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INHIBITION OF PROTEIN SYNTHESIS INDUCED BY ADENINE NUCLEOTIDES REQUIRES THEIR METABOLISM INTO ADENOSINE

SANDRINE TINTON and PEDRO BUC-CALDERON*

Unité de Biochimie Toxicologique et Cancérologique, Département des Sciences Pharmaceutiques, Université Catholique de Louvain, 1200 Bruxelles, Belgium

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Abstract—Adenine nucleotides and adenosine inhibit the incorporation of radiolabelled leucine into proteins of isolated hepatocytes. Impairment occurred with nucleotides which can be converted into 9-β-D-ribofuranosyladenine (adenosine) but was not observed after treatment with adenine or AMPCPP (the α,β-methylene analogue of ATP). Metabolism into adenosine was further suggested by the increase in cellular ATP levels following treatment of hepatocytes with ATP, adenosine or AMPPCP (the β,γ-methylene ATP analogue) while AMPCPP was without any significant effect. The inhibition of protein synthesis caused by adenosine was not due to a lytic effect nor to a general disturbance in hepatic functions and was reversed when the cells were washed and transferred to a nucleoside-free medium. This impairment, however, was not coupled to the activation of adenylate cyclase, as preincubation of hepatocytes with P_1 purinoceptor antagonists failed to prevent protein synthesis inhibition. In contrast, L-homocysteine enhanced the inhibitory effect of adenosine on the incorporation of radiolabelled leucine into proteins. Our results thus suggest that the inhibition of protein synthesis caused by adenine nucleotides requires their conversion into adenosine. They also indicate that the inhibitory effect of adenosine does not involve a receptor-mediated effect but may be related to an increase in *S*-adenosylhomocysteine content and a subsequent low level of macromolecule methylation.

Key words: extracellular adenine nucleotides; adenosine; ATP-analogues; P_1 and P_2 purinoceptors; protein synthesis; L-homocysteine

The key role of adenine nucleotides and adenosine as regulators of many physiological processes is now well established. In addition to their intracellular effects, these substances can interact with cell surface receptors first classified by Burnstock [1] as P_1 and P_2 according to their high specificity either for adenosine and AMP or for ATP and ADP, respectively. Neither P_1 nor P_2 receptors form a homogeneous group. According to the rank order of potencies of different ATP analogues, the P_2 receptors are subdivided into four main classes: P_{2x}, P_{2y},P_{2z},P_{2t} [2]. More recently, evidence of a P_{2u} class has also been reported [3, 4].

As for the adenosine-mediated effects, pharmacological studies have characterized two subtypes of P_1 receptors coupled to the inhibition (A_1 subclass) or activation (A_2 subclass) of adenylate cyclase [5, 6]. Evidence of an A_3 receptor coupled to a calcium channel has also been reported [7]. Nevertheless, in perfused liver as well as in isolated hepatocytes, only the A_2 receptor has been described, the cellular response being associated with an increase in cAMP [8, 9].

We have recently reported that adenine nucleotides and adenosine inhibit the incorporation of radiolabelled leucine into cellular proteins of isolated hepatocytes [10]. The present study was therefore undertaken to investigate the mechanism by which these compounds inhibit protein synthesis.

ATP-γ-S† a P_{2y} agonist, the methylene-ATP analogues (AMPPCP and AMPCPP) that preferentially interact with P_{2x} receptors [3] as well as suramin, a well-known P_2-purinoceptor antagonist [11], were used to investigate the role of a P_2 receptor-mediated effect. The use of a P_1 antagonist and the measurement of cAMP level were also carried out to determine whether the inhibition of protein synthesis induced by adenosine could be mediated via P_1-purinoceptors.

MATERIALS AND METHODS

Animals. Male Wistar rats weighing 250–280 g were purchased from Iffa-Credo (Les Oncins, France). Animals were housed in individual cages in a temperature- and light-controlled room. They received a standard diet (AO3, U.A.R., France) and water *ad libitum*.

Chemicals. BSA (fraction V), adenine, adenosine, L-homocysteine thiolactone, dipyridamole, adrenaline, NBTI, ATP-γ-S, AMPCPP, and AMPPCP

* Corresponding author: Tel. 32-2-764 73 66; FAX #-32-2-764 73 59; E-mail Calderon@bctc.ucl.ac.be.

