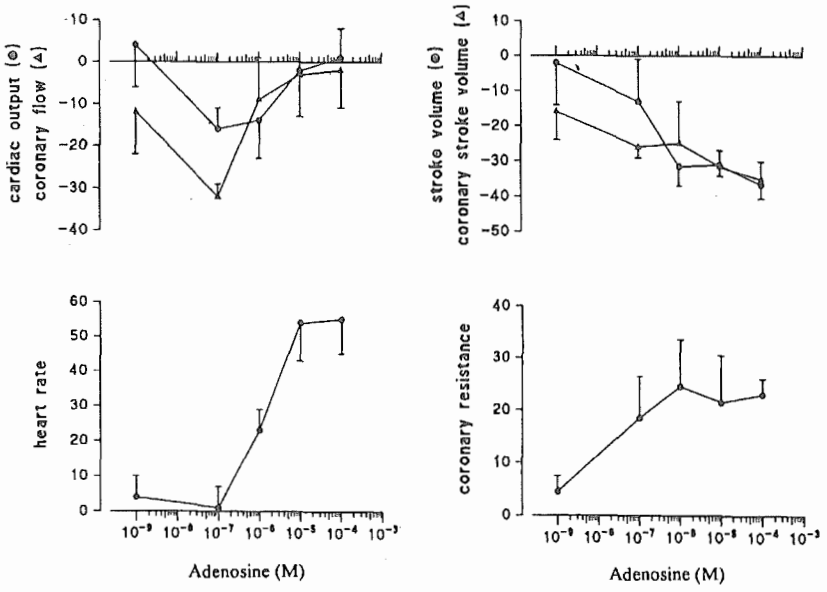

Fig. 2. Performance of the isolated systemic heart of *Octopus vulgaris* as a function of adenosine concentration in the perfusate. Percent changes of heart rate, cardiac output, coronary output, aortic and coronary stroke volumes, and coronary resistance are reported as a function of the drug concentration. Data are means (± S.E.) of 6 experiments.

response curves of adenosine and AMP. These values are comparable with those previously reported for similar preparations (Foti *et al.*, 1985; Houlihan *et al.*, 1987; Agnisola *et al.*, 1989, Agnisola *et al.*, 1994).

Table 1. Control performance parameters (mean ± SE) of the isolated systemic heart of octopus perfused with adenosine or AMP.

	Number of animals	Preload kPa	Afterload kPa	Heart rate beats min-1	Aortic output ml min^{-1} g^{-1}	Coronary flow ml min^{-1} g^{-1}	Coronary resistance GPa s m^{-3}
Adenosine	16	0.15±0.02	2.56±0.04	23±1	16.63±0.85	3.32±0.37	15.12±3.07
AMP	22	0.15±0-02	2.66±0.05	22±2	16.18±0.98	3.58±0.32	12.92±1.63

As shown in Fig. 2, adenosine had a powerful chronotropic effect, with a threshold concentration at 10-7 M. Cardiac output did not change significantly over the concentration range used, so that the chronotropic effect was accompanied by a reduction of stroke volume, which was significant at concentrations higher than 10-7 M. This result may indicate an inverse relationship between heart rate and stroke volume rather than a negative inotropic effect and was in accord with the results reported in fig. 1a.

Coronary flow was reduced by concentrations lower than 10 7 M. A significant 32% reduction was observed at 10-7 M. At higher concentrations this negative effect was compensated by an heart rate increase, so that the coronary flow came back to control values. The negative effect on coronary flow was accompanied by an increase in coronary resistance, which, however, was significant at concentrations higher than 10-7 M only. As Pmax and ventricular dP/dtmax did not change significantly (data not shown), the resistance increase must be accompanied by an increase in the mean ventricular pressure. This implies a change in the shape of the ventricular pressure wave. Fig. 3 shows the effect of increasing concentrations of adenosine (b and c, compared with the control, a) on the duration of the intraventricular pressure wave: a higher duration of pressure wave was observed, with a delay in the relaxation (as attested by the delay in the maximum negative dP/dt).

