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Influence of 2-chloroadenosine on the nucleotide content of isolated rat hepatocytes

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2-Chloroadenosine is presumably a non-metabolizable analogue of adenosine; however, this compound induced an increase in the enzymatically measured nucleotide content of isolated rat hepatocytes. HPLC separation and spectral analysis of the peaks showed that this increase may be related to the formation of 2-chloro nucleotides and that the 2-chloro nucleotides appeared in the first minutes of the incubation period. These results demonstrate that 2-chloroadenosine may be metabolized by phosphorylation in rat liver cells.

2-Chloroadenosine; Adenosine; Nucleotide triphosphate; HPLC; Hepatocyte

1. INTRODUCTION

2-Chloroadenosine is a presumably non-metabolizable derivative of adenosine [1]. It is characteristic of an external Ra receptor [2–5], which stimulates adenylate cyclase activity and so increases cyclic AMP concentration. In isolated hepatocytes, this adenosine derivative has been used in metabolic studies to distinguish effects due to direct action of adenosine on the adenylate cyclase system from those caused by metabolization of adenosine [6–8]. However, 2-chloroadenosine induces changes in gluconeogenesis and ketone-body release which cannot be explained by increased cAMP concentration: i.e. a decrease in gluconeogenesis from alanine or increase in ketone-body release in the presence of alanine or lactate (Lavoinne, A., unpublished). Such paradoxical metabolic modifications could be explained by the metabolization of 2-chloroadenosine through pathways involving either adenosine kinase or adenosine deaminase.

Here, we have studied the influence of 2-chloroadenosine on the nucleotide content of isolated hepatocytes. Our results demonstrate that 2-chloroadenosine may be metabolized by rat liver cells via a nucleoside kinase but not adenosine deaminase. Hepatocytes incubated with 2-chloroadenosine were found to contain 2-chloro-ADP and 2-chloro-ATP.

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2. MATERIALS AND METHODS

2.1. *Rats*

Male Wistar rats (200–250 g) were obtained from Charles River (France). Animals were fed ad libitum or starved for 24 h before use.

2.2. *Reagents*

Enzymes, coenzymes and adenosine were obtained from Boehringer Mannheim (France). 2-Chloroadenosine was from Sigma. Nucleotides were from Boehringer or Sigma, and Nembutal from Abbott (France). Deoxycoformycin was generously provided by Dr H.E. Machamer (Warner-Lambert, Parke-Davis Pharmaceutical Research Division, Detroit, MI), 5-iodotubercidin by Professor L.B. Townsend (College of Pharmacy, University of Michigan, Ann Arbor, MI) and

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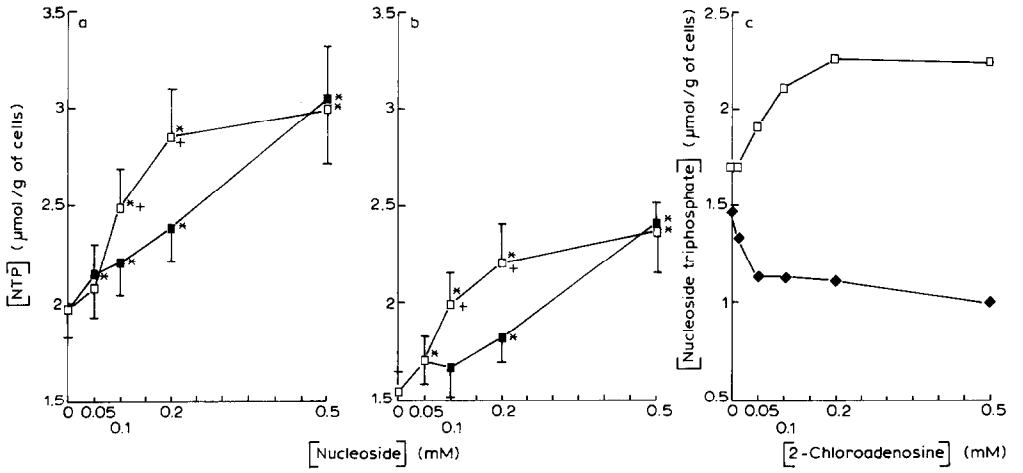