† Abbreviations: ATP-γ-S, adenosine 5′-*O*-(3-thiotriphosphate); AMPCPP, α,β-methyleneadenosine 5′-triphosphate; AMPPCP, β,γ-methyleneadenosine 5′-triphosphate; Ado, adenosine; Hcy, L-homocysteine; AdoHcy, *S*-adenosylhomocysteine; NBTI, *S*-(*p*-nitrobenzyl)-6-thioinosine; DMEM, Dulbecco's modified Eagle medium; IBMX, 3-isobutyl-1-methylxanthine; LDH, Lactate dehydrogenase; PCA, perchloric acid; HPLC, high-performance liquid chromatography.

were purchased from Sigma (St Louis, MO, U.S.A.). Caffeine and ATP were from Merck (Darmstadt, Germany). Suramin was from Biomol (Plymouth, PA, U.S.A.). Glucagon was obtained from Novo Nordisk A/S (Denmark). Collagenase was from Boehringer-Mannheim (Germany). DMEM was purchased from Flow Laboratories (Irvine, U.K.). IBMX was from Janssen Chimica (Geel, Belgium). [U-^{14}C]Leucine and cAMP kit TRK 432 were purchased from the Radiochemical Centre (Amersham, U.K.). [1-^{14}C]Palmitic acid was from Dupont NEN (Boston, MA, U.S.A.). All other chemicals were of the purest grade available.

L-Homocysteine was prepared according to Duerre and Miller [12] by cleavage of L-homocysteine thiolactone with 5N NaOH.

Palmitate-albumin-bound solution: stock solutions of [^{14}C]palmitate (0.5 mM)-BSA (0.75 mM) and unlabelled palmitate (1.70 mM)-BSA (1.13 mM) were each prepared, according to Capuzzi *et al.* [13], by addition of 10% solution of BSA to a warmed (±50°) and sonicated solution of palmitic acid.

Preparation of isolated hepatocytes. Hepatocytes were isolated using the standard procedure described by Berry and Friend [14] and slightly modified by Krack *et al.* [15]. Briefly, animals were anaesthetized with an i.p. injection of pentobarbital (60 mg/kg), and cells were isolated by liver perfusion with Krebs–Ringer solution containing collagenase. Approximately 85–95% of the freshly-isolated cells routinely exclude erythrosine B. Hepatocytes (0.5×10^6 cells/mL) were resuspended in DMEM supplemented with 0.3% BSA and incubated at 37° in a thermoregulated shaking water-bath (100 oscillations/min) under a continuous flow of O_2/CO_2 (95%/5%).

Assays.

- Hepatocyte viability was estimated by measuring the activity of LDH according to the procedure of Wroblesky and Ladue [16] both in the culture medium and in the cell pellet obtained after centrifugation as described elsewhere [15]. The results are expressed as a ratio of released activity to total activity.
- Protein synthesis was estimated by measuring the incorporation of radiolabelled leucine (specific activity: 94 μCi/mmol, 0.8 mM unlabelled leucine) into the pelleted material obtained by PCA precipitation as described by Seglen [17]. Results are expressed as dpm/mg protein. The amount of proteins was determined by the method of Lowry *et al.* [18] using BSA as standard.
- Triglyceride content and secretion were estimated by measuring [^{14}C]palmitic acid incorporation (spec. act.: 710 μCi/mmol, 0.04 mM unlabelled palmitate) into intra- and extracellular triglycerides. Labelled and unlabelled palmitate were added to the incubation medium in an albumin-bound form (see the Chemicals section). The incorporation was evaluated in 1 mL of cell suspension as described by Deboyser *et al.* [19].
- Glycogen content was evaluated as described by Goethals *et al.* [20]. Briefly, intracellular glycogen was extracted by KOH (1 N) for 10 min at 100°. After protein precipitation with acetic acid, the glucose (generated by incubating supernatants with amylo-1,4–1,6-glucosidase) was quantified using the glucose oxidase method [21].
- Intracellular cAMP levels were determined in duplicate using the Amersham kit TRK 432. Hepatocytes (5×10^6 cells/mL) were incubated for 30 min prior to addition of adenosine, ATP or glucagon. Exactly two min later aliquots of cell suspension were taken and deproteinized by precipitation with trichloracetic acid (5%, final concentration). Supernatants were neutralized, and the cAMP [^{3}H] assay was carried out following the instructions provided with the kit.
- Cellular ATP was measured by HPLC (LKB Pharmacia) equipped with a 4.7 × 125 mm anion exchange column (Partisphere SAX, Whatman, particle size 5 μm). Separation was achieved by the use of an isocratic elution mode with $NH_4H_2PO_4$ 0.45 M (pH 3.7) at a flow rate of 1.5 mL/min. Determination of ATP content was performed on neutralized perchloric acid extracts [22] of cells first separated from extracellular medium containing nucleotides or nucleosides by centrifugation onto a layer of silicone oil (115 cst, Serva; Germany). The results are expressed as nmol ATP/mg proteins.