The concentration-response curves of AMP were similar to those of adenosine (Fig. 4). However there were many quantitative differences. All the effects occurred at lower concentrations. The chronotropic effect was about 25% lower and its threshold was at 10 13 M. There was no significant effect on the stroke volume, so that cardiac output was significantly increased at 10-5 M. The effect of AMP on the coronary resistance was about 20% higher than that of adenosine.

DISCUSSION

Adenosine elicited on the octopus systemic heart both positive chronotropic and inotropic effects. This is opposite to what is known to occur in mammals, and further stresses the highly species-related specificity of the physiological role of this substance in mollusca, as suggested by Knight *et al.* (1992). Very little can be said on the mechanisms of the chronotropic effect of adenosine in octopus. The systemic heart is known to

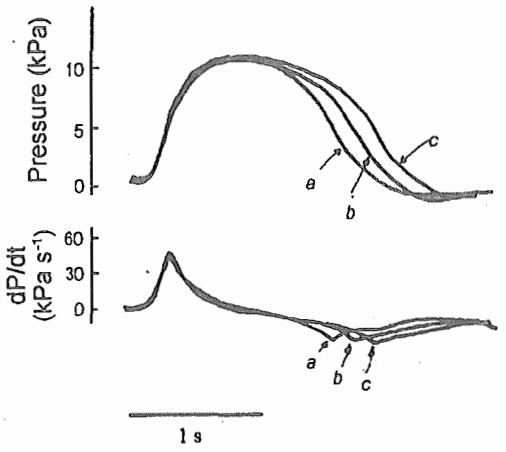

Fig. 3. Effect of adenosine perfusion on the shape of ventricular pressure wave. a) control perfusion; b) adenosine 10-6 M; c) adenosine 10-4 M.

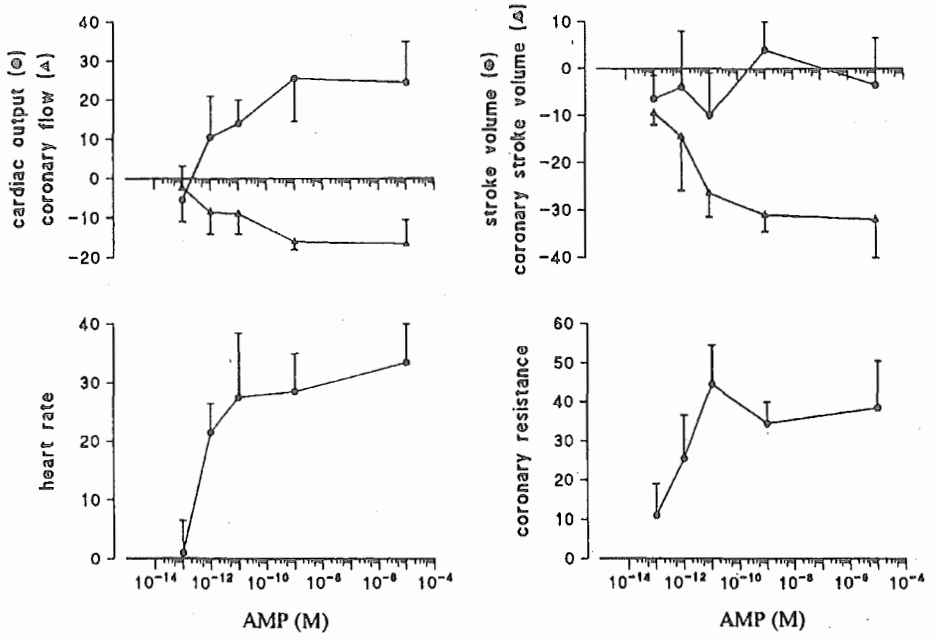

Fig. 4. Performance of the isolated systemic heart of *Octopus vulgaris* as a function of AMP concentration in the perfusate. Percent changes of heart rate, cardiac output, coronary output, aortic and coronary stroke volumes, and coronary resistance are reported as a function of the drug concentration. Data are means (± S.E.) of 6 experiments.

