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Modulation of maximal glycogenolysis in perfused rat liver by adenosine and ATP

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Rat livers perfused at constant flow via the portal vein with dibutyryl cyclic AMP produced glucose equivalents at a steady maximal rate (6 μmol/min per g of liver). Addition of adenosine (150 μM) caused a biphasic effect. (i) First, the glycogenolytic rate rose transiently, to a mean peak of 150% of control levels after 2 min. This glycogenolytic burst was reproduced by two P_1-receptor agonists, but not by ATP, and was blocked by a P_1-antagonist (8-phenyltheophylline), as well as by inhibitors of eicosanoid synthesis (indomethacin, ibuprofen or aspirin). It did not occur in phosphorylase-kinase-deficient livers. The adenosine-induced glycogenolytic burst coincided with moderate and transient changes in portal pressure (+6 cmH_2O) and O_2 consumption (−20%), but it could not be explained by an increase in cytosolic P_i, since the n.m.r. signal fell precipitously. (ii) Subsequently, the rate of glycogenolysis decreased to one-third of the pre-adenosine value, in spite of persistent maximal activation of phosphorylase. The decrease could be linked to the decline in cytosolic P_i: both changes were prevented by the adenosine kinase inhibitor 5-iodotubercidin, whereas they were not affected by ibuprofen or 8-phenyltheophylline, and were not reproduced by non-metabolized adenosine analogues. In comparison with adenosine, ATP caused a slower decrease of P_i and of glycogenolysis. The fate of the cytosolic P_i was unclear, especially with administered ATP, which did not increase the n.m.r.-detectable intracellular ATP.

INTRODUCTION

Hepatic glycogen degradation is catalysed by the phosphorylated *a*-form of glycogen phosphorylase. Phosphorylation is brought about by cyclic-AMP-dependent activation of phosphorylase kinase, or by a direct stimulation of the latter enzyme by increased cytosolic [Ca^{2+}]. Phosphorylase *a* activity is also non-covalently regulated, e.g. by the availability of the substrate P_i [1].

Since the discovery of ATP as a neurotransmitter, purinergic receptors have been identified in many tissues [2]. With respect to the activation of phosphorylase in hepatocytes, two purinergic mechanisms have been characterized. ATP interacts with P_{2Y}-receptors, and evokes a transient Ca^{2+} response through a GTP-dependent InsP_3-mediated mechanism [3,4]. In the intact organ, ATP also stimulates thromboxane and prostaglandin release by the Kupffer cells [5,6], which may be involved in mediating the glycogenolytic response [5,7–12]. Adenosine interacts with P_1-receptors that activate adenylate cyclase [13–15]. However, its glycogenolytic response is also attenuated by cyclo-oxygenase inhibitors [16], and this is correlated with a decreased release of prostaglandin D_2 [17]. Furthermore, portal administration of the purinergic mediators results in transient vasoconstriction [7,15,16], which may stimulate glycogenolysis by causing regional hypoxia [18], possibly through elevation of the cytosolic P_i concentration.

Nucleosides and nucleotides are subject to extensive hepatic metabolism. Extracellular ATP is extremely short-lived, owing to hydrolysis by ecto-pyrophosphatases and phosphatases [19,20], and generation of adenosine may recruit secondary P_1 effects. Adenosine in turn is subject to extensive hepatic metabolism. Adenosine administration results in accumulation of extra ATP [21–23] and a drop in cellular P_i [23,24]. In contrast with some non-metabolized analogues, adenosine decreases the glycogenolytic effects of glucagon and catecholamines [22,25], and it has been suggested [25] that this might be due to P_i trapping in the synthesis of ATP. Adenosine-induced changes in ATP/ADP ratios and in cytosolic P_i have been observed, and their potential consequences for metabolic regulation have been discussed [24].

