Biochem. J. (1986) 237, 773–780 (Printed in Great Britain)

Stimulation of glycogenolysis by adenine nucleotides in the perfused rat liver

Denis B. BUXTON,* Susan M. ROBERTSON and Merle S. OLSON†
Department of Biochemistry, University of Texas Health Science Center, San Antonio, TX 78284-7790, U.S.A.

Infusion of adenine nucleotides and adenosine into perfused rat livers resulted in stimulation of hepatic glycogenolysis, transient increases in the effluent perfusate [3-hydroxybutyrate]/[acetoacetate] ratio, and increased portal vein pressure. In livers perfused with buffer containing 50 μM-Ca^{2+}, transient efflux of Ca^{2+} was seen on stimulation of the liver with adenine nucleotides or adenosine. ADP was the most potent of the nucleotides, stimulating glucose output at concentrations as low as 0.15 μM, with half-maximal stimulation at approx. 1 μM, and ATP was slightly less potent, half-maximal stimulation requiring 4 μM-ATP. AMP and adenosine were much less effective, doses giving half-maximal stimulation being 40 and 20 μM respectively. Non-hydrolysed ATP analogues were much less effective than ATP in promoting changes in hepatic metabolism. ITP, GTP and GDP caused similar changes in hepatic metabolism to ATP, but were 10–20 times less potent than ATP. In livers perfused at low (7 μM) Ca^{2+}, infusion of phenylephrine before ATP desensitized hepatic responses to ATP. Repeated infusions of ATP in such low-Ca^{2+}-perfused livers caused homologous desensitization of ATP responses, and also desensitized subsequent Ca^{2+}-dependent responses to phenylephrine. A short infusion of Ca^{2+} (1.25 mM) after phenylephrine infusion restored subsequent responses to ATP, indicating that, during perfusion with buffer containing 7 μM-Ca^{2+}, ATP and phenylephrine deplete the same pool of intracellular Ca^{2+}, which can be rapidly replenished in the presence of extracellular Ca^{2+}. Measurement of cyclic AMP in freeze-clamped liver tissue demonstrated that adenosine (150 μM) significantly increased hepatic cyclic AMP, whereas ATP (15 μM) was without effect. It is concluded that ATP and ADP stimulate hepatic glycogenolysis via P_2-purinergic receptors, through a Ca^{2+}-dependent mechanism similar to that in α-adrenergic stimulation of hepatic tissue. However, adenosine stimulates glycogenolysis via P_1-purinoreceptors and/or uptake into the cell, at least partially through a mechanism involving increase in cyclic AMP. Further, the hepatic response to adenine nucleotides may be significant in regulating hepatic glucose output in physiological and pathophysiological states.

INTRODUCTION

Adenine nucleotides have been demonstrated to regulate many physiological processes by acting at extracellular purinergic receptors, and it has been proposed that ATP may act as a neurotransmitter in peripheral systems, after release from purinergic nerve terminals (Burnstock, 1978). Purinergic receptors have been divided into two classes, one preferring adenine nucleotides and designated P_2-purinergic receptors, whereas P_1 receptors are preferentially stimulated by adenosine (Burnstock, 1978; Daly, 1982).

Several laboratories have demonstrated effects of ATP on metabolism and ion fluxes in mammalian hepatic systems. ATP causes K^+ efflux from rabbit and guinea-pig hepatocytes (Burgess *et al.*, 1979, 1981; DeWitt & Putney, 1984), whereas rat hepatocytes accumulate K^+ in response to ATP (Burgess *et al.*, 1979, 1981). Glucose release (DeWitt & Putney, 1983) and decrease of $^{45}Ca^{2+}$ content of guinea-pig hepatocytes equilibrated with the radioisotope (Burgess *et al.*, 1981) were also observed in response to treatment with this nucleotide. Prpic *et al.* (1982) demonstrated Ca^{2+}-dependent phosphatidylinositol breakdown and activation of phosphorylase in rat hepatocytes in response to ATP and also reported a net uptake of Ca^{2+}. More recently, it has been shown that ATP stimulates formation of *myo*-inositol trisphosphate in rat hepatocytes (Charest *et al.*, 1985*a*) and causes transient elevation of cytosolic [Ca^{2+}], reduction of nicotinamide nucleotides, and activation of phosphorylase (Charest *et al.*, 1985*b*). Inositol trisphosphate production occurs in a variety of cells in response to a wide range of external stimuli, including the stimulation of hepatic glycogenolysis by Ca^{2+}-dependent hormones such as vasopressin, angiotensin and α-adrenergic agents; inositol trisphosphate is believed to act as a second messenger to mobilize intracellular Ca^{2+} (Berridge, 1984; Berridge & Irvine, 1984; Williamson *et al.*, 1985).

The aim of the present study was to characterize more completely the metabolic consequences of adenine nucleotide stimulation of the perfused rat liver, and to compare the nucleotide responses to those observed with α-adrenergic agonists.

EXPERIMENTAL

ATP, ADP, GTP, GDP, UTP and adenosine 5′-[α,β-methylene]triphosphate (sodium salts) were obtained from Pharmacia P-L Biochemicals, Piscataway, NJ,

* Present address: Division of Nuclear Medicine and Biophysics, Department of Radiological Sciences, UCLA Medical School, Los Angeles, CA 90024, U.S.A.
† To whom reprint requests should be addressed.