be myogenic, and there are indications of the existence of nodal areas at the atrio-ventricular junction (Agnisola, 1994), but the electrophysiological basis of its automatism is not known. Both the bolus injection experiments and the concentration-response curves indicate the existence of a complex interaction between the inotropic and chronotropic effects. In general, it appears that the inotropic effects are hidden by the chronotropic ones, owing to a negative force-frequency relationship, previously demonstrated to occur in the octopus heart (Agnisola *et al.*, 1994). This interpretation is also in agreement with the higher negative effect on stroke volume and in particular on coronary stroke volume induced by adenosine, whose chronotropic effects were higher than those induced by AMP.

The lack of effects of the adenosine transport inhibitor dipyridamole (Marangos *et al.*, 1985) on the adenosine action in the bolus injections experiments indicated that adenosine acted extracellularly, probably via membrane specific receptors, as it is well known to occur in vertebrates (Burnstock, 1981). This was confirmed by the fact that effects similar to those of adenosine were elicited by 2-chloroadenosine, an adenosine analogue which is a weak substrate for adenosine uptake and/or degradative processes (Muller and Paton, 1979).

The adenosine precursor AMP was also able to elicit effects similar to those of adenosine. Concerning the mechanism of AMP effects, there are two possibilities: (i) AMP may act on specific receptors, or (ii) it may be converted to adenosine by the 5'-nucleotidase, an ecto-enzyme known to be present in the octopus heart (Sciurba *et al.*, 1985). In mammals, inhibition of this enzyme induces an increase in the extracellular AMP and a reduction in the adenosine levels, suggesting a main role of AMP as extracellular source of adenosine (Imai *et al.*, 1989). The lower threshold of AMP effects with respect to adenosine, may suggest the involvement of purinergic receptors with a relatively low specificity for adenosine, or, alternatively, a strict interaction between 5'-nucleotidase and the adenosine receptor. This enzyme is thought to be closely associated with the nucleoside carriers in the mammalian heart (as adenosine formed from infused AMP is more rapidly transported than infused adenosine itself, Sparks and Bardenheuer, 1986), while a preferential activation of adenosine excitatory receptors A2 by adenosine originating from catabolism of released adenine nucleotides has been reported in hyppocampal and neuromuscolar synapses in rat (Cunha *et al.*, 1996).

An interesting result of the present study is the increased coronary perfusion in presence of AMP, despite the increased coronary resistance. In the octopus heart a peculiar organization of the coronary circulation has been described (Agnisola, 1990), with the coronary flow driven by the intraventricular pressure through the besian-like vessels that connect directly the intraventricular chamber and the coronary capillaries, which in turn drain into epicardial veins. Owing to this organization, any increase in contractile force or heart rate will result in an increase in resistance to the coronary flow, which can be compensated (as is the case with adenosine perfusion), or overcome (as is the case with AMP) by the consequent increase in intraventricular pressure. In definitive, despite the opposite effects on the heart with respect to mammals, andenylic compounds may help to increase coronary perfusion and oxygen supply to the heart, as in the mammalian heart.

REFERENCES

Agnisola, C. 1990. Functional morphology of the coronary supply of the systemic heart of *Octopus vulgaris*. *Physiological Zoology* 63:3-11

Agnisola, C. 1994 The myogenic automatism of the systemic heart of *Octopus vulgaris* (Cephalopoda: Coleoidea): evidence for localized stretch sensitivity and pacing activity. *Pakistan Journal of Marine Sciences* 3: 1-7

Agnisola, C., L. Cariello, A. De Santis, A. Miralto and B. Tota, 1989. Chronotropic and inotropic effects of atrial peptides on the isolated systemic heart of *Octopus vulgaris*. *Journal of Comparative Physiology* B 158:637-641

