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A biphasic response to adenosine in the coronary vasculature of the K^+-arrested perfused rat heart

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Abstract

Biphasic vasodilatory responses to adenosine and 5'-*N*-ethylcarboxamidoadenosine (NECA) were observed in the coronary vasculature of K^+-arrested perfused rat hearts. Dose-response data for both agonists were best represented by two-site models. For adenosine, two sites with negative log ED_{50} (pED_{50}) values of 8.1 ± 0.1 (mean ± S.E.M) and 5.2 ± 0.1 were obtained, mediating 31 ± 2% and 69 ± 2% of the total response. In the presence of 8-phenyltheophylline, the vasodilatory response to adenosine remained best fitted to a two-site model with pED_{50} values of 7.0 ± 0.2 and 5.4 ± 0.2. The relative contribution of each site to the total response remained unchanged. For NECA, pED_{50} values of 9.6 ± 0.1 and 6.8 ± 0.2 were obtained, representing 48 ± 3% and 52 ± 3% of the sites, respectively. In contrast, ATP produced a monophasic response with a pED_{50} value of 8.8 ± 0.1. These results provide evidence of adenosine receptor and response heterogeneity in the in situ coronary vasculature.

Keywords: Adenosine A_{2A} receptor; Adenosine A_{2B} receptor; Adenosine A_3 receptor; P_3 purinoceptor; Coronary vasculature, heart, perfused, rat

1. Introduction

Adenine compounds produce profound effects in cardiovascular tissue (Drury and Szent-Gyorgyi, 1929). The role of adenosine as a vasodilator has been examined in a variety of tissues and organs from a number of different animal species and it is now widely recognised that adenosine causes vasodilation in all vascular beds except those of the placenta and kidney (Olsson and Pearson, 1990). In the coronary circulation, the vasodilator effect of adenosine has long been attributed to adenosine A_2 receptors (Kusachi et al., 1983; Olsson and Pearson, 1990). Interestingly, there is considerable evidence of adenosine receptor heterogeneity in coronary and aortic vascular tissues, with evidence supporting the occurrence of A_1, A_{2B}, A_3 and a putative P_3 purinoceptor sensitive to adenosine (Collis and Brown, 1983; Collis et al., 1986; Collis and Hourani, 1993; Chinellato et al., 1992; Martin, 1992). Furthermore, there is evidence that different endothelial and smooth muscle receptors or responses can contribute to the overall vascular response to vasoregulatory adenosine (Nees et al., 1989; Headrick and Berne, 1990; Rose'Meyer and Hope, 1990; Martin, 1992). One difficulty in examining potential response heterogeneity is that significant levels of endogenous adenosine complicate interpretation. Moreover, the limited range for dilation in hearts maintained at intrinsic coronary tone limits the sensitivity of conventional dose-response analysis. In an attempt to more accurately determine the mechanisms, or receptors, responsible for adenosine mediated relaxation of rat coronary vessels, we have examined coronary vascular responses to adenosine and the adenosine A_2 receptor agonist NECA (5'-*N*-ethylcarboxamidoadenosine) in a K^+-arrested perfused rat heart model.

2. Materials and methods

Male Wistar rats (300–450 g) were anaesthetised with an intraperitoneal injection of sodium pentobarbitone (100 $mg \cdot kg^{-1}$). Hearts were excised into ice-cold Krebs-

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Henseleit buffer (KHB) containing in mM: NaCl 118, KCl 4.7, $CaCl_2$ 1.75, $MgSO_4$ 1.2, glucose 11, EDTA 0.5, $NaHCO_3$ 25. Following aortic cannulation, hearts were perfused in an isovolumic, non-recirculating Langendorff mode at a constant flow rate of 10 ml · min^{-1} as described in Harrison et al. (1996). Briefly, perfusion pressure was monitored via a Gould-Statham pressure transducer attached to a water-filled tube inserted into a side arm of the aortic cannula. Left ventricular developed pressure was monitored via a second Gould-Statham pressure transducer attached to a water-filled latex balloon inserted into the left ventricle. The left ventricle was vented with an apical drain. Following an equilibration period of 30 min, hearts were arrested by switching to a modified KHB (MKHB) perfusion medium containing in mM; NaCl 88, KCl 35, $CaCl_2$ 1.75, $MgSO_4$ 1.2, Glucose 11, EDTA 0.5, $NaHCO_3$ 25.