The interaction in the intact organ of the mechanisms described above is poorly understood. To single out effects of adenosine that are unrelated to a covalent modification of phosphorylase, we adopted the following experimental protocol [1]. Phosphorylase was clamped in its *a*-form by continuous perfusion of the liver with dibutyryl cyclic AMP. From this vantage point, we looked at time-dependent effects of the administration of adenosine and non-metabolized analogues thereof. The appearance of glucose and lactate in the perfusate was monitored to assess the rate of glycogenolysis. We used ^{31}P-n.m.r. to investigate whether changes in the catalytic efficiency of phosphorylase could be linked to changes in cytosolic P_i. Furthermore, ^{31}P-n.m.r. allowed us to monitor the cytosolic pH.

MATERIALS AND METHODS

Materials

Adenosine and indomethacin were purchased from Aldrich. ATP, 2-chloroadenosine, Arg-vasopressin (grade V), phenylephrine, propranolol and ibuprofen were from Sigma, 5′-(*N*-Ethyl)carboxamidoadenosine (NECA) was from Boehringer Mannheim, and 5-iodotubercidin was from Research Biochemicals Inc. (Natick, MA, U.S.A.). Acetylsalicylic acid was of standard pharmaceutical grade.

Handling of animals and liver perfusion

Animal care, and liver preparation and perfusion have been previously described [1]. The animals were well-fed male Wistar-derived Pfd rats (about 250 g) and 4-month-old *gsd/gsd* rats. Surgery was started at 09:00 h. The basic perfusion medium consisted of a balanced salt solution gassed with O_2/CO_2 (19:1).

Abbreviations used: FID, free induction decay; NECA, 5′-(*N*-ethyl)carboxamidoadenosine.

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During the first 0.5 h of the experiment, the organ was perfused under constant pressure (flow approx. 5 ml/min per g) in recirculating mode with the perfusate supplemented with 15 mM-glucose, 2.7 mM-lactate, 0.3 mM-pyruvate and 40 μM-glycerol. During the subsequent experimental phase, the liver was perfused at a constant flow rate of 50 ml/min in single-pass mode with nutrient-free buffer containing 100 μM-dibutyryl cyclic AMP. Changes in transhepatic hydrostatic pressure were read from an open-loop manometer inserted in the inflow tubing. O_2 extraction was monitored with a Clark electrode inserted in the caval outflow path. Hepatic production of hexose equivalents was estimated from the appearance of glucose [26], lactate [27] and pyruvate [28] in the perfusate. At the end of the experiments (usually about 15 min after administration of test compounds) the livers were freeze-clamped for the determination of the degree of activation of glycogen phosphorylase [29], and of residual glycogen on alkaline extracts [30].

^{31}P-n.m.r. spectroscopy

Spectra were obtained in a 4.7 T, 30 cm-wide bore magnet, equipped with a Biospec spectrometer (Bruker, Spectrospin). ^{31}P-n.m.r. spectra were acquired at 81.1 MHz. Per spectrum (1.5 min), we accumulated 128 scans, using 60 μs (72°) pulses with a 0.69 s repetition time. The latter conditions essentially saturate P_i from extracellular origin [1,31].

The FIDs (free induction decays) consisted of 2048 data points; the spectral width was 5200 Hz. The accumulated FIDs were processed by convolution difference [32] to remove the broad hump typical of liver spectra. The FIDs were multiplied by a damped exponential, corresponding to 700 Hz line broadening, and the result was subtracted from the original FID. Then, in order to improve the spectral resolution, Lorentz–Gauss filtering was applied to the FIDs [33]. The FID was multiplied by a positive exponential corresponding to a line narrowing of 50–70 Hz, and then by a Gaussian function corresponding to a line broadening of 30–50 Hz.

Relative changes in P_i and ATP levels were estimated from changes in the height of the spectral peaks of P_i and of the β-phosphate of nucleoside triphosphates (NTPs) respectively. Peak linewidths remained unchanged throughout the experiments. The intracellular pH was calculated from the spectral shift between the P_i and NTP α-phosphate signals, using reported chemical shift reference values and dissociation constants [34].

Statistics

Data are presented as means ± S.E.M., with the numbers of observations in parentheses. Vertical bars in the Figures represent S.E.M.

