# Stimulation of protein synthesis, glucose uptake and lactate output by insulin and adenosine deaminase in the rat heart

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In the anterogradely perfused rat heart, physiological concentrations of insulin stimulated the rates and efficiencies of protein synthesis in both ventricles and atria. Half-maximal stimulation of ventricular protein synthesis was obtained at about 35 μU/ml. Glucose uptake and lactate release were also stimulated over this range of insulin concentrations. Adenosine deaminase increased protein synthesis rates in ventricles and atria in the presence of submaximally stimulating insulin concentrations (40 μU/ml) but had no effect in the absence of insulin or in the presence of maximally stimulating concentrations. The insulin sensitivities of glucose uptake and lactate release were also increased by adenosine deaminase. Adenosine may be a modulator of insulin sensitivity in the heart.

*Protein synthesis* *Insulin sensitivity* *Adenosine* *Adenosine deaminase*

## 1. INTRODUCTION

Insulin stimulates ventricular protein synthesis in retrogradely perfused rat hearts (review [1]) at physiological concentrations [2]. In other tissues, the insulin sensitivities of several processes have been shown to be altered by AdoDA, presumably acting by removal of Ado. In adipose tissue, AdoDA decreased the insulin sensitivity of glucose uptake, lipolysis and pyruvate dehydrogenase activation [3,4]. In contrast, in incubated soleus muscle, AdoDA increased the sensitivity of glycolysis to insulin [5]. These findings indicate a possible role for Ado in the modulation of insulin sensitivity. In addition, Ado has multiple physiological effects on the heart (review [6,7]). Thus, in this report, we first established the sensitivity of protein synthesis to insulin in greater detail than in [2] in both atria and ventricles of the anterogradely perfused rat heart. Second, because of the interaction between insulin and Ado in other tissues, we investigated whether the insulin sensitivities of protein synthesis and other processes were altered by AdoDA. To our knowledge, there have been no previous investigations into the modulation of the insulin sensitivity of protein synthesis by Ado.

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*Abbreviations:* Ado, adenosine; AdoDA, adenosine deaminase (EC 3.5.4.4); $k_s$, rate of protein synthesis (pmol phenylalanine incorporated/mg protein per 2 h); $k_{RNA}$, efficiency of protein synthesis (pmol phenylalanine incorporated/μg RNA per 2 h)

## 2. EXPERIMENTAL

AdoDA (75 units/mg protein, 1.5 mg protein/ml in 50% glycerol) was from Sigma. Sources of all other materials are given in [8]. Male Sprague-Dawley rats (225–275 g on arrival) were fed and housed as in [8]. Food was withdrawn at 17:00 on the day before experiments were performed.

Hearts were anterogradely perfused for 2 h at a filling pressure of 0.5 kPa and an aortic pressure of 7 kPa essentially as in [9]. The recirculated perfusate (100 ml) was as in [10] containing addi-



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tionally 5 mM glucose, 0.4 mM [U-$^{14}$C]phenylalanine (spec. act. about 0.1 Ci/mol), all other amino acids necessary for protein synthesis each at 0.2 mM and, where indicated, insulin (see text for concentrations) and/or 10 $\mu$g/ml AdoDA. The incorporation of [U-$^{14}$C]phenylalanine into protein was measured as described in [11] except that tissue was homogenised and washed with 0.56 M $HClO_4$. The specific radioactivities of [U-$^{14}$C]phenylalanine in perfusates were measured as in [11].

Perfusate glucose and lactate concentrations were measured as in [9]. For glucose, results refer to the linear rate of glucose uptake, as determined from time courses. Dry weights were estimated from wet weights using a dry wt/wet wt ratio of 0.2045 ± 0.0017 ($n$ = 39). Protein was measured as in [12] using bovine serum albumin as standard, RNA as in [13] and AdoDA as in [14]. Ado was measured by HPLC by Dr G. Fleetwood, Section of Vascular Biology, MRC Clinical Research Centre, Northwick Park. Results are presented as means ± SE. Statistical significance was assessed by a two-tailed unpaired Student's $t$-test with $P <$ 0.05 being significant.