Statistics. The results represent mean values ± standard error of the mean (SEM) of at least three separate experiments. Analysis of variance (two-way ANOVA with the interaction time–treatment) was used to compare the concentration–response curves. For statistical comparison at a given time point, data were analysed using Student's *t*-test. A P value less than 0.05 was set as the minimum level of significance.

RESULTS

Effects of adenine derivatives on protein synthesis and LDH leakage

Figure 1 illustrates the effect of several adenine derivatives (1 mM) on both protein synthesis and LDH leakage after 120 min of incubation. Compared to control hepatocytes, adenosine, ATP and ATP-γ-S inhibited the incorporation of radiolabelled leucine into proteins by approximately 40–60%. Adenosine was without any significant effect on LDH leakage, while ATP and ATP-γ-S increased LDH leakage two-fold as compared to untreated cells. Most probably due to this increased cell lysis, the inhibitory effect of ATP (and ATP-γ-S) on protein synthesis was greater than that of adenosine.

As previously reported, ATP at a final concentration of 0.5 mM also inhibited protein synthesis but was without any significant effect on cell viability [10]. The possible involvement of P_2 receptors in ATP-mediated protein synthesis inhibition was nevertheless ruled out by using the P_2 antagonist suramin [11]. Indeed, when hepatocytes were preincubated for 10 min in the presence of suramin at either 0.05, 0.3 or 0.5 mM and further treated

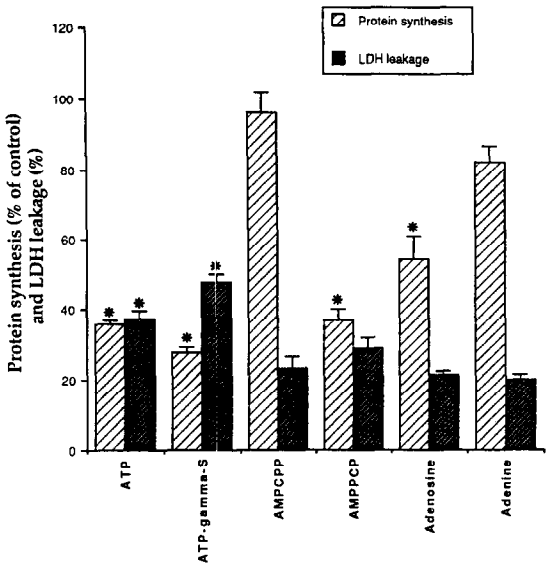

Fig. 1. Effect of adenine derivatives on protein synthesis and LDH leakage. Isolated hepatocytes were incubated for 120 min with adenine, adenosine, ATP, and the ATP analogues ATP-γ-S, AMPPCP and AMPCPP at a final concentration of 1 mM. At the end of the incubation, LDH leakage and protein synthesis were measured as described in the Materials and Methods section. Protein synthesis was expressed as a percentage of control values (2888 ± 119 dpm/mg prot). LDH leakage was 23 ± 1% for control hepatocytes. Values are means ± SEM of three to seven separate experiments. *Student's *t*-test: significance level $P < 0.05$ as compared to control hepatocytes.