Prior to acquiring dose-response curves, the perfusion pressure of equilibrated hearts was 48 ± 1 mm Hg ($n = 34$). Following perfusion with MKHB, this increased to 143 ± 4 mm Hg. Dose-dependent reductions in coronary perfusion pressure by adenosine, NECA and ATP were examined by bolus injections of the drugs into a side arm of the aortic cannula. Cumulative dose-response curves were constructed and perfusion pressure was allowed to stabilise prior to the next injection. One agonist was examined in each preparation. To examine the effects of 8-phenyltheophylline on the adenosine response, a dose-response curve for adenosine was determined in five hearts before and after the addition of the antagonist to the MKHB. Hearts were equilibrated for 30 min with 5 μM 8-phenyltheophylline prior to acquiring agonist dose-response curves.

Adenosine, NECA, ATP and 8-phenyltheophylline were obtained from Sigma Chemical Co. (St Louis, MO). Adenosine and ATP were dissolved in perfusate to give stock concentrations of 100 mM and 200 μM, respectively. NECA was dissolved in 10% DMSO in buffer to give a stock concentration of 10 mM. 8-phenyltheophylline was dissolved in 0.2 M NaOH in 20% methanol in water to give a stock concentration of 10 mM. All subsequent dilutions of 8-phenyltheophylline and NECA were performed in perfusate.

2.1. Data analysis

All responses are expressed as a percentage of the maximal increase in coronary perfusion pressure produced by MKHB. All data points represent geometric means of at least five individual determinations from different hearts. One and two-site models were fitted to dose-response data using the non-linear regression analysis protocol from the Prism program (GraphPad). The equation used to fit data to the one-site competition curve was:

$$y = a + (b - a)/(1 + 10^{x-c})$$

where y is the response, x is the log of the agonist dose, a is the bottom and b is the top plateau of the curve and c is the log ED_{50}.

For the two site model, the following equation was used:

$$y = a + (b - a) \cdot \left[c/(1 + 10^{x-d}) + (1 - c)/(1 + 10^{x-e})\right]$$

where x, y, a, and b are as defined above, c is the fraction of high potency sites and d and e are the log ED_{50} values for the high and low potency sites respectively. In these analyses, Hill slope factors were assumed to be unity. When fitting the curves to the data, the top of the curve (b) was set to 0% whereas the bottom of the curve (a) was allowed to float as a variable.

An F test was employed to compare the appropriateness of one and two-site models:

$$F = [(ss_1 - ss_2)/(df_1 - df_2)]/[(ss_2/df_2)]$$

where ss is the sum of the squares, df is the degrees of freedom and the subscripts 1 and 2 refer to the one and two site models, respectively.

Statistical comparisons of negative log ED_{50} (pED_{50}) values were performed using unpaired Student's t tests. A P value of less than 0.05 was considered significant for the F tests and t tests.

3. Results

3.1. The effects of adenosine, NECA and ATP on perfusion pressure in the coronary vasculature of the isolated perfused rat heart

Adenosine, NECA and ATP all caused dose-dependent reductions in coronary perfusion pressure in the arrested hearts (Fig. 1). Adenosine and NECA exhibited more complex dose-response curves than ATP. F test analysis of the adenosine and NECA data indicated that a two-site model provided a statistically better fit than a one-site model ($P < 0.0001$). In contrast, F test analysis of ATP dose-response data indicated that the single site model provided the best fit. Data obtained for the one and two-site curve fits are shown in Table 1. The proportion of high and low potency sites for the two-site model are also shown. The 100% response to adenosine could not be determined experimentally because doses of adenosine in excess of 15 μmol produced constriction rather than further dilation of the preparation. The maximum response calculated from the curve fitting program was −84 ± 6%.