## 3. RESULTS

Since only a restricted concentration dependence for insulin stimulation of protein synthesis in the ventricles of the heart has previously been published [2], we first showed there was significant stimulation of atrial and ventricular $k_s$ and $k_{RNA}$ values at added insulin concentrations of

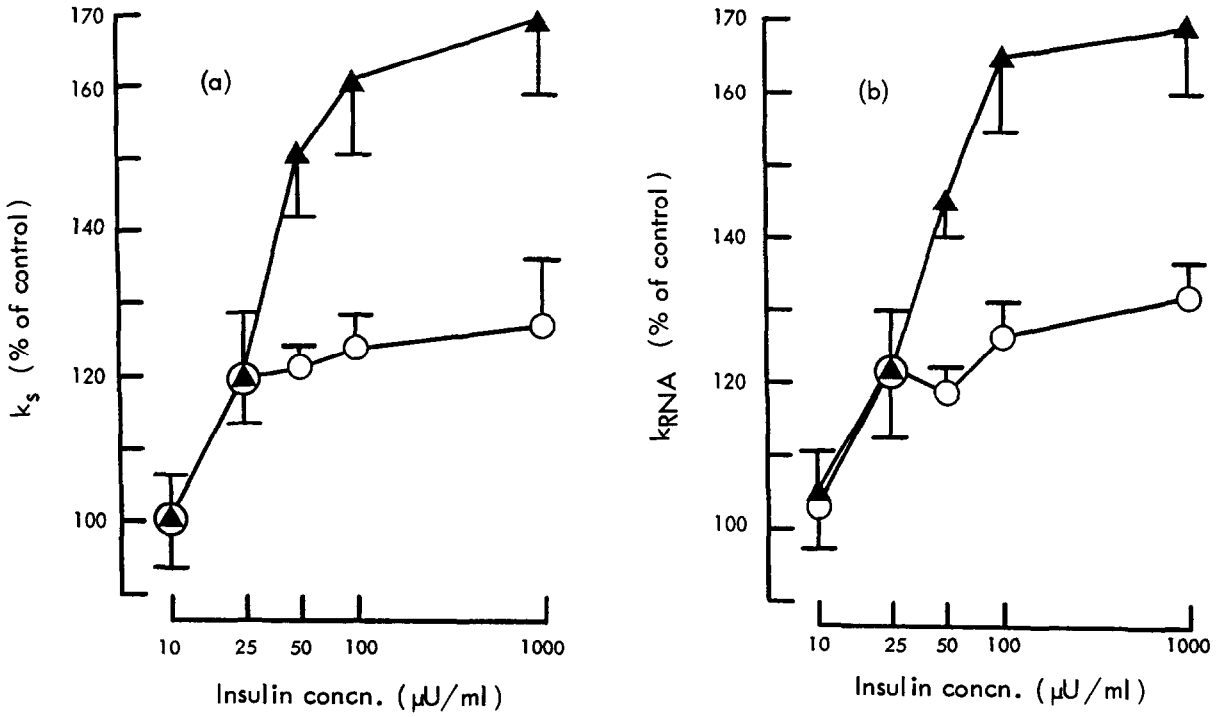


Fig.1. Effect of insulin concentration on atrial and ventricular $k_s$ and $k_{RNA}$ in the perfused rat heart. Atrial (○) and ventricular (▲) $k_s$ (a) and $k_{RNA}$ values (b) are shown relative to controls in the absence of added insulin. The graphs were constructed from individual experiments in which the effect of a single insulin concentration was compared with simultaneously perfused controls in its absence with 4–6 observations at each point. The mean absolute $k_s$ (pmol Phe incorporated/2 h per mg protein) and $k_{RNA}$ (pmol Phe incorporated/2 h per $\mu$g RNA) values from 23 perfusions in the absence of added insulin for atria were 2390 ± 47 and 164.0 ± 3.7, respectively, and for ventricles 1127 ± 29 and 142.3 ± 4.4, respectively. Except for perfusions with 10 $\mu$U/ml insulin, all results were significantly different from controls at $P < 0.05$ or, more frequently, at $P < 0.01$ or 0.001. The percentage stimulation of protein synthesis by insulin in ventricles was significantly greater ($P < 0.05$ or, more often, $P < 0.01$ or 0.001) than in atria at insulin concentrations of 50, 100 and 1000 $\mu$U/ml.

25 $\mu$U/ml (but not at 10 $\mu$U/ml) and that stimulation was maximal at 100 $\mu$U/ml (fig.1). Stimulation was greater on a percentage basis in ventricles than in atria. However, because atrial $k_s$ was twice ventricular $k_s$ (fig.1 [11]), the increases in absolute terms were similar in the two compartments. From fig.1, half-maximal stimulation of ventricular $k_s$ by insulin was at 35 $\mu$U/ml in this experiment. Similar estimates for atrial $k_s$ are more difficult but it is apparent that the response occurred over a physiological range.

Because the insulin concentrations refer to those added to the perfusates and because of the known propensity of insulin for binding to glass, the insulin concentration dependence of $k_s$ was compared with that of glucose uptake and lactate output as in [15]. Significant stimulation of glucose uptake and lactate output was observed at added insulin concentrations of 50 and 70 $\mu$U/ml, respectively (not shown). Thus protein synthesis is at least as sensitive to insulin as other insulin-stimulated processes.