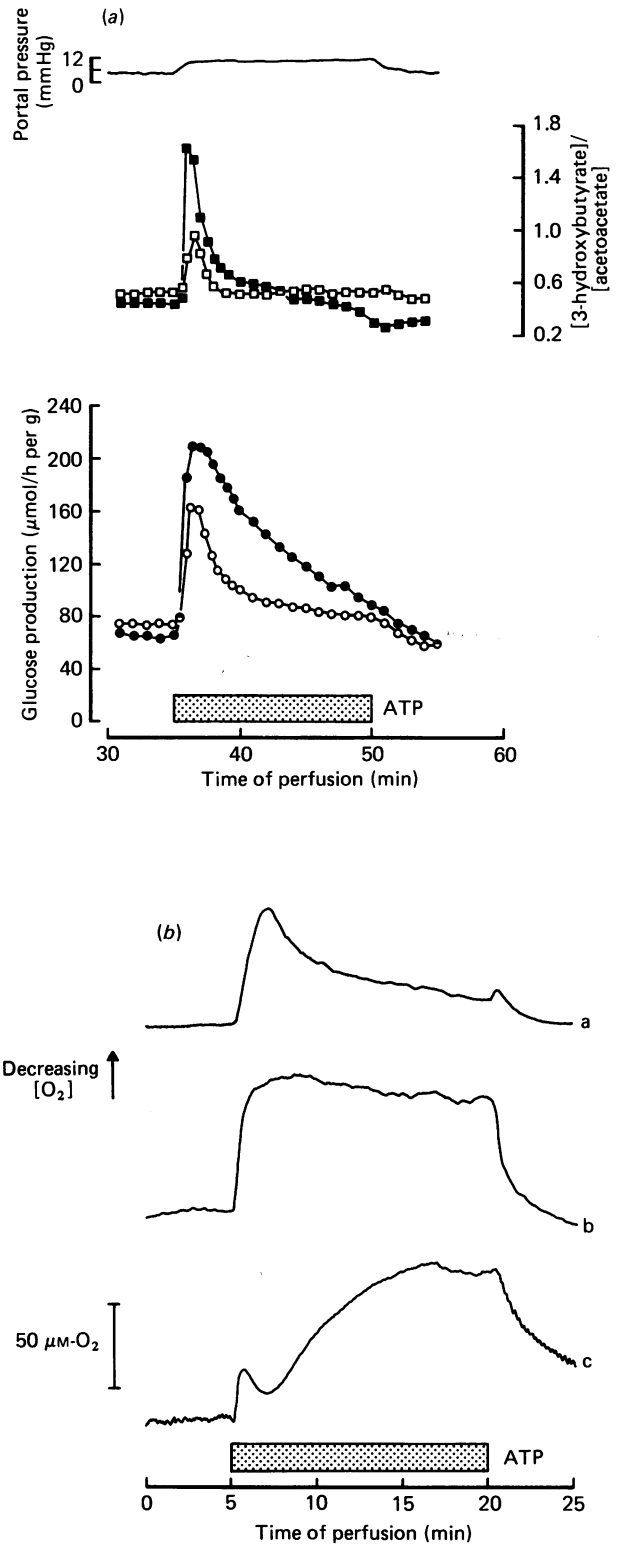

Fig. 1. Effect of ATP infusion on (*a*) hepatic glucose output, effluent perfusate [3-hydroxybutyrate]/[acetoacetate] ratio and portal-vein pressure, and (*b*) on hepatic O_2 consumption

Livers were perfused as described in the Experimental section. (*a*) 5 μM-ATP (○, □); 150 μM-ATP (●, ■); 4-methyl-2-oxopentanoate (1 mM) was infused throughout as a source of ketone bodies. (*b*) Trace a, 5 μM-ATP; trace b, 50 μM-ATP; trace c, 150 μM-ATP. In (*a*) and (*b*) traces shown are representative of three or four perfusions.

U.S.A. Adenosine 5′-[β,γ-imido]triphosphate and adenosine 5′-[β,γ-methylene]triphosphate (lithium salts) were purchased from Boehringer Mannhein Biochemicals, Indianapolis, IN, U.S.A. AMP (sodium salt) and adenosine were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. All other chemicals were of analytical grade and were obtained from standard commercial sources.

Livers from male Sprague–Dawley rats (Harlan, Houston, TX, U.S.A.) (160–200 g body wt., fed *ad libitum*) were perfused *in situ* with a non-recirculating constant-flow haemoglobin-free perfusion sysem (Scholz *et al.*, 1973), at a flow rate of 35 ml/min. The perfusion medium was Krebs–Henseleit (1932) bicarbonate buffer, pH 7.4, saturated with O_2/CO_2 (19:1), and maintained at 37 °C. The Ca^{2+} concentration was 1.25 mM, except where stated otherwise in the Figure legends. Hepatic O_2 consumption was monitored with a Clark-type oxygen electrode placed in the perfusion circuit immediately after the liver. Portal-vein pressure, an index of intrahepatic pressure (Greenway, 1983), was monitored with a Statham P 23 ID pressure transducer in conjunction with a Grass model 7 polygraph, connected to the portal cannula line. Effluent perfusate was collected for 30 s intervals for measurements of metabolites. Livers were perfused for 30 min before infusion of agonists to ensure removal of endogenous hormones and to stabilize glucose output. In most experiments, 4-methyl-2-oxopentanoate (0.5 mM) was infused to facilitate measurement of ketone bodies; infusion was initiated 5 min before the start of sample collection.

Glucose was measured by the method of Bergmeyer *et al.* (1974). The ketone bodies 3-hydroxybutyrate and acetoacetate were measured as described by Williamson & Corkey (1969) and Mellanby & Williamson (1974) respectively. Ca^{2+} was measured by atomic-absorption spectroscopy. For measurement of cyclic AMP, livers were freeze-clamped as rapidly as possible (approx. 2 s after removal from the rat) with aluminium tongs cooled in liquid N_2, and powdered with a porcelain pestle and mortar. The pestle and mortar were first cooled in a solid-CO_2/acetone bath, and kept on solid CO_2 during grinding. The powdered tissue was extracted as described by Michal & Wunderwald (1974) and assayed with a binding protein assay kit from Diagnostic Products, Los Angeles, CA, U.S.A.

Metabolite production rates are expressed per wet wt. of tissue. Results are presented as means ± S.E.M. Significance of differences was tested by Student's *t* test. Perfusion experiments illustrated in the various Figures are representative experiments which were performed at least three times with essentially identical results.

