INCREASED ANTILIPOLYTIC EFFECT OF THE ADENOSINE 'R-SITE' AGONIST N^6-(PHENYLISOPROPYL)ADENOSINE IN ADIPOCYTES FROM ADRENALECTOMIZED RATS

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Received 21 April 1980

1. Introduction

Adenosine, at extremely low concentrations, appears to be a potent endogenous inhibitor of adenylate cyclase and possibly of other unknown hormonal effector systems in rat adipocytes [1]. These effects appear to be exerted through two types of receptors, the most potent involving an 'R-type' of adenosine receptor having a strict requirement for an unsubstituted ribose moiety [1]. Adenosine greatly diminishes the responsiveness of rat adipocytes to lipolytic hormones and increases the effects of insulin upon these cells (for references see [1,2]). Effects of endogenous adenosine can be abolished or minimised by addition of adenosine deaminase to cell incubations. Adrenalectomy and hypothyroidism are conditions associated with decreased responsiveness of adipocytes to lipolytic hormones. It has been observed that addition of adenosine deaminase can restore the responsiveness to lipolytic hormones of cells from adrenalectomized rats [2]. This finding suggested that endogenous adenosine might play a part in the changes brought about by adrenalectomy. It was suggested [2] that adrenalectomy might result in increased formation of adenosine, decreased degradation of adenosine, increased adenosine release by the cells, or increased sensitivity to adenosine. The latter possibility can be tested by removing endogenous adenosine (the quantity of which is unknown) by adenosine deaminase and then observing the effects of the adenosine 'R-site' agonist N^6-(phenylisopropyl)adenosine. This drug is not a substrate for adenosine deaminase. Using this method it has been shown that adipocytes from hypothyroid rats are more sensitive to N^6-(phenylisopropyl)adenosine [3]. It is shown here that this agent also has a greater effect upon cells from adrenalectomized rats as measured by its ability to inhibit noradrenaline-stimulated lipolysis.

2. Materials and methods

Chemicals were obtained and treated as in [2]. In addition, N^6-(phenylisopropyl)adenosine was obtained from Boehringer. Adrenalectomy and sham-adrenalectomy was performed on 130 g male Sprague-Dawley rats by Olac Ltd (Bicester, England). On day 4 after operation the animals were transported to University College London and maintained with constant access to food and water. Adrenalectomized rats were given 0.9% (w/v) NaCl solution to drink. The rats were sacrificed on days 11,12 or 14 at which time they weighed (mean ± SEM of 8 animals) 216 ± 3 g (sham-operated) and 178 ± 10 g (adrenalectomized). The success of the adrenalectomy operation was verified by checking for the absence of the adrenal glands. From days 6–11 sham-operated rats grew at the rate of 9.7 ± 0.2 g/day and adrenalectomized rats at 7.0 ± 0.5 g/day (mean ± SEM of 12 animals). Adenosine deaminase (EC 3.5.4.4) was dialysed overnight against 100 vol. ice-cold 0.9% (w/v) NaCl to remove ammonium sulphate and then standardised spectrophotometrically [4] just prior to experiments. Adipocytes were isolated [5] simultaneously from the epididymal adipose tissues of 2 sham-operated and 2 adrenalectomized rats. Each preparation was diluted to 20 ml with Krebs-Ringer bicarbonate containing fatty acid-poor albumin (10 mg/ml) and 1.0 ml portions of these stock suspensions then added to incubation flask contents to give 4.0 ml final vol. The final contents of the flasks are shown in the figure legend. Incubation conditions and preparation of deproteinised extracts were as in

[2]. Glycerol release and adipocyte DNA content were measured as in [6] and [7], respectively.