In the experiment described in table 1, perfusion with media containing 40 $\mu$U/ml insulin stimulated ventricular $k_s$ by 38% of the maximal stimulation (rates in the presence of 5 mU/ml insulin were maximal). Inclusion of AdoDA significantly stimulated ventricular $k_s$ to 81% of the maximum. There was no effect of AdoDA in the absence of added insulin or in the presence of 5 mU/ml insulin. Over the 2 h of perfusion, perfusate AdoDA activity declined by only 20–30%. The response of atrial $k_s$ was more equivocal, but the pattern was similar to that in ventricles. Thus, although 40 $\mu$U/ml insulin did not significantly stimulate atrial $k_s$ compared with basal conditions in this experiment, addition of AdoDA significantly ($P < 0.01$) stimulated $k_s$ compared with basal conditions (table 1). The difficulty with atria is that the insulin stimulation of $k_s$ is much smaller on a percentage basis than in ventricles (fig.1). AdoDA also stimulated glucose uptake in the presence of 40 $\mu$U/ml insulin (table 1) but was without significant effect under basal conditions or when insulin concentrations were saturating.

Lactate release was significantly stimulated by AdoDA at 40 $\mu$U/ml insulin and also, in contrast to $k_s$ and glucose uptake, under basal conditions (fig.2). There was no effect of AdoDA at 5 mU/ml insulin. Stimulation of lactate release by AdoDA under basal conditions could have resulted from hypoxia caused by vasoconstriction [6] as Ado was removed by AdoDA. Coronary flow under basal conditions was significantly ($P < 0.001$) lower in the presence of AdoDA than in its absence [14.2 ± 0.9 ($n$ = 10) vs 18.2 ± 0.6 ($n$ = 14) ml/min per g wet wt, respectively]. Since vasoconstriction was detected, it can also be inferred that Ado was being removed by AdoDA. Although AdoDA did not significantly increase glucose uptake under basal

Table 1

Effects of AdoDA on the insulin stimulation of protein synthesis and glucose uptake

| Perfusion condition | $k_s$ (pmol Phe incorporated/ 2 h per mg protein) | | $k_{RNA}$ (pmol Phe incorporated/ 2 h per $\mu$g RNA) | | Glucose uptake ($\mu$mol/h per g dry wt) |
|---|---|---|---|---|---|
| | Ventricles | Atria | Ventricles | Atria | |
| Basal | 1083 ± 58 (14) | 2327 ± 96 (14) | 133 ± 5 (14) | 152 ± 5 (14) | 267 ± 17 (13) |
| + 10 $\mu$g/ml AdoDA | 1075 ± 58 (10) | 2261 ± 114 (10) | 131 ± 5 (10) | 153 ± 6 (10) | 314 ± 39 (14) |
| + 40 $\mu$U/ml insulin | 1292 ± 50 (14)[a] | 2549 ± 111 (14) | 158 ± 6 (14)[b] | 170 ± 5 (14)[a] | 378 ± 17 (11)[c] |
| + 40 $\mu$U/ml insulin + 10 $\mu$g/ml AdoDA | 1523 ± 43 (14)[e] | 2774 ± 85 (14) | 182 ± 5 (14)[e] | 177 ± 5 (13) | 483 ± 40 (14)[d] |
| + 5 mU/ml insulin | 1627 ± 153 ( 4)[c] | 2742 ± 275 ( 4) | 174 ± 15 ( 4)[b] | – | 589 ± 15 ( 4)[c] |
| + 5 mU/ml insulin + 10 $\mu$g/ml AdoDA | 1577 ± 97 ( 4) | 2926 ± 184 ( 4) | 170 ± 9 ( 4) | – | 567 ± 16 ( 4) |

Results are means ± SE. Statistical significance: [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$ for effect of insulin alone vs basal conditions (no insulin or AdoDA added); [d] $P < 0.05$, [e] $P < 0.01$ for the effect of AdoDA vs equivalent perfusions in its absence