RESULTS AND DISCUSSION

Biphasic modulation of maximal glycogenolysis by adenosine

Basic characteristics of the perfusion system. Continuous exposure of the liver to 100 μM-dibutyryl cyclic AMP causes full activation of phosphorylase within 10 min [1]. The glycogenolytic rate at that moment was 6.07 ± 0.23 μmol of hexose equivalents released/min per g of liver ($n = 38$). We assume that full activation of phosphorylase persisted throughout the experiments, since the degree of activation was 89 ± 1.3 % ($n = 38$) at the time of removal of the livers from the magnet. It was also ascertained that glycogen stores sufficed to sustain maximal glycogenolysis, since the residual glycogen concentration at the end of the experiments was 35 ± 3 mg/g of liver ($n = 35$).

Time- and concentration-dependent effects of adenosine and ATP. When added 15 min after exposure to dibutyryl cyclic AMP, a high concentration (150 μM) of adenosine, but not of ATP, elicited an immediate transient burst of glycogenolysis (Fig. 1). Lower concentrations of adenosine (20–80 μM) had little or no effect (Table 1). The glycogenolytic burst seemed to be superimposed on a slower, but progressive, inhibition of glycogenolysis, which was observed with both adenosine and ATP (Fig. 1). The inhibition occurred notwithstanding conservation of phosphorylase *a* levels. The drop in glycogenolytic efficacy may be related to a drop in cytosolic P_i (Fig. 1). For adenosine, but not for ATP, the drop in P_i may be due in part to trapping in steadily accumulating ATP.

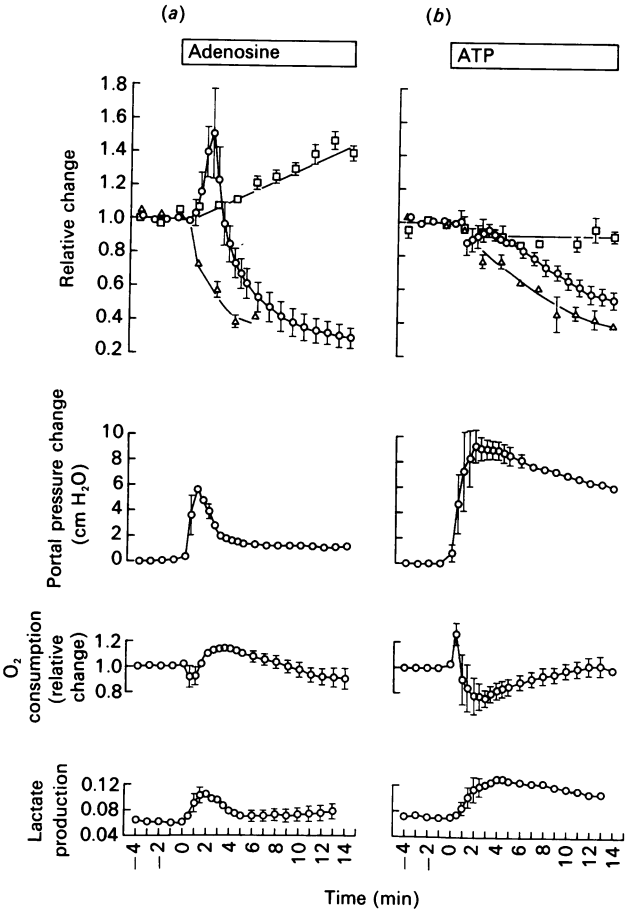

Fig. 1. Time course of hepatic effects induced by adenosine and ATP

Livers were continuously perfused with 100 μM-dibutyryl cyclic AMP. After 15 min (time zero), 150 μM-adenosine ($n = 5$; *a*) or ATP ($n = 3$; *b*) was added. In the top panels, the rate of output of hexose equivalents (○) and the n.m.r. signal amplitudes of P_i (△) and of ATP (□) are expressed relative to values measured in the same experiments over the last 5 min before zero time (= 1). The evolution of cytosolic P_i has been omitted when the n.m.r. signal could no longer be discerned from noise in all experiments. The middle panels show absolute changes in portal pressure and relative changes in O_2 extraction. The bottom panels show lactate production as a fraction of total hexose equivalents released from the liver.