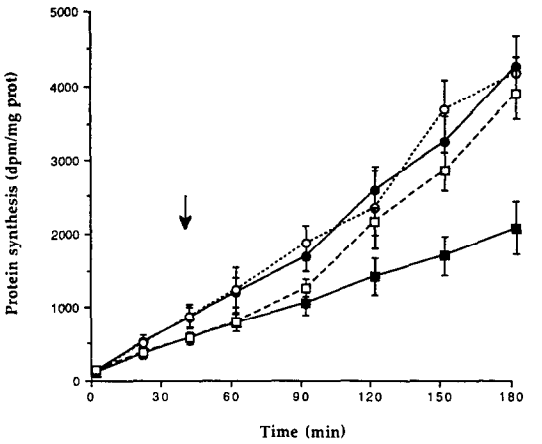

Fig. 2. Reversible inhibition of protein synthesis by adenosine. Hepatocytes were incubated either for 180 min (continuous lines, filled symbols) or for 40 min (dashed lines, open symbols) in the absence (circles) or in the presence of 0.5 mM adenosine (squares). After 40 min of incubation, cells were washed and resuspended in an adenosine-free medium as indicated by the arrow. Protein synthesis was estimated as described in the Materials and Methods section. Values are means ± SEM of three separate experiments. ANOVA test: two-factor interactions (time–treatment) significance level $P < 0.05$ for the comparison between both adenosine treatments.

with ATP (0.5 mM), we found an inhibition of protein synthesis similar to that induced by ATP alone (data not shown). Moreover, suramin (0.5 mM) by itself did not modify the incorporation of [^{14}C]leucine into proteins as compared to untreated cells.

AMPPCP inhibited protein synthesis, while adenine and AMPCPP failed to significantly inhibit the incorporation of radiolabelled leucine into proteins or to increase LDH leakage (Fig. 1). AMPCPP and AMPPCP differ only by the existence of a methylene group rather than an oxygen at the α,β and β,γ position, respectively. Yet, their effects on protein synthesis were different, possibly because AMPPCP can be hydrolysed to AMP and further to adenosine whereas this is not the case for AMPCPP [9].

The inhibitory effect of adenosine on protein synthesis seems to be saturated at concentrations higher than 0.5 mM. Indeed, when hepatocytes were incubated in the presence of 0.05 mM and 0.5 mM adenosine, the incorporation of [^{14}C]leucine represented approx. 70% (2044 ± 145 dpm/mg prot) and 50% (1547 ± 131 dpm/mg prot) of control values, respectively. Incubation of hepatocytes with 1 mM adenosine did not further inhibit protein synthesis since the incorporation of [^{14}C]leucine represented 1566 ± 165 dpm/mg prot and was not statistically different from that observed with 0.5 mM adenosine.

Reversible protein synthesis inhibition induced by adenosine

Compared to untreated cells, adenosine (0.5 mM) significantly reduced the incorporation of radiolabelled leucine into proteins (two-way ANOVA $P < 0.05$). Protein synthesis was inhibited by 36% and 52% after 40 min and 180 min of incubation respectively (Fig. 2). Nevertheless, when cells were first incubated for 40 min with adenosine, then washed and transferred to a nucleoside-free medium, protein synthesis was restored, becoming essentially indistinguishable from that observed in washed control-cells (two-way ANOVA, $P > 0.05$).

P_1 purinoceptors are not involved in adenosine-mediated protein synthesis inhibition

The reversibility of the inhibition of protein synthesis by adenosine raises the possibility of a receptor-mediated effect. As illustrated in Table 1, the addition of adenosine and ATP at 0.5 mM and 2.5 mM did not significantly modify the cAMP level as compared to untreated cells. Even the preincubation of hepatocytes with 0.5 mM IBMX, an inhibitor of phosphodiesterases, for 10 min prior to adenosine did not increase the cAMP level (8 pmol/10^6 cells) compared to cells which received IBMX alone (7.9 pmol/10^6 cells). Moreover, when hepatocytes were treated for 120 min with glucagon (10 nM), which increased the cAMP content by seven-fold (Table 1), or adrenaline (10 μM), the incorporation of radiolabelled leucine into proteins represented 92 ± 10% and 89 ± 6%, respectively, of that observed in control cells (mean ± SEM of three separate experiments).