RESULTS

The effects of infusion of ATP into perfused rat livers from fed rats on hepatic metablism are shown in Fig. 1. ATP infusion caused a dose-dependent increase in glucose output from the liver, and led to a transient increase in the [3-hydroxybutyrate]/[acetoacetate] ratio in the effluent perfusate, indicating that the mitochondrial nicotinamide adenine dinucleotides were reduced transiently (Williamson *et al.*, 1967). A similar transient reduction of the mitochondrial oxidation–reduction state is also observed in response to α-adrenergic agonists and vasopressin (Sugano *et al.*, 1978*a*,*b*; Buxton

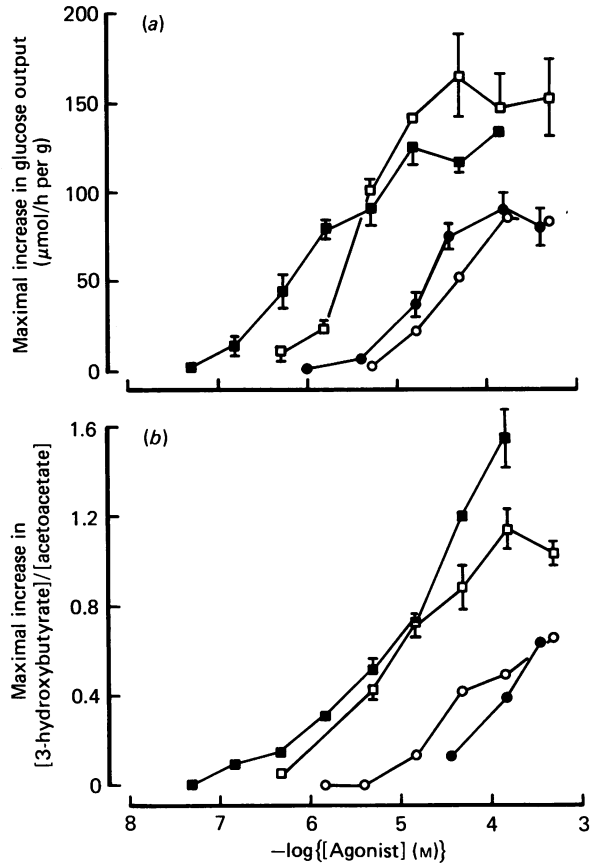

Fig. 2. Dose–response curves for hepatic responses to adenine nucleotides and adenosine

(*a*) Maximal stimulation of hepatic glucose output. (*b*) Maximal increase in [3-hydroxybutyrate]/[acetoacetate] ratio. □, ATP; ■, ADP, ○, AMP; ● adenosine.

et al., 1982; Balaban & Blum, 1982; Blackmore *et al.*, 1983; Reinhart *et al.*, 1984). Although in the experiments shown 4-methyl-2-oxopentanoate was infused as a ketone-body precursor to facilitate measurement of the [3-hydroxybutyrate]/[acetoacetate] ratio, similar changes were observed when endogenous ketone bodies alone were measured to monitor the mitochondrial redox state. Infusion of ATP also caused a stable elevation in the portal-vein pressure, which was reversed on removal of the nucleotide.

Fig. 1(*b*) illustrates the effect of infused ATP on hepatic O_2 consumption. At lower ATP concentrations (e.g. 5 μM) a transient increase in hepatic O_2 consumption was observed in response to the nucleotide. Increasing the ATP concentration to 50 μM resulted in a stable increase in O_2 consumption, which was reversed on removal of the nucleotide. A further increase in the ATP concentration to 150 μM or higher led to a biphasic increase in O_2 consumption.

In Fig. 2, the dose–response curves for the increases in hepatic glucose output and [3-hydroxybutyrate]/[acetoacetate] ratio in response to adenine nucleotides and to adenosine are shown. ATP stimulated glucose output over a concentration range of 0.5–500 μM-ATP, with half-maximal stimulation occurring at approx. 4 μM. ADP was more potent at lower concentrations, the lowest dose causing stimulated glucose output being 0.15 μM, but gave a shallower dose–response curve and slightly lower maximal stimulation (e.g. half-maximal stimulation was observed at about 1 μM). AMP and adenosine were much less effective in stimulating glucose output, with half-maximal stimulation occurring at approx. 40 and 20 μM respectively. Maximal stimulation of glucose output in response to these two agonists also was much lower than for ATP and ADP.

Fig. 3 demonstrates that the dose–response curves for maximal glucose output in response to ATP and ADP were essentially unchanged by reducing the perfusate Ca^{2+} concentration from 1.25 mM (Fig. 2) to 50 μM. The change in the mitochondrial oxidation–reduction state, as indicated by the [3-hydroxybutyrate]/[acetoacetate] ratio, was diminished markedly at a lower Ca^{2+} concentration, however, as has been observed for α-adrenergic agonists (Buxton *et al.*, 1982). Both glycogenolytic and oxidation–reduction-state responses to AMP and adenosine were impaired at a lower Ca^{2+} concentration (Fig. 3).

A transient efflux of Ca^{2+} from the liver was observed in response to adenine nucleotides. α-Adrenergic agonists, vasopressin and angiotensin have been shown by several laboratories to cause similar Ca^{2+} effluxes (Friedmann & Park, 1968; Blackmore *et al.*, 1978; Chen *et al.*, 1978; Jakob *et al.*, 1978). ADP was the most effective of the