We will further refer to the early burst of glycogenolysis as a 'phase 1' effect, and to the ensuing inhibition of glycogenolysis as a 'phase 2' effect.

Phase 1: receptor-mediated transient stimulation of glycogenolysis

P_1-receptor characteristics. Addition of 20 μM-8-phenyltheophylline, a P_1-receptor antagonist [2], inhibited the early stimulatory effect of adenosine (Fig. 2). In keeping with P_1-

Table 1. Hepatic effects of extracellular nucleosides and nucleotides

Experiments were performed as described in the legends to Figs. 1–3. Control rates of glycogenolysis and O_2 consumption refer to mean activities measured over the last 5 min of cyclic AMP pre-treatment. Responses in glycogenolysis and O_2 consumption are expressed relative to control values. The captions 'peak' and 'dip' values refer to local maxima and minima, as also illustrated in Fig. 1; 'absent' means that no peak or dip occurred. The caption 'phase 2' refers to the mean activity measured between 10 and 15 min after administration of the test substances. Changes in portal pressure are expressed as absolute increments with respect to the basal portal pressure during the control period. Lactate production is expressed as a fraction of hexose equivalents released from the liver. Fractional lactate production under control conditions was 5.9±0.2% (n = 38). According to the number of experiments reported, ranges of individual values ($n \leqslant 2$) or means±S.E.M. ($n > 2$) are given.

			Glycogenolysis				O_2 consumption			Lactate production
Compounds	Concn. (μM)	(n)	Control (μmol/min per g)	Peak or dip value (% of control)	Phase 2 (% of control)	Pressure increment maximum (cmH_2O)	Control (μmol/min per g)	Dip value (% of control)	Peak value (% of control)	peak value (% of hexose release)
Wistar rats										
Adenosine	20	3	6.03±0.05	Absent	65±3	1.3±0.6	2.64±0.22	Absent	118±5	Absent
	80	3	6.95±0.64	107±5	40±3	0.9±0.2	2.45±0.12	Absent	126±2	7.3±0.3
	150	5	6.23±0.58	158±24	33±6	6.6±0.8	2.58±0.32	79±10	1'5±3	11.4±0.9
ATP	150	5	5.69±0.72	83±3	60±8	8.4±1.4	1.86±0.28	78±7	1: ?±6	12.5±0.5
2-Chloroadenosine	150	3	7.02±0.66	147±31	84±1	3.9±0.3	2.90±0.23	78±11	112±6	10.4±0.4
NECA	100	2	4.63–8.77	127–197	83–116	7.4–13.0	1.98–2.70	48–86	105–111	8.6–13.3
Adenosine	150									
+Phenyltheophylline	20	2	6.25–6.67	104–106	28–39	0.5–0.8	2.67–2.68	Absent	120–124	7.3–13.3
+Ibuprofen	20	2	4.66–7.15	Absent	17–37	0.7–1.3	1.90–2.37	Absent	110–115	12.2–18.2
+Indomethacin	50	2	4.03–5.50	Absent	19–25	0.8–1.5	2.00–3.07	Absent	110–125	9.3–14.1
+Aspirin	2000	2	3.25–6.35	Absent	18–23	0.3–0.5	2.05–2.12	Absent	133–141	7.5–8.2
+I-tubercidin	10	2	4.28–4.98	128–130	73–103	1.4–3.6	1.96–2.20	85–98	110–124	8.4–10.2
Phenylephrine	10									
+Propranolol	100	2	5.55–8.09	Absent	79–86	3.1–3.9	2.57–2.85	Absent	110–114	4.3–7.9
Vasopressin	0.01	2	5.14–7.99	Absent	83–86	0.9–1.0	1.93–2.29	Absent	103–117	8.3–8.4
gsd/gsd rats										
Adenosine	150	1	0.69	Absent	44	2.3	1.14	95	133	–*
NECA	150	1	0.65	Absent	112	12.0	1.27	83	108	–*

* Lactate output too low.