Fig.1. Dose response of the effect of adenosine and 2-chloroadenosine on nucleoside triphosphate content in hepatocytes. (a,b) Hepatocytes were isolated from fed (a) or fasted (b) rats. After 20 min incubation without substrate, adenosine (■) or 2-chloroadenosine (□) was added at various concentrations and the incubation continued for 60 min. Values are means ± SE for 5 cell preparations. NTP, enzymatically measured. * Significance vs control; + significance for chloroadenosine vs adenosine. (c) Procedure as in (a). (□) Nucleoside triphosphate measured with the hexokinase reaction (NTP); (◆) ATP measured using HPLC. Values are means for 2 cell preparations.

R-51469 by Dr H. Van Belle (Janssen Pharmaceuticals Research Laboratories, Beerse, Belgium). For HPLC, ultrapure KH_2PO_4 from Merck was used.

2.3. *Isolated liver cells*

Preparation and incubation of isolated liver cells were performed as in [9], except that no albumin was added to the incubation medium; briefly, hepatocytes (50 mg wet wt/ml) were incubated for 20 min in the absence or presence of substrates before addition of effector(s).

All results are expressed as means ± SE. Statistical significance of differences was assessed by Student's paired *t*-test with $P < 0.05$ taken as the threshold of probability.

2.4. *Nucleotide measurements*

Nucleoside triphosphates were measured by both enzymatic determination and HPLC. The hexokinase method used for the measurement of nucleoside triphosphate is not completely specific for ATP [10] and we use here the term 'nucleoside triphosphate' (NTP) rather than ATP for this enzymatic determination. HPLC (Waters) was performed as described [11] using 20 mM KH_2PO_4/40 mM KCl (pH 3.8) as the initial eluant and 250 mM KH_2PO_4/500 mM KCl (pH 5.1) as final eluant. The column packing used was SAX10 (Waters Associates). Integration of peak areas was automatically effected by an ICAP-10 (Delsi) integrator.

3. RESULTS AND DISCUSSION

2-Chloroadenosine induced an increase in nucleoside triphosphate (NTP) content of isolated

Table 1

Influence of nucleoside transporter and adenosine kinase inhibitors on the 2-chloroadenosine effect on nucleotide content in hepatocytes from fed rats

	Control	+ Ado (0.1 mM)	+ 2-ClAdo (0.1 mM)	+ R-51469			+ 5-Iodotubercidin	
				Control	+ Ado (0.1 mM)	+ 2-ClAdo (0.1 mM)	Control	+ 2-ClAdo (0.1 mM)
NTP content (μmol/g)	2.03 ± 0.18	2.44[a] ± 0.17	2.31[a] ± 0.20	1.76 ± 0.18	1.74 ± 0.20	1.67 ± 0.22	1.97 ± 0.18	1.90 ± 0.19

[a] Significance vs control

After 20 min incubation without substrate, effectors were added for 30 min: 0.5 mM R51469, an inhibitor of the nucleoside transporter; 10 μM 5-iodotubercidin, an adenosine kinase inhibitor. Values are means for 4 cell preparations

hepatocytes (fig.1a) and chloroadenosine in the range 0.1–0.2 mM was more effective than adenosine at increasing NTP content. This increase in NTP was also observed in hepatocytes isolated from fasted rats (fig.1b) and appeared in the presence of various energetic substrates such as glucose or pyruvate (not shown). Therefore, the effect of 2-chloroadenosine on the NTP content of hepatocytes was independent of the nutritional status of the animals.