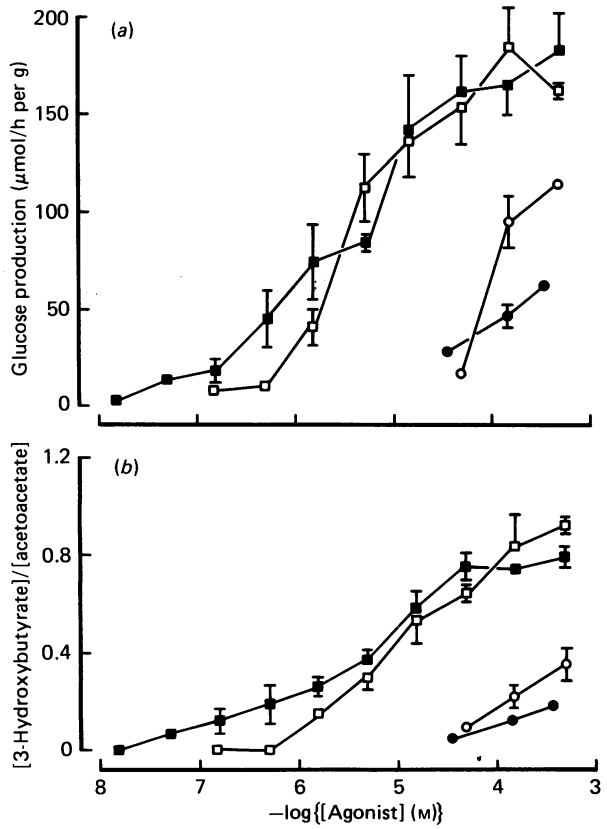

Fig. 3. Dose–response curves for hepatic responses to adenine nucleotides and adenosine at lower perfusate Ca^{2+} concentration (50 μM)

Each point represents mean ± S.E.M. for three to six livers, except where no error bar is shown, where the point is the mean for two livers. □, ATP; ■, ADP; ○, AMP; ●, adenosine.

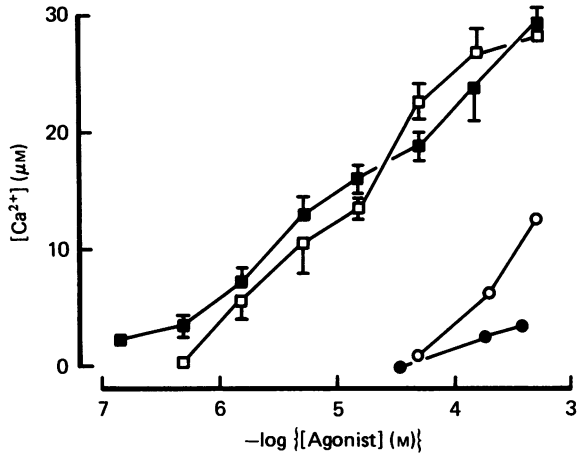

Fig. 4. Maximal hepatic Ca^{2+} output in response to adenine nucleotides and adenosine in livers perfused at low Ca^{2+} concentration (50 μM)

□, ATP; ■, ADP; ○, AMP; ●, adenosine. Values for ATP and ADP represent means ± S.E.M. for three to six livers; for AMP and adenosine, means for two livers are presented.

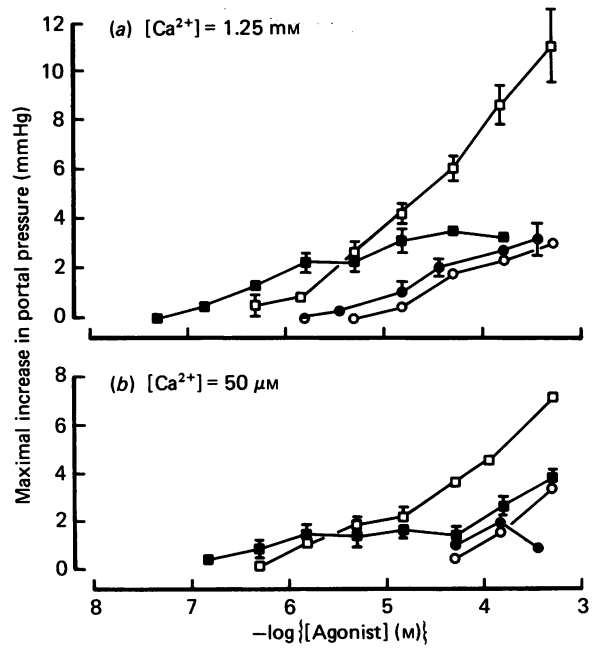

Fig. 5. Dose–response curves for the increase in portal-vein pressure during infusion of adenine nucleotides in livers perfused with (*a*) 1.25 mM-Ca^{2+} or (*b*) 50 μM-Ca^{2+}

Results are means for two to six livers; where shown, bars represent S.E.M. □, ATP; ■, ADP; ○, AMP; ●, adenosine.

adenine nucleotides in promoting Ca^{2+} efflux, with ATP being slightly less effective at low concentrations (Fig. 4). AMP was approximately two orders of magnitude less effective in promoting Ca^{2+} efflux, and adenosine was less potent yet. A similar order of potency was observed in isolated rat hepatocytes for the increase in cytosolic Ca^{2+} by adenine nucleotides and adenosine (Charest *et al.*, 1985*b*).

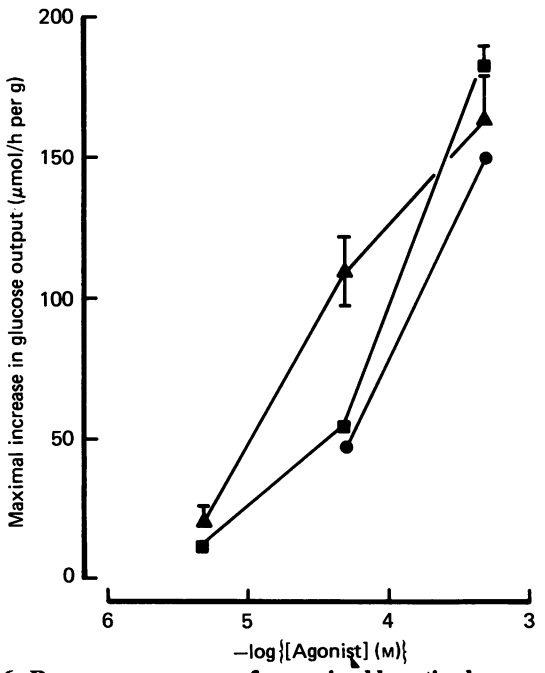

Fig. 6. Dose–response curves for maximal hepatic glucose output stimulation in response to GTP, GDP and ITP

Livers were perfused at 50 μM-Ca^{2+}. Results are means for two or three livers. ▲, ITP; ■, GTP, ●, GDP.