Table 1. Effect of adenosine and ATP on cAMP content

Treatments	cAMP content (pmol/10^6cells)
Control	3.15 ± 0.50
Adenosine (0.5 mM)	3.37 ± 0.40
Adenosine (2.5 mM)	3.67 ± 0.27
Glucagon (10 nM)	22.70 ± 4.90*
ATP (0.5 mM)	3.80 ± 0.21
ATP (2.5 mM)	3.56 ± 0.53

Hepatocytes (5×10^6 cells/mL) were incubated for 30 min before addition of adenosine (0.5 mM and 2.5 mM), ATP (0.5 mM and 2.5 mM) and glucagon (10 nM). Two minutes later, aliquots of the cell suspension were taken and cAMP was measured as described in the Materials and Methods section. Results (means ± SEM of three to four separate experiments) are expressed as pmol/10^6 cells.

* Student's *t*-test: significance level $P < 0.05$ as compared with control values.

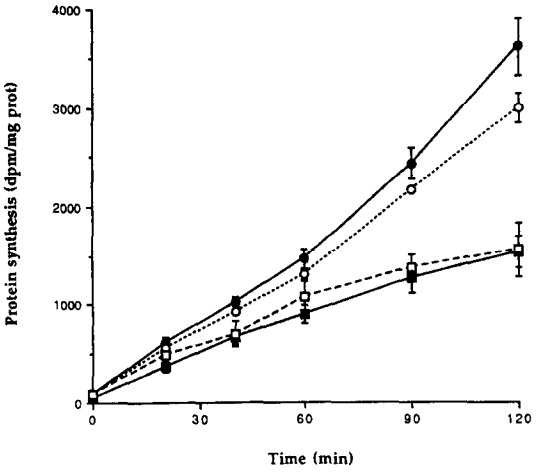

Fig. 3. Effect of caffeine on protein synthesis inhibition induced by adenosine. Hepatocytes were first preincubated for 10 min in the absence (continuous lines) or in the presence of 0.5 mM caffeine (dashed lines). Cell suspensions preincubated under both conditions were further treated for 120 min in the absence (circles) or in the presence of 0.5 mM adenosine (squares). Control cells (filled circles) did not receive caffeine or adenosine. Protein synthesis was estimated as described in the Materials and Methods section. Values are means ± SEM of three separate experiments. ANOVA test: two-factor interactions (time–treatment) significance level $P < 0.05$ as compared with both control and treatment with caffeine alone.

Preincubation of hepatocytes with caffeine (0.5 mM), a P_1 receptor antagonist [1], for 10 min prior to adenosine (0.5 mM) failed to protect the cells against the significant inhibition of protein synthesis caused by adenosine (Fig. 3). Both curves, adenosine plus caffeine and adenosine alone, showed a very similar inhibition profile. Similar results were obtained when 8-phenyltheophylline (10 μM) was used instead of caffeine and cellular viability was not modified under any experimental conditions

Table 2. Effects of adenosine transport inhibitors on protein synthesis inhibition induced by adenosine

Treatments	Incorporation of [^{14}C]Leu (dpm/mg protein)
Control	2227 ± 216
Adenosine (0.5 mM)	1384 ± 140*
NBTI (400 μM)	1528 ± 66*
Dipyridamole (50 μM)	1348 ± 128*
Adenosine (0.5 mM) + NBTI (400 μM)	1459 ± 89*
Adenosine (0.5 mM) + dipyridamole (50 μM)	1194 ± 112*

Hepatocytes (0.5×10^6 cells/mL) were incubated for 15 min in the absence or in the presence of NBTI (400 μM) or dipyridamole (50 μM). Cells were further incubated for 120 min in the presence or in the absence of adenosine (0.5 mM). Protein synthesis was estimated by measuring the incorporation of radiolabelled leucine into proteins as described in the Materials and Methods section. Results are means ± SEM of three to five separate experiments.

* Student's *t*-test: significance level $P < 0.05$ as compared to control values.

where both methylxanthines were added (data not shown).