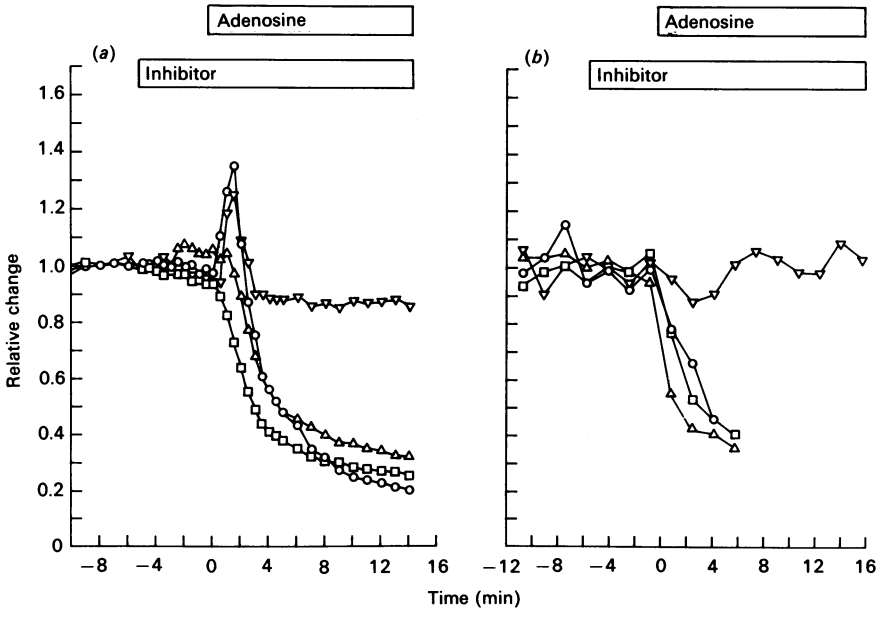

Fig. 2. Effects of inhibitors of the P_1-receptor, of cyclo-oxygenase and of adenosine kinase

Livers were continuously perfused with 100 μM-dibutyryl cyclic AMP. The tested inhibitors were added 15 min after the onset of the cyclic AMP treatment, and 5 min before challenge with 150 μM-adenosine (at time zero). The time course of glycogenolysis (*a*) and of P_1 (*b*) are shown for the P_1-receptor antagonist 8-phenyltheophylline (△, 20 μM), the cyclo-oxygenase inhibitor ibuprofen (20 μM, □), the adenosine kinase inhibitor 5-iodotubercidin (10 μM, ▽) and for dimethyl sulphoxide (3.75 mM, control, ○), the vehicle for the poorly water-soluble compounds. Each result is the mean of two experiments.