The dose–response curves for increases in hepatic portal pressure in response to adenine nucleotides and adenosine in livers perfused with normal (1.25 mM) or low (50 μM) Ca^{2+} are shown in Fig. 5. Again, ADP was the most potent nucleotide at low concentrations, whereas at higher concentrations (> 50 μM) ATP was much more effective. AMP and adenosine were much less potent. In all cases, the haemodynamic responses were impaired at the lower concentration of perfusate Ca^{2+}.

The specificity of the responses to adenine nucleotides was tested with GTP, GDP and ITP. These three nucleotides each stimulated glycogenolysis in a manner similar to the adenine nucleotides, with ITP being the most potent. However, compared with ATP and ADP, these nucleotides were 1–2 orders of magnitude less potent in stimulating glycogenolysis (Fig. 6). Similarly, changes in mitochondrial redox and Ca^{2+} efflux resembling those seen with ATP were observed with GTP, GDP and ITP, and again the order of potency was ITP > GTP > GDP, with ITP approximately an order of magnitude less potent than ATP (results not shown).

The ATP analogues adenosine 5′-[β,γ-methylene]-triphosphate, adenosine 5′-[α,β-methylene]triphosphate and adenosine 5′-[β,γ-imido]triphosphate were tested for their ability to promote metabolic changes in the perfused liver. At a concentration that promoted almost maximal stimulation of hepatic glycogenolysis when ATP or ADP were used as agonists (5 μM), the imido analogue gave only a small stimulation of hepatic glycogenolysis, Ca^{2+} efflux and mitochondrial redox change, similar to that observed with GTP or ITP at this concentration. The methylene analogues were even less effective, perturbing hepatic metabolism only slightly at this concentration (results not shown).

To investigate further whether the Ca^{2+} pool mobilized by adenine nucleotides was the same as that mobilized

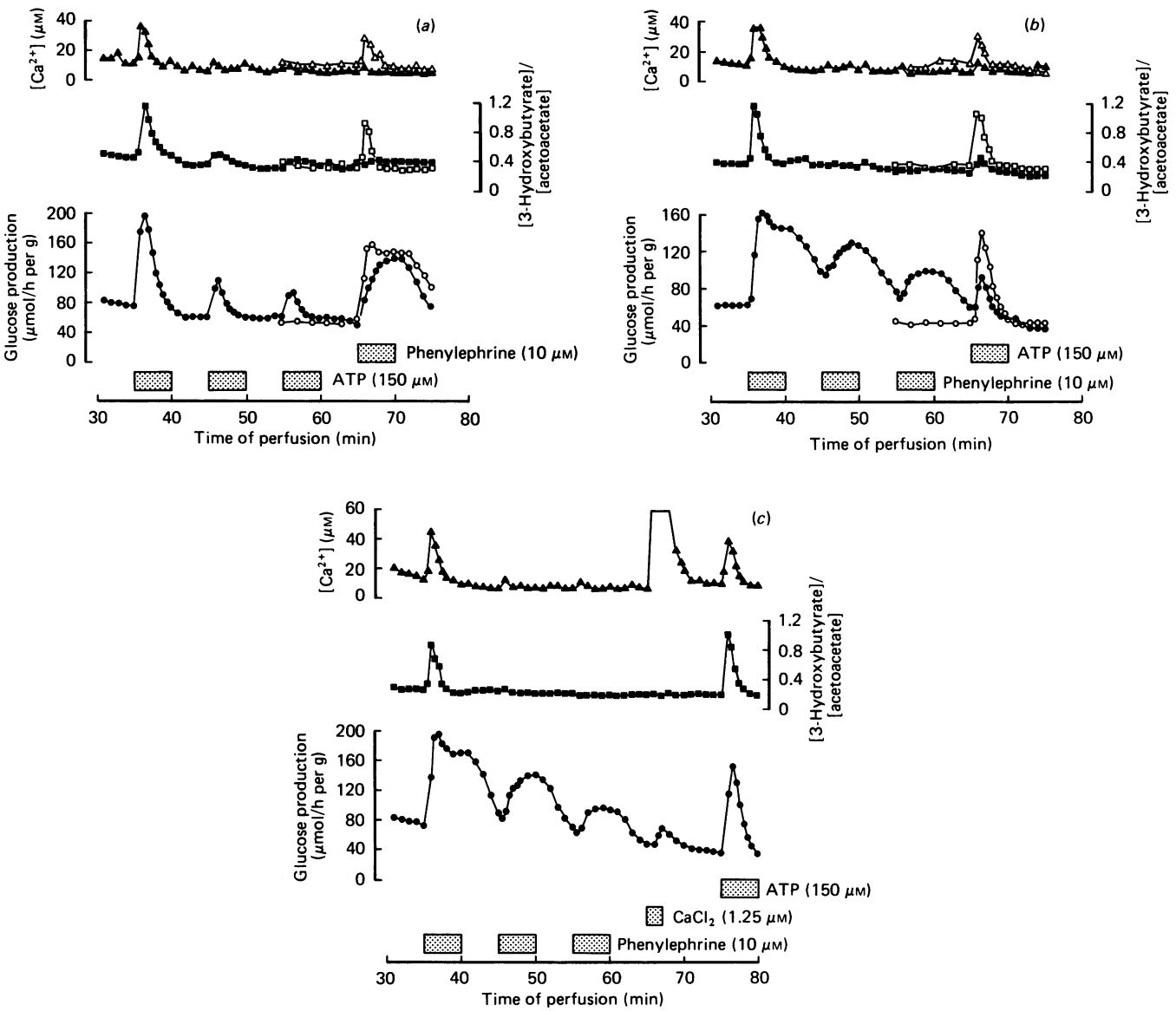

Fig. 7. Heterologous desensitization of hepatic responses to ATP and phenylephrine in livers perfused with buffer containing low (7 μM) Ca^{2+}