3. Results and discussion

Adenosine deaminase was added to cell incubations at 20 munits/ml since this amount of the enzyme is just sufficient to maximally increase basal lipolysis, (see fig.3 of [2]), and presumably decreases endogenous adenosine to a low level. As found [2] this basal lipolysis seen with adenosine deaminase present was lower after adrenalectomy. Noradrenaline at 0.2 μM was added as a dose of lipolytic hormone sufficient to give a maximal hormone-stimulated rate of lipolysis in the presence of adenosine deaminase. As found in [2] (fig.6) this is similar in the adrenalectomized and sham-operated conditions.

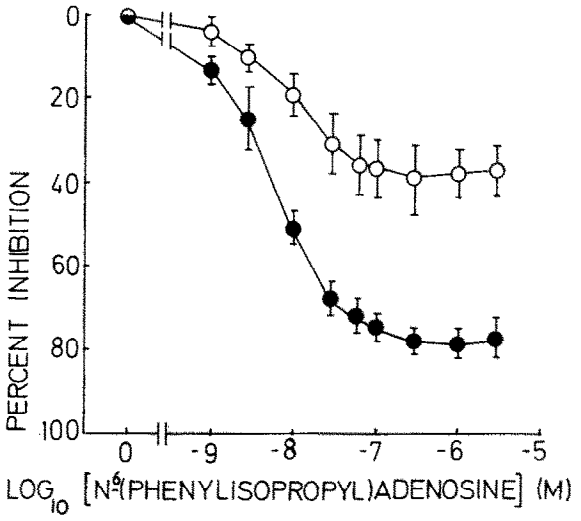

Fig.1. Inhibition of noradrenaline-stimulated lipolysis by N^6-(phenylisopropyl)adenosine in adipocytes from adrenalectomized and sham-adrenalectomized rats. The values are means ± SEM of 4 separate experiments and refer to percentage inhibition of glycerol release obtained when the cells were incubated for 60 min in Krebs-Ringer bicarbonate containing 5 mM glucose, fatty acid-poor albumin (40 mg/ml), adenosine deaminase (20 munits/ml) and 0.2 μM noradrenaline. These rates were 35.9 ± 1.1 and 31.1 ± 5.3 μmol . h^{-1} . 100 μg DNA^{-1} for the sham-operated and adrenalectomized conditions, respectively. Basal lipolytic rates with adenosine deaminase but no noradrenaline were 5.7 ± 0.9 and 2.3 ± 0.6 μmol . h^{-1} . 100 μg DNA^{-1} for the sham-operated and adrenalectomized conditions, respectively and were significantly different ($P<0.02$). The mean adipocyte DNA/ml flask contents was: sham-operated, 4.1 ± 0.2 μg; adrenalectomized, 4.4 ± 0.4 μg. (○) sham-operated; (●) adrenalectomized.

decreased lipolysis by ~80% in the adrenalectomized state whereas in normal cells this decrease was only 40% (fig.1). Half-maximal effect was seen with 10 nM N^6-(phenylisopropyl)adenosine in the control condition which is the same as that observed with normal cells [3]. N^6-(phenylisopropyl)adenosine at 5 nM produced 50% of the larger maximal effect in cells from adrenalectomized rats. Hence, the maximal response to N^6-(phenylisopropyl)adenosine was appreciably increased after adrenalectomy with a small increase in sensitivity to the drug. This may be contrasted with the hypothyroid state where there is at least a 10-fold increase in sensitivity to N^6-(phenylisopropyl)adenosine [3].

Provided N^6-(phenylisopropyl)adenosine satisfactorily mimics all the effects of adenosine that can be removed by addition of adenosine deaminase, it is quite likely that an increased effect of adenosine contributes to the apparent diminution of effects of noradrenaline and other lipolytic hormones after adrenalectomy [2]. This does not preclude the possibility that adenosine release or metabolism by the cells might also be altered. The molecular basis of the altered response to N^6-(phenylisopropyl)adenosine (and presumably adenosine) is unknown at present. It could presumably involve altered binding to, or altered numbers of, putative adenosine receptors [8] or alterations in the interaction of these receptors with adenylate cyclase or other effector systems.

Acknowledgement

I am indebted to Miss C. A. Carpenter for skilled technical assistance.

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