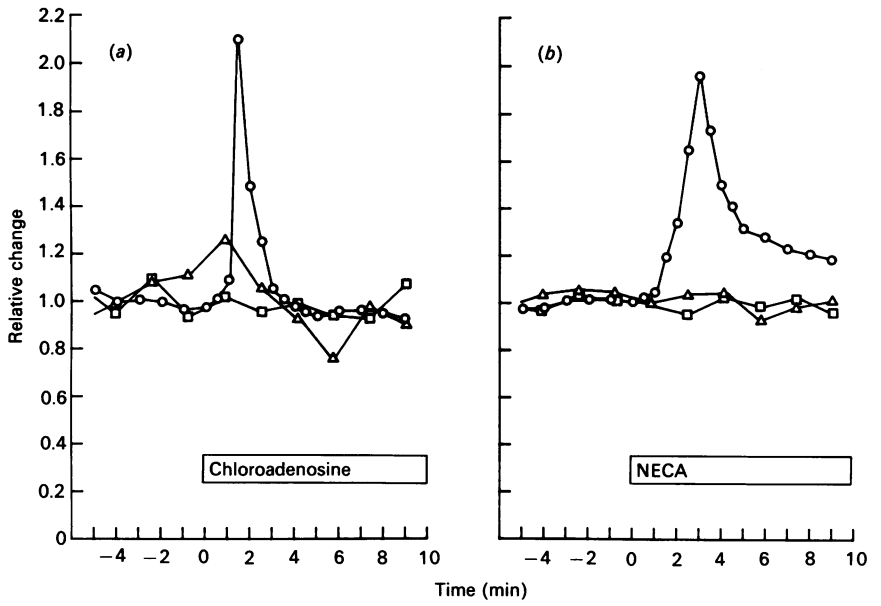

Fig. 3. Effects of non-metabolized adenosine analogues

Livers were continuously perfused with 100 μM-dibutyryl cyclic AMP. After 15 min (time zero), 150 μM-2-chloroadenosine (*a*) or NECA (*b*) was added. The rate of hepatic output of hexose equivalents (○) and the n.m.r. signal amplitudes of P_i (△) and of ATP (□) are expressed as in Fig. 1. Typical experiments are shown.

receptor involvement, the adenosine analogues 2-chloroadenosine and NECA elicited a pronounced glycogenolytic burst (Fig. 3 and Table 1), but ATP at comparable concentrations remained ineffective (Fig. 1).

Hepatocellular P_1-receptors are believed to exert their effect through cyclic-AMP-dependent activation of phosphorylase [13–15]. However, the continuous exposure of the liver to dibutyryl cyclic AMP would seem to pre-empt any mechanism depending on the stimulation of cyclic-AMP-dependent protein kinase. A Ca^{2+}-mediated mechanism at the hepatocellular level seems to be excluded also, since neither vasopressin nor an α-agonist modulated the maximal glycogenolysis (Table 1).

Eicosanoid involvement. In the intact organ, adenosine may activate glycogenolysis in part through eicosanoids, generated by non-parenchymal cells [16,17]. Perfusion with any one of the cyclo-oxygenase inhibitors ibuprofen, indomethacin or acetylsalicylic acid prevented the glycogenolytic burst (Fig. 2 and Table 1). Therefore, transient stimulation of maximal glycogenolysis by adenosine is apparently eicosanoid-mediated.

Dependence on phosphorylase *a*. Whereas the glycogenolytic burst is not due to additional conversion of phosphorylase *b* into *a* (see above), it required the presence of active phosphorylase *a*. Indeed, as shown in Table 1, 150 μM-adenosine or -NECA was unable to stimulate the very low glucose release from phosphorylase-kinase-deficient livers of *gsd/gsd* rats, where only 6–10% of phosphorylase was present in the *a* form. Transient pressure responses were preserved (Table 1).

Our results indicate that actual induction of hepatic glycogenolysis by adenosine is a two-component process: (i) hepatocellular P_1-receptor-mediated cyclic-AMP-dependent activation of phosphorylase would be permissive with respect to (ii) a transient stimulation of the catalytic efficiency of phosphorylase *a* in the intact organ. The latter requires high concentrations of adenosine, is eicosanoid-dependent and is very transient in nature, as is the full-sized glycogenolytic effect of adenosine in perfused liver [16]. The proposed scenario can explain why adenosine-induced hepatic glycogenolysis is substantially suppressed by cyclo-oxygenase inhibitors [17].

A role for hypoxia? Portal vasoconstriction can result in redistribution of the intrahepatic microcirculation [35,36], and in turn in regional hypoxia and in stimulation of glycogenolysis. A likely link between hypoxia and stimulation of glycogenolysis would be an increase in cytosolic P_i [1].

The adenosine-induced glycogenolytic burst indeed coincided with a transient vasoconstriction and diminished O_2 consumption (Fig. 1 and Table 1). Loss of the vasoconstrictive response, be it by lowering the adenosine concentration, by P_1-receptor blockade or by cyclo-oxygenase inhibition, was correlated with loss of the glycogenolytic response. Preferential release of lactate reflected faithfully the vasoconstrictive response of adenosine (Fig. 1). The increase in lactate coincided with a sharp and persistent drop in pyruvate, which became quasi-undetectable (results not shown). A persistently increased lactate/pyruvate ratio might be secondary to the P_i-dependence of respiration [37].