(*a*) Effect of short-interval infusions of ATP (150 μM) on subsequent responses to phenylephrine (10 μM). (*b*) Effect of prior infusions of phenylephrine on subsequent responses to ATP. (*c*) Restoration of hepatic responses to ATP after phenylephrine infusions by a short-interval infusion of Ca^{2+} (1.25 mM). Livers were perfused with buffer containing normal (1.25 mM) Ca^{2+} until 5 min before sample collection was initiated, at which time Ca^{2+} infusion was terminated, lowering the perfusate Ca^{2+} concentration to approx. 7 μM. In (*a*) and (*b*), the open symbols show results obtained from livers perfused for the same length of time with low-Ca^{2+} buffer, but without infusions of the desensitizing agent. Results are means obtained from three or four livers.

during α-adrenergic stimulation of the liver, the experiments shown in Fig.7 were performed. Removal of added perfusate Ca^{2+}, which lowered the perfusate Ca^{2+} concentration to approx. 7 μM as measured by atomic-absorption spectroscopy, at 10 min before infusion of ATP (150 μM) did not affect significantly the maximal glycogenolytic response to the nucleotide, compared with perfusions at 1.25 mM-Ca^{2+}; however, the return to the basal glucose output rate was greatly accelerated. Interestingly, the responses to subsequent infusions of ATP were greatly inhibited, and infusion of phenylephrine (10 μM) after the third infusion of ATP gave almost no increase in the mitochondrial redox or hepatic Ca^{2+} efflux. An increase in glucose output was observed in response to phenylephrine; this probably represents a cyclic AMP-dependent stimulation, which has been shown to occur via α-adenergic receptors in rats of approx. 200 g weight, particularly after Ca^{2+} depletion (Chan & Exton, 1977; Blair *et al.*, 1979; Morgan *et al.*, 1983). In control experiments, perfusion of livers with buffer containing 7 μM-Ca^{2+} for 35 min without addition of ATP inhibited hepatic responses to phenylephrine only slightly compared with addition of phenylephrine at a point 10 min after changing to the low-Ca^{2+} perfusion buffer.

The converse experiment, in which livers were treated

Table 1. Effect of adenosine and ATP on cyclic AMP contents in the perfused rat liver

Livers were perfused for 30 min before being freeze-clamped immediately or 90 s after infusion of adenosine (150 μM) or ATP (15 μM). Cyclic AMP was assayed in extracts of the frozen livers as described in the Experimental section.

Perfusion contents	Cyclic AMP content (nmol/g wet wt.)
No addition	0.46 ± 0.03
+ ATP (15 μM), 90 s	0.54 ± 0.04
+ Adenosine (150 μM), 90 s	1.61 ± 0.14*

* $P > 0.001$ versus no addition.

with phenylephrine in the presence of low (7 μM) Ca^{2+} before infusion of ATP, produced similar results; Ca^{2+} efflux and mitochondrial redox responses to phenylephrine were desensitized, as demonstrated by Reinhart *et al.* (1984) A slower, presumably cyclic AMP-dependent, stimulation of glycogenolysis was observed, but the Ca^{2+} efflux, redox and glycogenolytic responses to ATP were greatly inhibited under these conditions. Again, perfusion of livers with the low-Ca^{2+} buffer for 35 min in the absence of phenylephrine inhibited the subsequent response to ATP only modestly (Fig. 7*b*). A brief infusion of Ca^{2+} (1.25 mM), before infusion of ATP, was able to reverse the desensitization of hepatic responses to ATP caused by prior infusion of phenylephrine (Fig. 7*c*), in a manner analogous to the reversal of homologous desensitization of hepatic responses to phenylephrine by Ca^{2+} (Reinhart *et al.*, 1982, 1984).

Since adenosine has been demonstrated to increase cyclic AMP in isolated hepatocytes (Bartrons *et al.*, 1984), the effects of adenosine and ATP on hepatic cyclic AMP concentrations were investigated. Table 1 shows that, 90 s after infusion of adenosine (150 μM), cyclic AMP in extracts of freeze-clamped livers was increased significantly, whereas infusion of ATP (15 μM) had no significant effect on hepatic cyclic AMP concentrations.

DISCUSSION

Infusion of adenine nucleotides into perfused livers led to a dose-dependent stimulation of hepatic glycogenolysis. Concomitantly, enhanced O_2 consumption, a transient increase in the [3-hydroxybutyrate]/[acetoacetate] ratio and a stable increase in hepatic portal-vein pressure were observed. At lower Ca^{2+} concentrations (7 or 50 μM), transient efflux of Ca^{2+} from the liver was found in response to stimulation with ATP or ADP. Charest *et al.* (1985*b*) and Staddon & McGivan (1985) have demonstrated a transient increase in cytosolic Ca^{2+} in isolated hepatocytes in response to adenine nucleotides. Also, production of inositol trisphosphate, the putative intracellular second messenger believed to act via release of intracellular Ca^{2+} (Berridge, 1984; Berridge & Irvine, 1984; Williamson *et al.*, 1985), has been shown in response to ATP (Charest *et al.*, 1985*a*), suggesting involvement of intracellular Ca^{2+} in the responses to adenine nucleotides. This type of mechanism was supported by the present study; depletion of intracellular Ca^{2+} by repeated infusions of either phenylephrine or ATP during perfusion with medium containing 7 μM-Ca^{2+} inhibited hepatic responses to ATP, and a short infusion of Ca^{2+} (1.25 mM), allowing replenishment of intracellular stores, restored hepatic responsiveness to ATP. Prior infusions of ATP also were able to desensitize Ca^{2+}-dependent responses to phenylephrine, in experiments performed at 7 μM-Ca^{2+}. Thus it is apparent that adenine nucleotides and α-adrenergic agonists mobilize a similar pool of intracellular Ca^{2+}.