Cellular uptake of adenosine as a key event leading to protein synthesis inhibition

Besides its extracellular action, adenosine is rapidly taken up by mammalian cells where it is further metabolized into ATP, inosine or AdoHcy [23–26]. To investigate whether the cellular uptake of adenosine was involved in the inhibition of protein synthesis, we tested the effect of both NBTI (400 μM) and dipyridamole (50 μM). The use of such adenosine transport inhibitors [25] was unsuccessful, however, since they themselves reduced the incorporation of radiolabelled leucine into proteins (Table 2), thereby masking the inhibitory effect observed when added in the presence of adenosine.

The uptake of adenosine was, however, supported by a change in cellular ATP content. Indeed, adenosine (0.5 mM) induced a rapid and significant increase in the ATP level (Fig. 4) as compared to control hepatocytes (two-way ANOVA $P < 0.05$). Extracellular ATP (1 mM) and AMPPCP (1 mM) also raised cellular ATP content significantly. At the end of the experiment the ATP level was 50 nmol/mg prot in adenosine- or ATP-treated hepatocytes and was 44 nmol/mg prot in cells incubated with AMPPCP. In contrast to these compounds, AMPCPP (1 mM) was unable to significantly increase ATP content (two-way ANOVA, $P > 0.05$).

Although an elevation in the cellular ATP level might be correlated with the inhibition of protein synthesis, it is important to point out that after its transport into the cells adenosine is metabolized by several pathways. Adenosine can be phosphorylated into AMP by adenosine kinase but can also be degraded into inosine by adenosine deaminase

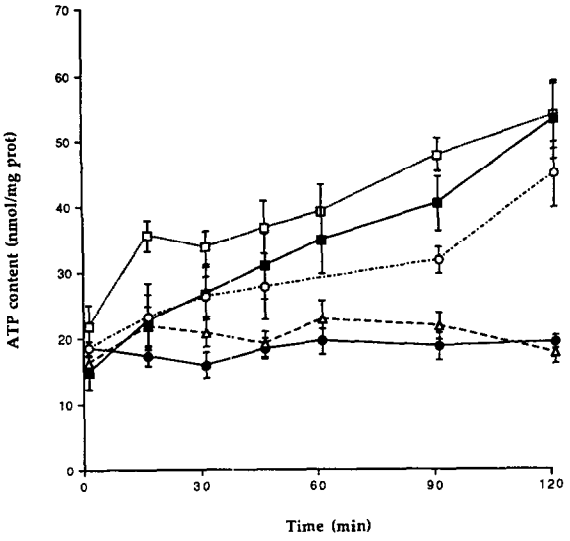

Fig. 4. Effect of adenosine, ATP and the methylene ATP analogues on the intracellular ATP level. Hepatocytes were incubated for 120 min in the absence (filled circles) or in the presence of 0.5 mM adenosine (open squares), 1 mM ATP (filled squares), and the two methylene ATP analogues, AMPPCP (open circles) and AMPCPP (open triangles) at a final concentration of 1 mM. At the times indicated, aliquots of the cell suspension were taken and intracellular ATP levels measured by HPLC as described in the Materials and Methods section. Values are means ± SEM of four to 11 separate experiments. ANOVA test: two-factor interactions (time–treatment) significance level $P < 0.05$ for AMPPCP-, ATP-, and adenosine-treated cells as compared with control values.

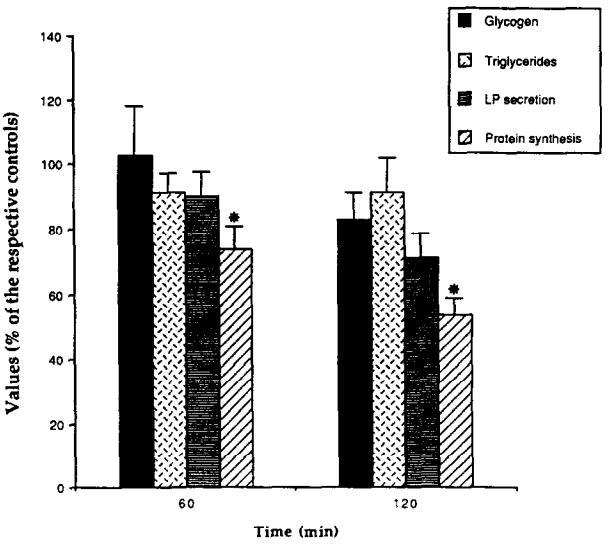

Fig. 5. Comparative effect of adenosine on several hepatocellular functions. Hepatocytes were incubated for 120 min with or without adenosine (0.5 mM). At the time indicated, glycogen content, radiolabelled triglyceride content and their secretion as lipoproteins (LP secretion) and protein synthesis were measured as described in the Materials and Methods section. Results are expressed as a percentage of the control values for each corresponding time. Values are means ± SEM of three to four separate experiments. *Student's *t*-test: significance level $P < 0.05$.