Whereas the observations described above point to the possibility that a transient hypoxic spell could mediate the adenosine-induced glycogenolytic burst, other data do not support this hypothesis. The increase in the proportion of hexose equivalents released as lactate after adenosine administration was not specifically linked to vasoconstriction, as this increase was also observed after abolition of the latter by treatment with cyclo-oxygenase inhibitors (Table 1). Furthermore, ATP did not stimulate glycogenolysis, although it provoked a more pronounced and sustained vasoconstriction and decrease in O_2 consumption than did adenosine. Finally, our n.m.r. spectra revealed an immediate and precipitous decline in P_i after adenosine administration. No signs of peak-broadening or of the appearance of a hypoxic acidifying compartment were observed. The cytosolic pH before adenosine administration was 7.32 ± 0.02 ($n = 18$), and it did not alter significantly afterwards.

It is unlikely that the stimulation of glycogenolysis by adenosine was due to accumulation of AMP, be it as a result of hypoxia or of phosphorylation of adenosine. AMP is expected to have only a very moderate effect on the catalytic efficiency of phosphorylase *a* [38]. Secondly, stimulation of glycogenolysis was also observed in the presence of the adenosine kinase

inhibitor 5-iodotubercidin and with non-metabolized adenosine analogues (Figs. 2 and 3 and Table 1).

Phase 2: progressive inhibition of glycogenolysis

The gradual drop in P_i explains the inhibition of glycogenolysis. After the phase 1 effect had dissipated, inhibition of glycogenolysis became apparent. The extent and rate of inhibition differed depending on whether it was induced by adenosine or ATP, and was correlated with the evolution of cytosolic P_i (Fig. 1). The adenosine-induced inhibition of glycogenolysis was dose-dependent, but required lower concentrations of adenosine than did the glycogenolytic burst (Table 1). The decrease in P_i suffices to explain the inhibition of glycogenolysis, in view of the known P_i-dependence of the catalytic efficiency of phosphorylase *a* [1]. The decline in P_i and the proportional inhibition of maximal glycogenolysis can explain the observed negative co-operativity between the glycogenolytic effects of adenosine or ATP, and of other glycogenolytic agents [22,25]. A persistent drop in P_i might also contribute to homologous desensitization to adenosine and ATP [15,16].

Dependence on adenosine metabolism. The progressive inhibition of glycogenolysis is not receptor-mediated, but is of metabolic origin. The phenomenon is observed with both adenosine and ATP, and is not affected by 8-phenyltheophylline or cyclo-oxygenase inhibitors, which obliterate the adenosine-induced glycogenolytic burst (Fig. 2 and Table 1). One can postulate that adenosine might exert metabolic effects secondary to the accumulation of phosphorylated nucleotides [39] and/or trapping of cytosolic P_i [24].

Conversion of adenosine to ATP, and concurrent trapping of P_i, can be prevented by blockade of adenosine phosphorylation or precluded by the use of non-metabolized adenosine analogues. When adenosine was tested in livers treated with 10 μM-5-iodotubercidin, an adenosine kinase inhibitor [40], the changes in ATP and in cytosolic P_i were absent, and accordingly the drop in glycogenolysis was not observed (Fig. 2). However, the early transient glycogenolytic burst was preserved, in keeping with its postulated P_1-receptor-mediated origin. The kinase inhibitor, itself an adenosine analogue, was devoid of obvious direct P_1-type effects at the tested concentration. The same results were obtained with non-metabolized adenosine analogues (Fig. 3). It has been shown that 2-chloroadenosine (100 μM) increases progressively the NTP pool in isolated hepatocytes over a 1 h period, apparently by conversion to 2-chloro-ATP [41]. However, we could not detect such an effect within the time scale of our experiments (Fig. 3).