3.2. The effect of 8-phenyltheophylline on the biphasic response to adenosine in the perfused rat heart

The effect of the adenosine receptor antagonist 8-phenyltheophylline on the adenosine dose-response curve can

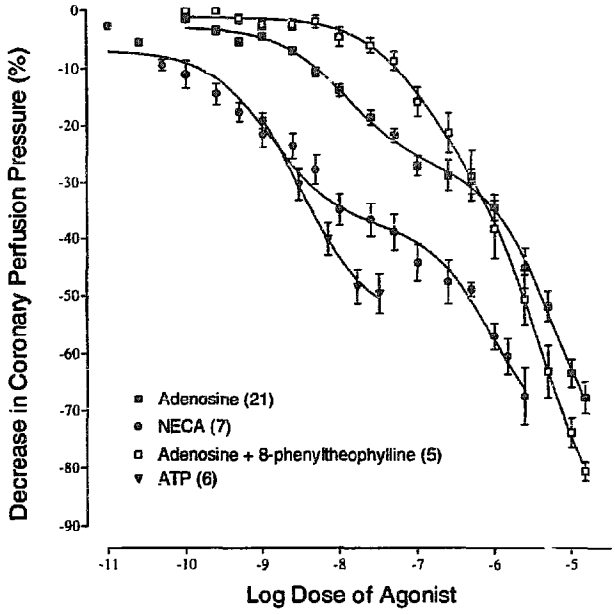

Fig. 1. The decrease in coronary perfusion pressure induced in the K^+-arrested perfused rat heart by NECA, ATP and adenosine alone and in the presence of 8-phenyltheophylline. Each point represents the mean ± S.E.M. of the number of experiments shown in parenthesis. Adenosine and NECA data sets were best fitted to a two-site model and the ATP data set to a single-site model.

be seen in Fig. 1. A right-ward shift of the curve is seen at lower adenosine concentrations and 8-phenyltheophylline significantly ($P < 0.05$) increased the pED_{50} for the high sensitivity site by an order of magnitude (Table 2). The low-sensitivity site was unaffected by antagonist treatment, and the proportion of the total response attributable to each site was also unchanged by 8-phenyltheophylline. Thus, 8-phenyltheophylline antagonised the effects of adenosine

Table 1
Summary of the dose-response data for the effects of adenosine (Ado), NECA, and ATP on the coronary perfusion pressure of K^+-arrested perfused rat heart

Agonist	High potency site pED_{50} (% site)	Low potency site pED_{50} (% site)	Single site pED_{50}
Ado	8.1 ± 0.1	5.2 ± 0.1	
$n = 21$	(31 ± 2)	(67 ± 2)	
NECA	9.6 ± 0.1	6.8 ± 0.2	
$n = 7$	(48 ± 3)	(52 ± 3)	
ATP	–	–	8.8 ± 0.1
$n = 6$			

Values were generated by fitting data to one- or two-site models as described in the text. Negative log ED_{50} values are the geometric mean ± S.E.M. of the indicated number of experiments (n). The percentage of high and low affinity sites is shown in parenthesis ± S.E.M.

Table 2
Summary of the dose-response data for the effects of adenosine (Ado) alone and in the presence of 5 μM 8-phenyltheophylline (8-pt) on the coronary perfusion pressure of K^+-arrested perfused rat heart

Agonist	High potency site pED_{50} (% sites)	Low potency site pED_{50} (% sites)
Ado	7.9 ± 0.1 [a]	5.1 ± 0.2 [b]
$n = 5$	(35 ± 4) [c]	(65 ± 4)
Ado + 8-pt	7.0 ± 0.2 [a]	5.4 ± 0.2 [b]
$n = 5$	(26 ± 5) [c]	(74 ± 5)

Values were generated by fitting data to a two-site model as described in the text. Negative log ED_{50} values are the geometric mean ± S.E.M of the indicated number of experiments. The percentage of high and low affinity sites is shown in parenthesis ± S.E.M. [a] $P < 0.01$. [b] Not significant (NS). [c] NS.

at the high but not the low sensitivity site. Dose-response data for adenosine in the presence of the antagonist remained best described by a two-site model ($P < 0.0001$). No significant difference ($P > 0.05$) was found between the adenosine dose-response data acquired in these experiments (Table 2), and the adenosine data shown in Table 1.