Using R-51469, a compound which is known to inhibit adenosine transport in hepatocytes [6], the effect of both nucleosides (adenosine and 2-chloroadenosine) on NTP content was suppressed (table 1). This result suggested that 2-chloroadenosine may enter hepatocytes using the nucleoside transporter as described for human erythrocytes [12]. Moreover, this increase in NTP content was suppressed by 5-iodotubercidin, an inhibitor of adenosine kinase [13,14] (table 1), suggesting metabolism of 2-chloroadenosine through adenosine kinase. However, because of the low

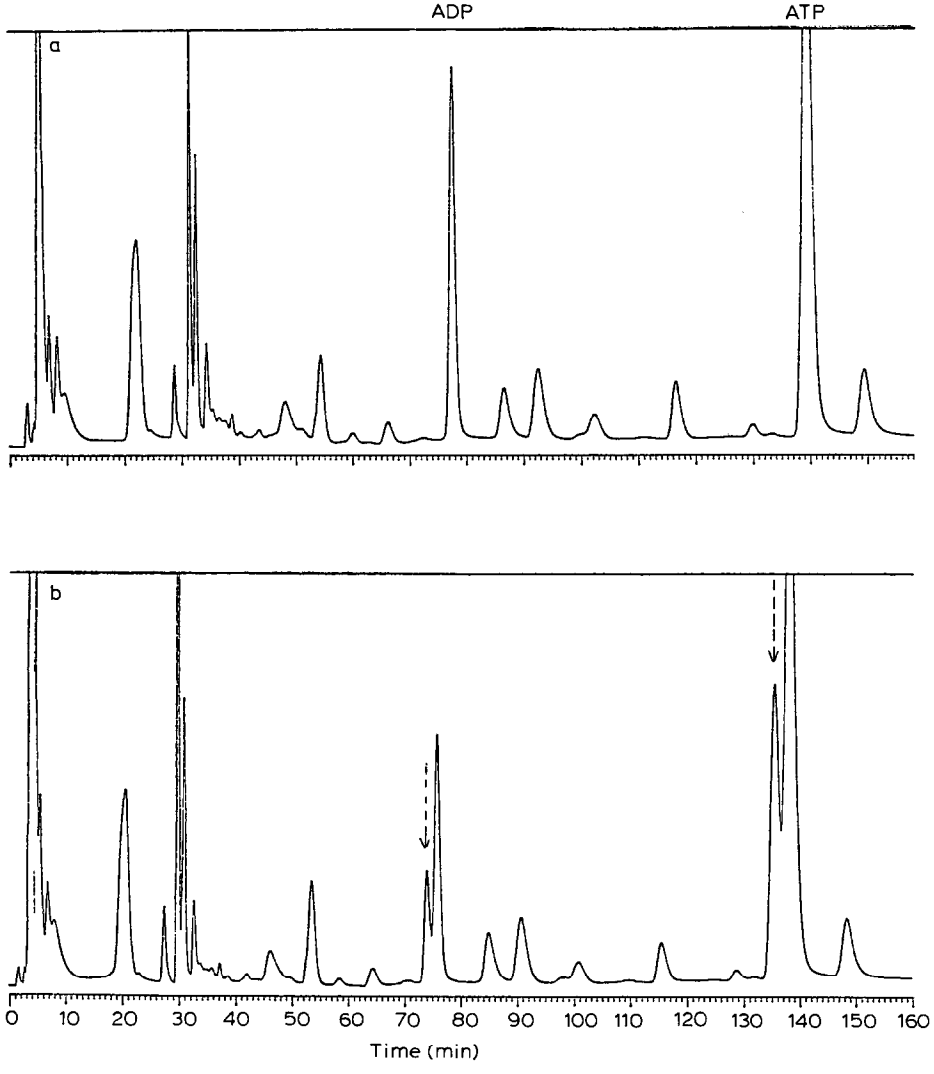

Fig.2. HPLC separation of the nucleotide content in hepatocytes incubated with adenosine or 2-chloroadenosine. Experimental procedure as in fig.1a. (a) 0.1 mM adenosine; (b) 0.1 mM 2-chloroadenosine. Supplementary peaks are indicated by an arrow.