Thus the adenosine-induced changes in cytosolic P_i depend on metabolism of adenosine, via a pathway involving phosphorylation of the nucleoside. N.m.r. data showed that the adenosine-treated livers steadily accumulated ATP during the 15 min perfusion with adenosine (Fig. 1). However, a number of observations were inconsistent with the hypothesis that trapping in the form of ATP accounts completely for the drop in P_i. Indeed the adenosine-induced drop in P_i occurred more swiftly than the steady increase in ATP (Fig. 1*a*). The dissociation between reciprocal changes in P_i and ATP was particularly obvious with ATP, which induced a drop in P_i without any observable growth of the ATP signal (Fig. 1*b*).

Trapping of P_i in an intracellular compartment. To investigate the fate of cytosolic P_i, livers were perfused with adenosine in a P_i-free perfusion system; this allowed us to monitor P_i leakage from the liver into the perfusate (Fig. 4). As previously observed [1], perfusion with P_i-free medium resulted in a slow but steady loss of cytosolic P_i, and in a gradual decrease in phosphorylase *a*-catalysed glycogenolysis. Adenosine elicited a transient glycogenolytic burst, in spite of an abrupt decline of n.m.r.-observable cytosolic P_i. The latter was correlated with an overall decrease in P_i release into the perfusate, as expected for the concentration-gradient-driven transmembrane movement of P_i [42].

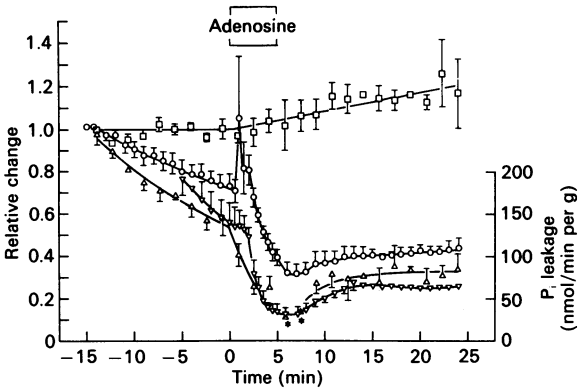

Fig. 4. Interrupted adenosine administration in a P_i-free perfusion system

Phosphorylase was activated by continuous perfusion with 100 μM-dibutyryl cyclic AMP. At −15 min, the perfusions ($n = 3$) continued with a P_i-free balanced salt solution, 15 min after onset of the dibutyryl cyclic AMP treatment, and 15 min before challenge with adenosine (150 μM), for 5 min. The evolution of glycogenolysis (○), the n.m.r. signal amplitudes for P_i (△) and ATP (□), as well as the rate of release of P_i into the perfusate (▽), are shown. At the indicated time points (*) the n.m.r. signal remained distinguishable from the noise in only one experiment.

A small transient shoulder in the P_i leakage curve at the onset of adenosine administration might reflect a transient increase in cytosolic P_i, e.g. secondary to local hypoxia. However, being far from proportional with the transient glycogenolytic burst, it falls short of explaining the latter.

The overall drop in cytosolic P_i did not stem from active P_i extrusion. Rather, P_i leakage passively followed cytosolic changes. After cessation of adenosine infusion, cytosolic P_i partially recovered. This P_i could not come from the P_i-free extracellular compartment, or from degradation of ATP, since the accumulation of ATP continued after withdrawal of extracellular adenosine, while the cytosolic P_i increased. Apparently, adenosine challenge or withdrawal triggers movement of P_i into or out of an intracellular, possibly metabolic, but n.m.r.-unobservable compartment. The existence of such a compartment has been postulated previously [1,43].

Conclusions

Induction of glycogenolysis by adenosine entails more than the conversion of phosphorylase *b* into phosphorylase *a*. Our results point to the operation of mechanisms for non-covalent control of phosphorylase *a* activity. Adenosine enhances transiently the catalytic efficiency of phosphorylase *a*, possibly through eicosanoid-mediated modulation of the concentration of a hitherto unrecognized stimulator or inhibitor. Subsequently, the activity of the enzyme drops, as a result of a decline in cytosolic P_i.

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