4. Discussion

The effects of adenosine, NECA and ATP on coronary tone were examined in the intact coronary vasculature of perfused rat hearts arrested with KCl. This model was employed in order to: (i) minimise or eliminate changes in coronary flow secondary to changes in cardiac function, (ii) to provide a greater functional range over which to examine the dilator response, and (iii) to minimise potential interference from endogenous adenosine levels. Under these conditions, we provide the first evidence of biphasic responses to adenosine and NECA in coronary vasculature. In contrast, the coronary response to ATP appears to be monophasic.

Two possibilities exist for the present observation of biphasic responses to adenosine and NECA but not ATP: (i) differential activation of similar receptor types located in different vascular 'compartments' (Lew and Duling, 1990; Headrick et al., 1992) or (ii) activation of two distinct receptor populations with varying sensitivities (e.g., A_{2A}, A_{2B} or A_3). With respect to the first possibility, previous studies have documented vascular compartmentation (functional, physical and metabolic) capable of altering dose-response relationships for adenosine and other agonists in intact perfused vessels (Nees et al., 1989; Lew and Duling, 1990; Headrick et al., 1992). To test which of the above-mentioned mechanisms is involved in the biphasic response, we perfused hearts with the competitive antagonist, 8-phenyltheophylline for a period of 30 min (a

period sufficient for equilibration throughout vascular and interstitial compartments). If the biphasic response is due to activation of the same receptor sub-type in different 'compartments' the antagonist should produce a parallel shift in the dose-response curve. On the other hand, if multiple receptor sub-types are involved, the antagonist should alter the shape of the dose-reponse curve. 8-phenyltheophylline significantly attenuated the response mediated at the high sensitivity site without altering the low sensitivity site. Therefore, the effect of the antagonist supports the existence of two receptor types with differing sensitivities to 8-phenyltheophylline and adenosine. A similar approach has been employed previously to characterise mechanisms underlying biphasic responses to adenosine agonists in rat kidney (Kenakin and Pike, 1987).

The present findings shed some light on the potential identity of the two apparent sites. The high sensitivity site is both xanthine sensitive and shows high affinity for adenosine and NECA. It is likely that this component of the response is produced by the A_{2A} class of adenosine receptor. The identity of the low sensitivity site is more difficult to determine. Since this site is xanthine insensitive it is unlikely to be either an adenosine A_{2A} or A_{2B} receptor. It cannot be the internal P site since NECA, a poor substrate for the nucleoside transport system (Collis, 1983; Collis and Brown, 1983) activated both the low and high sensitivity sites. Furthermore, as the P_2 receptor is 2–3 orders of magnitude more sensitive to ATP than adenosine or NECA, the low affinity site is unlikely to be the result of P_2 receptor activation.

The xanthine insensitivity of the low affinity site raises two possibilities. This site may represent the recently cloned adenosine A_3 receptor (Zhou et al., 1992) thought to mediate hypotension in angiotensin II supported rats (Carruthers and Fozard, 1993; Fozard and Carruthers, 1993), or it could reflect the putative P_3 purinoceptor reported in rabbit thoracic aorta (Chinellato et al., 1992). Both receptors are insensitive to xanthines. However, since the adenosine A_3 receptor exhibits a high affinity for NECA (Fozard and Carruthers, 1993), the low sensitivity of this site in the rat heart ($pED_{50} = 6.84$) may preclude this receptor type. Taken together, the xanthine insensitivity of the site, and its low sensitivity to NECA appears to preclude roles for adenosine A_1, A_{2A}, A_{2B} or A_3 receptors. The site displays characteristics which are similar to those reported for the putative P_3 purinoceptor (Chinellato et al., 1992).

In summary, a biphasic response to adenosine and NECA, but not ATP, was observed in a modified Langendorff perfused heart preparation. This response may represent a heterogenous population of A_{2A} and P_3 receptors within the rat coronary vasculature. Previous investigations of the effects of adenosine analogues in the coronary vasculature of perfused rat and guinea-pig hearts, employing changes in coronary flow as a measure of vasodilation, have not revealed the presence of two sites for adenosine (Headrick and Willis, 1988; Hutchison et al., 1989; Ueeda et al., 1991). This may be due to the presence of significant endogenous adenosine potentially masking high sensitivity sites in non-arrested preparations, and to reduced vasodilator sensitivity in buffer-perfused hearts maintained at intrinsic tone (Harrison et al., 1996).

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