Agnisola C., D.F. Houlihan, 1994. Some aspects of cardiac dynamics in *Octopus vulgaris* (LAM). *Marine and Freshwater Behaviour and Physiology* 25: 87-100

Agnisola, C., D.F. Houlihan, 1991. Oxygen supply and in vitro performance of the systemic heart of *Octopus vulgaris*: effects of haemocyanin. *Journal of Experimental Zoology* 157:523-541

Agnisola, C., R. Venzi, T. Mustafa and B. Tota, 1994. The systemic heart of *Octopus vulgaris:* effects of exogenous arachidonic acid and capability for arachidonic metabolism. *Marine Biology*120:47-53

Agnisola, C., G. Zummo and B. Tota, 1990. Coronary drainage in the *Octopus vulgaris* systemic heart. *Journal of Experimental Zoology* 253:1-6

Belardinelli L., S. Vogel, J. Linden and RM. Berne, 1982. Antiadrenergic action of adenosine on ventricular myocardium in embryonic chick hearts. *Journal of Molecular and Cellular Cardiology* 14:291-294

Berne R.M. 1980. The role of adenosine in the regulation of coronary blood flow. *Circulation Research* 47:807-813

Berne, R.M., R.M Knabb, S.W. Ely and R. Rubio, 1983. Adenosine in the local regulation of blood flow: a brief overview. *Federation Proceedings* 42:3136-3144

Bruckner R., A. Fenner, W. Meyer, T-M. Nobis, W. Schmitz, and H. Scholz, 1985. Cardiac effects of adenosine and adenosine analogs in guinae pig atrial and ventricular preparations: evidence against a role of cyclic AMP and cyclic GMP. *Journal Pharmacology and Experimental Therapeutics* 234:766-774

Burnstock, G. (ed.) 1981. *Purinergic receptors.* Chapman and Hall, London

Chiba S., N. Himori, 1975. Different inotropic responses to adenosine on the atrial and ventricular muscle of the dog heart. *Japanese Journal of Pharmacology* 25:489-491

Clemo H.F. and L. Belardinelli, 1986. Effect of adenosine on the atrioventricular conduction. I: Site and characterization of adenosine action in guinea pig atrioventricular node. *Circulation Research* 59:427-436

Cunha R.A., P. Correia-de-Sa, A.M. Sebastiao and J.A. Ribeiro. 1996. Preferential activation of adenosine receptors at rat hippocampal and neuromuscular synapses by adenosine formed from released adenine nucleotides. *British Journal of Pharmacology* 19: 253-260.

Daval, J.-L., A. Nehlig and F. Nicolas. 1991. Physiological and pharmacological properties of adenosine: therapeutic implications. *Life Sciences* 49: 1435-1453

Dobson, J.C. Jr. 1983. Mechanisms of adenosine inhibition of catecholamine-induced responses in the heart. *Circulation Research* 52: 151-160

Foti L., I. Trara Genoino, C. Agnisola. 1985. In vitro cardiac performance in *Octopus vulgaris*. *Comparative Biochemistry and Physiology* 82C:483-488

Hartzell H.C. 1979. Adenosine receptors in frog sinus venosus: slow inhibitory potentials produced by adenosine compounds and acetylcholine. *Journal of Physiology* 293:23-49

Headrick J.P. and R.M. Berne. 1990. Endothelium-dependent and independent relaxation to adenosine in the guinea pig aorta. *American Journal of Physiology* 259: H62-H67

Houlihan D.F., C. Agnisola, N.M. Hamilton and I. Trara Genoino. 1987. Oxygen consumption of the isolated heart of Octopus: effects of power output and hypoxia. *Journal of Experimental Biology* 131:137-157

Imai S., WP. Chin, H. Jin, M. Nakazawa. 1989. Production of AMP and adenosine in the interstitial fluid compartment of the isolated perfused normoxic guinea pig heart. *Pflugers Archiv. European Journal of Physiology* 414:443-449

Kling G. and R. Schipp. 1987. Comparative ultrastructural analysis of the cephalopod systemic heart and its innervation. *Experientia* 43:502-511

Knight, G.E., C.H.V. Hoyle and G. Burnstock. 1992. Effects of adenosine nucleosides and nucleotides on the isolated heart of the snail *Helix aspersa* and the slug *Arion ater*. *Comparative Biochemistry and Physiology* 101C: 175-181

Kuo, L. and J.D. Chancellor, 1995. Adenosine potentiates flow-induced dilation of coronary arterioles by activating ATP-sensitive potassium channels in endothelium. *American Journal of Physiology* 269:H541-H549.