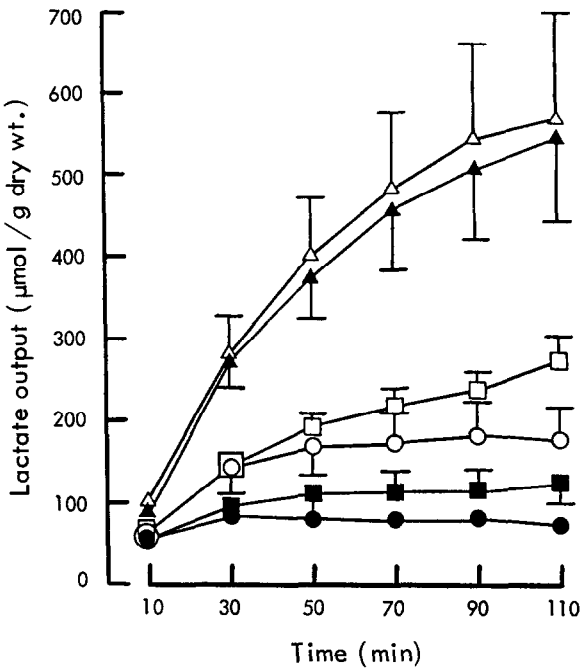


Fig.2. Effects of AdoDA on lactate release by the perfused heart. Hearts were perfused in the presence (open symbols) or in the absence (closed symbols) of 10 $\mu$g/ml AdoDA and in the absence (●,○) or presence of 40 $\mu$U/ml insulin (■, □) or 5 mU/ml insulin (▲, △). Results are means for 10 (●,○, ■, □) or 4 (▲, △) perfusions. When error bars are not shown, the SE was encompassed by the point. Insulin alone (5 mU/ml, ▲) significantly stimulated lactate release compared with controls (no added insulin, ●) at $P < 0.01$ or 0.001. In the absence of insulin (●,○), AdoDA (○) significantly stimulated lactate release at $P < 0.05$ compared with controls (●). In the presence of 40 $\mu$U/ml insulin (■, □), AdoDA significantly stimulated lactate release (□) at $P < 0.05$ (or, more often, at $P < 0.01$ or 0.001) compared with controls (■).

conditions (table 1), there were suggestions of an increase which, if real, would be sufficient to account for the AdoDA-stimulated production of lactate under basal conditions (82 $\mu$mol lactate/g dry wt between 10 and 70 min perfusion). In the presence of 40 $\mu$U/ml insulin, AdoDA stimulation of glucose uptake (table 1) was sufficient to provide approximately twice as much lactate as was released in the 10–70 min perfusion period (95 $\mu$mol lactate/g dry wt). Presumably the excess glucose was converted to glycogen or less likely (since work done by the hearts was similar in both cases), oxidized.

In an attempt to demonstrate inhibition of $k_s$ by Ado, we perfused with 70 $\mu$U/ml insulin in the presence and absence of 5 $\mu$M Ado. No inhibition of $k_s$ was detectable, but about 75% of the Ado had disappeared from the perfusate after 15 min of perfusion and none remained after 2 h. Inosine and uric acid appeared. Furthermore, non-hydrolysable Ado receptor agonists such as 2-Cl-Ado were cardioplegic at 0.3 $\mu$M or greater. We considered whether the production of inosine (or a metabolite thereof) was stimulating $k_s$ and glucose uptake. However, inosine (20 $\mu$M) did not stimulate protein synthesis or glucose uptake in the presence of 40 $\mu$U insulin/ml. Similarly, glycerol at a concentration identical with that present in the AdoDA addition was without effect.

## 4. DISCUSSION

Ado has been called a 'local hormone' [16] or a 'retaliatory metabolite' [17] which is being continually released and taken up by cells. After uptake, it is either rephosphorylated or deaminated. Although a weakness of our work is that we have not measured Ado concentrations, it is recognised that it may be very difficult to measure Ado concentrations at the requisite extracellular sites [6]. It is however difficult for us to see how AdoDA could be acting if it is not by destruction of extracellular Ado. The failure of Ado to affect protein synthesis directly could be the result of its rapid disappearance from the extracellular phase and/or of there being a sufficiently rapid release of endogenous Ado under normal conditions to produce a maximal decrease in insulin sensitivity.

Although vasoconstriction may account for increased lactate release in the presence of AdoDA, this is unlikely to account for the observed stimulation of $k_s$, which is inhibited by hypoxia [18,19]. It could be argued that the stimulation of $k_s$ by AdoDA (and insulin, for that matter) could be the indirect result of the stimulation of lactate output, because lactate is known to stimulate cardiac protein synthesis compared with glucose as sole fuel [20]. This seems to be unlikely since the rates of lactate production in the presence of AdoDA are similar in the presence or absence of 40 $\mu$U/ml insulin (fig.2), yet there is a significant ($P < 0.01$)

stimulation of $k_s$ only if insulin is present (table 1).

The effects of AdoDA we have shown are presumably extracellular and are the result of deamination of Ado to inosine. We propose that endogenous Ado interacts with extracellular Ado receptors (review [21]) and thereby inhibits the binding of insulin to its receptor and/or interferes with the transmission of the insulin signal. Such inhibition is removed by perfusion with AdoDA. There is a precedent for suggesting that Ado may affect insulin binding. In adipocytes, where Ado increases sensitivity of glucose uptake and lipolysis to insulin (i.e. the effect is opposite to its effect on glycolytic flux in muscle) and inhibits adenylate cyclase [22], isoproterenol inhibits insulin binding [23]. This effect is opposed by Ado [23].

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