[23, 24, 27]. Nevertheless, the incubation of hepatocytes with 5-iodotubercidin and/or coformycin, which are inhibitors of adenosine kinase and adenosine deaminase, respectively [28, 29], was unable to prevent the protein synthesis inhibition induced by adenosine [30].

The inhibition of protein synthesis induced by adenosine was quite specific. For example, several metabolic functions of liver cells such as the synthesis of triglycerides or the maintenance of glycogen content were not affected by adenosine, although the secretion of triglycerides as lipoproteins was slightly but not significantly decreased after 120 min of incubation (Fig. 5).

Effect of L-*homocysteine on protein synthesis inhibition induced by adenosine*

It has been reported that AdoHcy could play a role in adenosine-mediated toxicity to cultured mouse T-lymphoma cells [26]. We therefore investigated the effect of L-homocysteine on protein synthesis inhibition induced by adenosine.

Compared to control hepatocytes, L-homocysteine (2 mM) and adenosine (0.5 mM) reduced the incorporation of radiolabelled leucine into proteins after 60 min of incubation by 15% and 42%, respectively (Table 3). However, when L-homocysteine was added together with adenosine a stronger inhibition occurred, reaching 75% by the end of the experiment. Under such conditions, the level of AdoHcy was dramatically increased by 180-fold as compared to control cells [30].

Although the LDH leakage observed after 60 min following treatment of hepatocytes with both adenosine and L-homocysteine (23%) was significantly increased as compared to untreated cells, it was not significantly different from that measured when cells were incubated separately in the presence of adenosine or L-homocysteine (Table 3).

DISCUSSION

The impairment of protein synthesis represents an early and very sensitive event during chemical-induced toxicity [20] as well as under various pathological conditions such as hypoxia [22, 31] or during oxidative stress [31, 32]. We have recently reported that incubation of isolated hepatocytes in the presence of ATP or adenosine resulted in the inhibition of protein synthesis [10]. In the present study, we investigated the mechanism by which these compounds induced protein synthesis inhibition and examined if such impairment could involve a receptor interaction.

The lack of receptor P_1 interaction in protein synthesis inhibition induced by adenosine was confirmed by the fact that preincubation of hepatocytes with P_1 antagonists prior to adenosine

Table 3. Effect of L-homocysteine on LDH leakage and on protein synthesis inhibition induced by adenosine

	Incorporation of [^{14}C]Leu (dpm/mg prot)		LDH leakage (%)	
Time (min)	20	60	20	60
control	409 ± 24	1063 ± 59	12 ± 2	15 ± 1
Adenosine (0.5 mM)	319 ± 89	618 ± 16*	14 ± 1	18 ± 2
Hcy (2 mM)	420 ± 82	911 ± 86	12 ± 1	17 ± 2
Adenosine (0.5 mM) + Hcy (2 mM)	145 ± 19*	267 ± 44**	13 ± 2	23 ± 3*

Hepatocytes were incubated for 60 min in the absence or in the presence of adenosine (0.5 mM) or Hcy (2 mM) alone or in combination. Protein synthesis and LDH leakage were measured as described in the Materials and Methods section. Values are means ± SEM of three separate experiments.

Student's *t*-test: significance level $P < 0.05$ (*) and $P < 0.01$ (**) as compared to control values at each corresponding time.

treatment was unable to protect the cells against the impairment caused by the nucleoside.