Lazou A. 1989. Adenylate metabolizing enzymes in invertebrate tissues. *Comparative Biochemistry and Physiology* 92B:175-180

Lazou A., Is. Beis. 1986. The role of adenosine in the isolated *Rana ridibunda* heart. *Journal of Comparative Physiology* B 156:839-844

Linden J., C.E. Hollen and A. Patel. 1985. The mechanism by which adenosine and cholinergic agents reduce contractility in rat myocardium. Correlation with cyclic adenosine monophosphate and receptor densities. *Circulation Research* 56:728-735

Marangos P.J., M. Houston and P. Montgomery. 1985. [3H]-dipyridamole: a new ligand probe for brain adenosine uptake sites. *European Journal of Pharmacology* 117: 393-394

Muller M.J., and D.M. Paton, 1979. Presynaptic inhibitory actions of 2-substituted adenosine derivatives on neurotransmission in rat deferens: effects of inhibitors of adenosine uptake and deamination. *Naunyn Schmiedebergs Archiiv fuer Pharmacologie* 306: 23-28.

Olanrewaju., H.A.., F.T. Hargittai, E.A. Liebermen and S.J. Mustafa, 1995. Role of endothelium in hyperpolarization of coronary smooth muscel by adenosine and its analogues. *Journal of Cardiovascular Pharmacology* 25:234-239

Olsson RA., R. Bunger. 1987. Metabolic control of coronary blood flow. *Progressive Cardiovascular Disease* 29:369-387

Olsson R.A. and J.D. Pearson. 1990. Cardiovascular purinoceptors. *Physiological Reviews* 70:761-845

Ramagopal M.V., M. Nakazawa and S.J. Mustafa. 1989. Relaxing effects of adenosine in coronary artery in calcium-free medium. *European Journal of Pharmacology* 159:33-40

Schipp R. 1987. General morphological and functional chracteristics of the cephalopod circulatory system. An Introduction. *Experientia* 43:474-477

Sciurba M., C. Agnisola, L. Foti and I. Trara Genoino. 1985. Evidence for 5'-nucleotidase activity in the heart of Octopus vulgaris. *Comparative Biochemistry and Physiology* 80B:67-72

Sparks H.VJr. and H. Bardenheuer. 1986. Regulation of adenosine formation by the heart. *Circulation Research* 59:193-201

Verhaege RH. and P.M. Vanhoutte. 1977. Inhibition of sympathetic neurotrasmission in canine blood vessels by adenosine and adenine nucleotides. *Circulation Research* 40:208-215.

Wells M.J. and P.J.S. Smith 1987. The performance of the octopus circulatory system: a triumph of engineering over design. *Experientia* 43:487-499

West G.A., W. Giles and L. Belardinelli. 1987. The negative chronotropic effect of adenosine in sinus node cells. In Gerlach E., Becker BF (eds) *Topics and perspectives in adenosine research*, Springer-Verlag, Berlin, Pp. 336-343

Winbury N.M., D.H. Papierski, M.L. Hemmer and W.E. Hambourger. 1953. Coronary dilator action of the adenine ATP series. *Journal of Pharmacology and Experimental Therapeutics* 109:255-260

Yoshid S. and T. Aikawa. 1993. Partial characterization of adenosine deaminase purified from teh scallop (*Patinopecten yessoensis*) midgut gland. *Comparative Biochemistry and Physiology* 105B: 51-62.

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