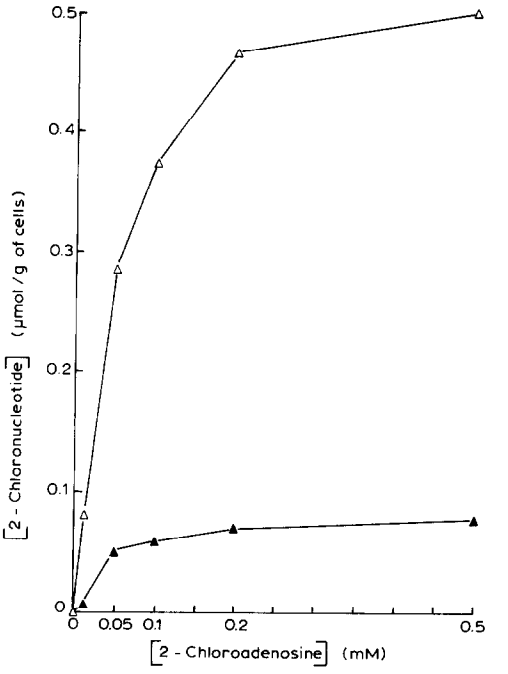

Fig.3. Dose response of the effect of 0.1 mM 2-chloroadenosine on the formation of 2-chloro-ADP and 2-chloro-ATP in hepatocytes. Experimental procedure as in fig.1a. (▲) 2-Chloro-ADP; (△) 2-chloro-ATP. Values are means for 2 cell preparations.

specificity of the hexokinase reaction (see section 2), we cannot conclude as to the nature of the nucleotide formed from 2-chloroadenosine.

Separation of nucleotides using HPLC showed that, in contrast to adenosine, intracellular ATP content was not increased in the presence of 2-chloroadenosine, but decreased (fig.1c). However, two new peaks were observed on the chromatogram in hepatocytes incubated with 2-chloroadenosine, with retention times close to those of ADP and ATP (fig.2). Maximum absorption was found at 259 nm for adenosine and 265 nm for 2-chloroadenosine, and absorption of the supplementary peaks observed after incubation