ATP and ADP were much more effective than adenosine and AMP in stimulating hepatic metabolism, both in the perfused liver in the present study and in the isolated hepatocyte (Charest *et al.*, 1985*b*), suggesting that adenine nucleotides act primarily through P_2-purinergic receptors (Burnstock, 1978; Daly, 1982) rather than through breakdown of the nucleotides by ecto-hydrolases (Pearson & Gordon, 1979; Krell *et al.*, 1983) and subsequent stimulation of the adenosine-preferring P_1-purinoreceptor. Since significant stimulation of hepatic glycogenolysis by adenosine occurs at concentrations of the nucleoside that do not mobilize hepatic Ca^{2+}, i.e. below 100 μM-adenosine, it is likely that a second mechanism exists, acting through P_1-purinergic receptors and/or uptake of adenosine. Studies using isolated rat liver plasma membranes have demonstrated stimulation of adenylate cyclase by adenosine (Londos *et al.*, 1980; Schutz *et al.*, 1982), and more recently transient increases in cyclic AMP and phosphorylase *a* activity have been demonstrated in hepatocytes stimulated with adenosine (Bartrons *et al.*, 1984), in contrast with a previous report in which no effect of adenosine on hepatic cyclic AMP concentration or glycogen phosphorylase activity was found (Fain & Shepherd, 1977). Measurement of cyclic AMP in freeze-clamped liver 90 s after infusion of adenosine (150 μM) or ATP (15 μM) showed a significant increase in cyclic AMP in response to adenosine (approx. 250%), whereas ATP did not alter cyclic AMP concentrations significantly. Also, it has been proposed that vasoconstriction in the perfused liver in response to adenosine, confirmed in the present study, may activate phosporylase through hypoxia and ischaemia (Ismail & Hems, 1978). The relative contributions of these two mechanisms to the glycogenolytic action of adenosine has yet to be determined. It would appear that vasoconstriction-induced hypoxia is not important in the P_2-purinergic receptor responses to ATP and ADP, however; at lower perfusate Ca^{2+} concentrations, the vasoconstrictive effect of these nucleotides was very much inhibited, but the maximal glycogenolytic response was unimpaired.

It is possible that ADP rather than ATP is the active species involved in binding to the P_2-purinergic receptor in liver, since ADP is more effective than ATP in provoking hepatic responses. Hepatocytes have been shown to possess an ecto-ATPase activity (Krell *et al.*, 1983) catalysing the sequential degradation of ATP to ADP and AMP. The slowly hydrolysed ATP analogues adenosine 5′-[α,β-methylene]triphosphate, adenosine 5′-[β,γ-methylene]triphosphate and adenosine 5′-[β,γ-imido]triphosphate were much less effective than ATP or ADP, which is consistent with ADP being the active species. It is noteworthy that the receptor involved does not display strict specificity for the adenine moiety of the nucleotide, since GTP, GDP and ITP were able to mimic

the adenine nucleotides, although 10–20 times less potently than ATP.

The results obtained in this study are difficult to reconcile with other studies demonstrating a rapid uptake of extracellular Ca^{2+} by hepatocytes in response to ATP (Krell *et al.*, 1983; Bellomo *et al.*, 1984). The nucleotide effects in the studies cited were also very specific for ATP, since GTP and adenosine 5'-[β,γ-methylene]triphosphate (Krell *et al.*, 1983) and ADP and AMP (Bellomo *et al.*, 1984) did not stimulate Ca^{2+} uptake. Extracellular Ca^{2+} appears to be necessary for maintaining the hepatic responses to ATP, which are most transient at lower Ca^{2+} concentrations, as has been demonstrated for α-adrenergic responses (Reinhart *et al.*, 1984). It is clear that mobilization of intracellular Ca^{2+}, leading to net Ca^{2+} efflux, is an initial event in the responses of the perfused liver to adenine nucleotides.

Release of ATP and/or ADP into the blood can occur through several mechanisms. ATP has been proposed as an autonomic neurotransmitter (Burnstock, 1979, 1981) and may be released from adrenergic nerves in the portal vein as co-transmitter (Burnstock *et al.*, 1984). Stimulation of human endothelial cells by thrombin causes the selective release of most of the ATP of the endothelial cell (Pearson & Gordon, 1979). Stimulation of platelets to secrete the contents of their dense granule releases both ATP and ADP; *in vitro*, collagen-stimulated human platelets in whole blood can secrete sufficient ATP to elevate the blood ATP concentration to 2 μM (Ingermann-Wojenski *et al.*, 1983). The responsiveness of the hepatic system to ADP is similar to that for human platelet activation and dense-granule secretion in whole blood, which occurs in response to 0.3–10 μM-ADP, with half-maximal responses at approx. 2 μM (Lumley & Humphrey, 1981).

In conclusion, the perfused liver responds to extracellular adenine nucleotides via two distinct mechanisms. The first is a P_2-purinergic receptor, linked to a Ca^{2+}-mobilizing mechanism similar to that activated by α-adrenergic stimulation of the liver. A second mechanism is preferentially stimulated by adenosine, and may involve both P_1-purinoreceptor stimulation and adenosine uptake (D. Buxton, unpublished work) and acts, at least partially, via increasing cyclic AMP. The P_2-receptor in particular responds to physiologically relevant concentrations of ADP and ATP, and may be important in stimulating energy supply to extra-hepatic tissues during altered physiological and patho-physiological states.

The expert technical assistance of Lynn Barron and Yvonne DeCento is gratefully acknowledged. This work was supported by grants from N.I.H. (AM-19473) and the Robert A. Welch Foundation (AQ-728).