In perfused livers as well as in isolated hepatocytes, various cellular responses such as glycogenolysis [8, 9] have been related to an increase in cAMP due to the stimulation by adenosine of the A_2 receptor coupled to the activation of adenylate cyclase. Several studies have led to conflicting results as to whether cAMP participates in the response of hepatocytes to adenosine [23, 33]. In the present study we showed that neither adenosine nor ATP was able to increase the intracellular content of cAMP. Conversely, glucagon and adrenaline, which are known to activate adenylate cyclase, failed to produce a significant inhibition of protein synthesis. The role of cAMP in the regulation of protein synthesis remains unclear [31] but our results suggest that interaction with the A_2 receptor is not involved.

Extracellular ATP regulates many physiological processes by interacting with cell surface P_2 purinoceptors [1–4]. In the present study we found that both ATP-γ-S and AMPPCP, which are P_{2y} and P_{2x} agonists respectively [3], induced a similar inhibition of protein synthesis as ATP. Since AMPCPP (another P_{2x} agonist) failed to inhibit the incorporation of radiolabelled leucine into proteins, a P_{2x}-mediated effect is unlikely to be involved in the impairment of protein synthesis. The lack of P_2 receptor stimulation is further suggested by the fact that suramin, a P_2 antagonist [11], was unable to prevent the inhibitory effect of ATP on protein synthesis. Supporting this view, we have recently reported that an increase in free cytosolic calcium concentration, which not only represents an important biochemical event following P_2 receptor stimulation [3, 4, 34] but also plays a major role in the control of the translation [31], was not involved in the impairment of protein synthesis observed in adenine nucleotide-treated cells [35].

It has been well established that the extracellular action of ATP and ADP is complex due to their rapid hydrolysis to adenosine by ecto-nucleotidases present on various cell types such as hepatocytes [36, 37]. Our results suggest that conversion into adenosine is required to produce an inhibition of protein synthesis. Three lines of evidence support this conclusion:

(a) amongst the different ATP analogues tested, only nucleotides which can be hydrolysed into AMP and further into adenosine were able to inhibit the incorporation of radiolabelled leucine into proteins while AMPCPP was without any significant effect;
(b) AMPPCP and ATP, but not AMPCPP, increase intracellular levels of ATP. This process necessarily requires conversion to adenosine and its uptake by the cell, where it is metabolized by adenosine kinase to AMP and further to ATP;
(c) the inhibitory effect on protein synthesis was not additive when hepatocytes were incubated in the presence of both adenosine and ATP despite their interaction with different P_1 and P_2 receptors. Indeed, adenosine and ATP (both at 0.5 mM) inhibited protein synthesis by 53% and 65%, respectively, and when associated by 70%, a value not significantly different from that of ATP alone (data not shown).

Our results lead us to propose that the inhibition of protein synthesis could represent an intracellular effect following the rapid uptake of adenosine into the cells. Such a hypothesis might thus explain why AMPCPP, which was unable to impair protein synthesis, also failed to increase cellular ATP content.

Treatment of human lymphocytes with an inhibitor of adenosine deaminase resulted in an impairment of the incorporation of tritiated leucine into proteins [38]. Kredich and Martin [26] showed that adenosine inhibits the growth of lymphoma cells deficient in adenosine kinase or treated with an inhibitor of adenosine deaminase. They suggested that the cytotoxic effect of adenosine involves the inhibition of AdoHcy hydrolase, since it was enhanced by Hcy and was correlated with an increase in AdoHcy. AdoHcy hydrolase catalyses the reversible cleavage of AdoHcy to adenosine and Hcy. The equilibrium of the reaction favours the synthesis of AdoHcy and

it seems likely that the direction of the biological flow depends on the removal of Hcy and adenosine. It has been reported that inhibition of AdoHcy hydrolase by adenosine or adenosine analogues in perfused liver as well as in various cell types [39] results in an increase in AdoHcy, a potent inhibitor of AdoHcy-dependent transmethylation reactions in numerous macromolecules such as proteins, lipids and nucleic acids [39, 40].

The mechanism by which adenosine inhibits protein synthesis in isolated hepatocytes remains unknown. Our results, however, show that the incorporation of radiolabelled leucine into proteins was further decreased when adenosine was associated with Hcy. Accumulation of AdoHcy and inhibition of transmethylation reactions could therefore play an important role in the inhibition of protein synthesis induced by adenosine and will be the object of further investigation.

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