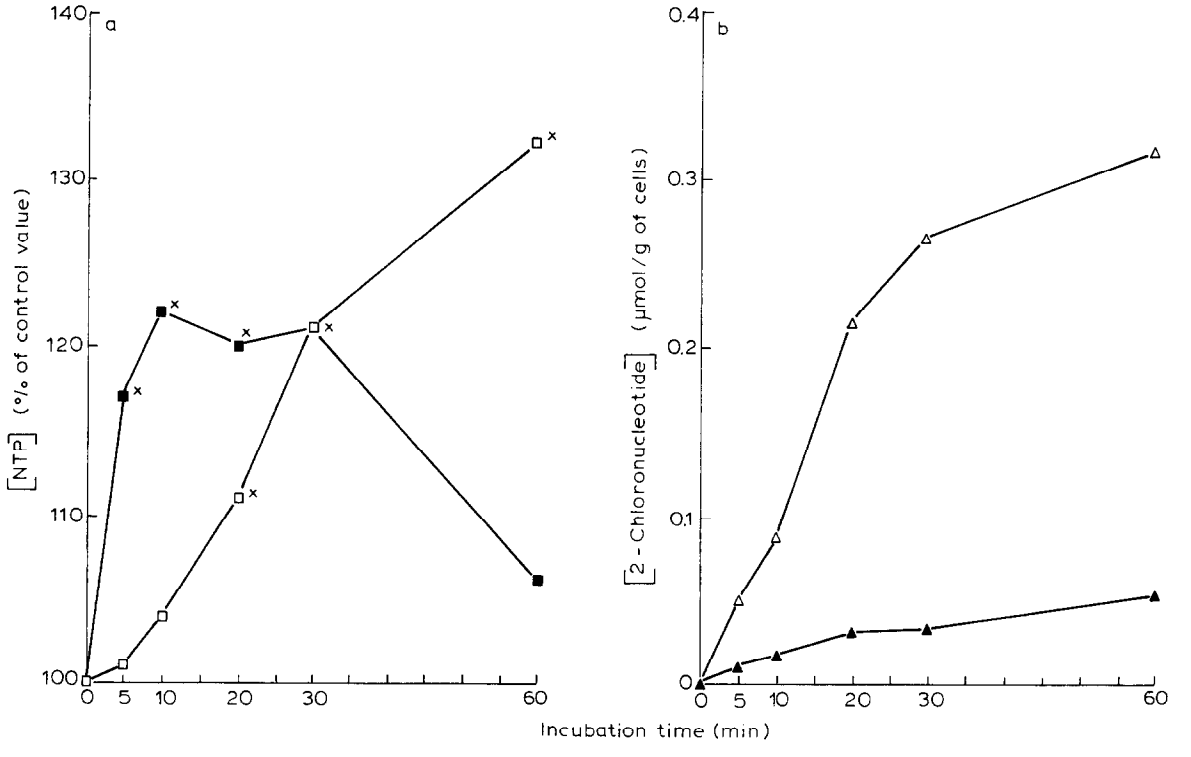

Fig.4. Time-course study of the influence of 0.1 mM adenosine or 2-chloroadenosine on the nucleotide content in hepatocytes. Experimental procedure as in fig.1a. (a) (■) Adenosine; (□) 2-chloroadenosine. Control value (0 min) was 2.15 ± 0.19 µmol/g cells for NTP. Values are means for 5 cell preparations. (b) (▲) 2-Chloro-ADP; (△) 2-chloro-ATP. Values are means for 2 cell preparations.

of hepatocytes with 2-chloroadenosine was maximal at 265 nm (spectra were recorded at the maximum elution of the peak using an HP 1040 A spectrophotometric detector). It may thus be concluded that these peaks correspond to the formation of 2-chloro-ADP and 2-chloro-ATP, respectively. Moreover, the increase in NTP content may be related to the formation of 2-chloro-ATP in the presence of 2-chloroadenosine (fig.3).

Investigation of the time course clearly showed that the increase in NTP content was more pronounced during the initial part of the incubation period in the presence of adenosine than with 2-chloroadenosine (fig.4a), which may be explained as being due to slower metabolization of the latter. However, 2-chloro-ADP and 2-chloro-ATP were found to appear in the first minutes of the incubation period (fig.4b).

In the presence of deoxycoformycin, an inhibitor of adenosine deaminase [15], the intracellular NTP content increased (fig.5a), however, the rise in 2-chloro-ATP concentration was not reinforced (fig.5b). The assumption that 2-chloroadenosine is a non-metabolizable analogue of adenosine is based mainly on the work of Clark et al. [1] who demonstrated that the 2-substituted analogues of adenosine were insusceptible to enzymatic deamination in the rat. Our results confirmed this insusceptibility of 2-chloroadenosine to deamination in the rat but demonstrated that it may be metabolized by phosphorylation. Adenosine kinase might be responsible for the phosphorylation of 2-chloroadenosine, since 5-iodotubercidin (an inhibitor of this enzyme) inhibits this process. However, to our knowledge, there are no data concerning the specificity of this inhibitor with respect to nucleoside kinases; moreover, 2-chloroadenosine is not a substrate of yeast adenosine kinase [16] and a study on the adenosine kinase purified from rabbit liver