REFERENCES

Balaban, R. S. & Blum, J. J. (1982) Am. J. Physiol. **242**, C182–C187

Bartrons, R., Van Schaftingen, E. & Hers, H.-G. (1984) Biochem. J. **218**, 157–163

Bellomo, G., Nicotera, P. & Orrenius, S. (1984) Eur. J. Biochem. **144**, 19–23

Bergmeyer, H. U., Bernt, E., Schmidt, F. & Stork, H. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), vol. 3, pp. 1144–1169, Academic Press, New York

Berridge, M. J. (1984) Biochem. J. **220**, 345–360

Berridge, M. J. & Irvine, R. F. (1984) Nature (London) **312**, 315–321

Blackmore, P. F., Brumley, F. T., Marks, J. L. & Exton, J. H. (1978) J. Biol. Chem. **253**, 4851–4858

Blackmore, P. F., Hughes, B. P., Charest, R., Shuman, E. A. & Exton, J. H. (1983) J. Biol. Chem. **258**, 10488–10494

Blair, J. B., James, M. E. & Foster, J. L. (1979) J. Biol. Chem. **254**, 7579–7584

Burgess, G. M., Claret, M. & Jenkinson, D. H. (1979) Nature (London) **279**, 544–546

Burgess, G. M., Claret, M. & Jenkinson, D. H. (1981) J. Physiol. (London) **317**, 67–90

Burnstock, G. (1978) in Cell Membrane Receptors for Drugs and Hormones: A Multi-Disciplinary Approach (Bolis, L. & Straub, R. W., eds.), pp. 107–118, Raven Press, New York

Burnstock, G. (1979) in Physiological and Regulatory Functions of Adenosine and Adenine Nucleotides (Baer, H. P. & Drummons, G. I., eds.), pp. 3–32, Raven Press, New York

Burnstock, G. (1981) J. Physiol. (London) **313**, 1–35

Burnstock, G., Crowe, R., Kennedy, C. & Torok, J. (1984) Br. J. Pharmacol. **82**, 359–368

Buxton, D. B., Barron, L. L. & Olson, M. S. (1982) J. Biol. Chem. **257**, 14318–14323

Chan, T. M. & Exton, J. H. (1977) J. Biol. Chem. **252**, 8645–8651

Charest, R., Prpic, V., Exton, J. H. & Blackmore, P. F. (1985*a*) Biochem. J. **227**, 79–90

Charest, R., Blackmore, P. F. & Exton, J. H. (1985*b*) Fed. Proc. Fed. Am. Soc. Exp. Biol. **44**, 1473

Chen, J.-L., Babcock, D. F. & Lardy, H. A. (1978) Proc. Natl. Acad. Sci. U.S.A. **75**, 2234–2238

Daly, J. W. (1982) J. Med. Chem. **25**, 197–207

DeWitt, L. M. & Putney, J. W., Jr. (1983) FEBS Lett. **160**, 259–263

DeWitt, L. M. & Putney, J. W., Jr. (1984) J. Physiol. (London) **346**, 395–407

Fain, J. N. & Shepherd, R. E. (1977) J. Biol. Chem. **252**, 8066–8070

Friedmann, N. & Park, C. R. (1968) Proc. Natl. Acad. Sci. U.S.A. **61**, 504–508

Greenway, C. V. (1983) Fed. Proc. Fed. Am. Soc. Exp. Biol. **42**, 1678–1684

Ingermann-Wojenski, C., Smith, J. B. & Silver, M. J. (1983) J. Clin. Lab. Med. **101**, 44–52

Ismail, N. A. & Hems, D. A. (1978) Biochem. Pharmacol. **27**, 1341–1345

Jakob, A., Salzmann, M. & Diem, S. (1978) Experientia **34**, 912

Krebs, H. A. & Henseleit, K. (1932) Hoppe-Seyler's Z. Physiol. Chem. **210**, 33–66

Krell, H., Ermish, N., Kasperek, S. & Pfaff, E. (1983) Eur. J. Biochem. **131**, 247–254

Londos, C., Cooper, D. M. F. & Wolff, J. (1980) Proc. Natl. Acad. Sci. U.S.A. **77**, 2551–2554

Lumley, P. & Humphrey, P. P. (1981) J. Pharmacol. Methods **6**, 153–166

Mellanby, J. & Williamson, D. J. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), vol. 4, pp. 1840–1843, Academic Press, New York

Michal, G. & Wunderwald, P. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.), vol. 4, pp. 2136–2144, Academic Press, New York

Morgan, N. G., Blackmore, P. F. & Exton, J. H. (1983) J. Biol. Chem. **258**, 5103–5109

Pearson, J. D. & Gordon, J. L. (1979) Nature (London) **281**, 384–386

Prpic, V., Blackmore, P. F. & Exton, J. H. (1982) J. Biol. Chem. **257**, 11323–11331

Reinhart, P. H., Taylor, W. M. & Bygrave, F. L. (1982) Biochem. J. **208**, 619–630

Reinhart, P. H., Taylor, W. M. & Bygrave, F. L. (1984) Biochem. J. **220**, 35–42

Scholz, R., Hanson, W. & Thurman, R. G. (1973) Eur. J. Biochem. **38**, 64–72
Schutz, W., Tuisl, E. & Kraupp, O. (1982) Naunyn-Schmiedeberg's Arch. Pharmacol. **319**, 34–39
Staddon, J. M. & McGivan, J. D. (1985) Biochem. Soc. Trans. **13**, 742–743
Sugano, T., Shiota, M., Tanaka, T., Miyamae, Y., Shimada, M. & Oshino, N. (1978*a*) J. Biochem. (Tokyo) **87**, 153–166
Sugano, T., Shiota, M., Khono, H., Shimada, M. & Oshino, N. (1978*b*) J. Biochem. (Tokyo) **87**, 465–472
Williamson, D. H., Lund, P. & Krebs, H. A. (1967) Biochem. J. **103**, 514–527
Williamson, J. R. & Corkey, B. E. (1969) Methods Enzymol. **13**, 434–513
Williamson, J. R. Cooper, R. H., Joseph, S. K. & Thomas, A. P. (1985) Am. J. Physiol. **248**, C203–C216

Received 9 December 1985/19 March 1986; accepted 8 April 1986