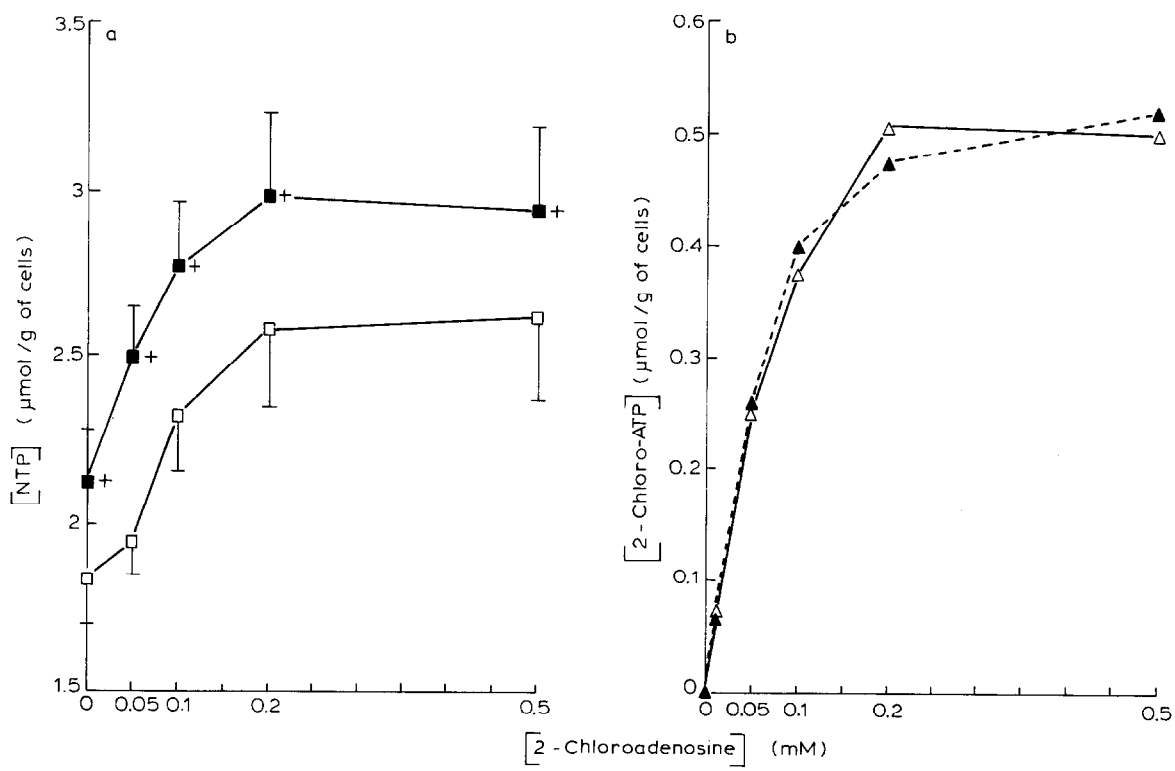

Fig.5. Influence of an inhibitor of adenosine deaminase on the 2-chloroadenosine effect on the nucleotide triphosphate content in hepatocytes from fed rats. After 20 min incubation without substrate, 2-chloroadenosine ± deoxycoformycin was added. (a) Influence on NTP content: (□) control; (■) 10 µM deoxycoformycin. + Significance vs control. (b) Influence on 2-chloro-ATP content: (△) control; (▲) 10 µM deoxycoformycin. Values are means for 2 cell preparations.

reported a substrate efficiency of 250 for adenosine and 0.003 for 2-chloroadenosine [17]. Thus, another nucleoside kinase is probably responsible for phosphorylation of 2-chloroadenosine.

In conclusion, 2-chloroadenosine may be metabolized by phosphorylation in rat liver cells. The formation of 2-chloro-ATP and (or) 2-chloro-ADP may be responsible for the unexpected metabolic changes that we have observed in the presence of this nucleoside. This metabolism of 2-chloroadenosine also suggests caution in the use of this adenosine analogue only to stimulate the adenylate cyclase system, at least in hepatocytes.

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