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EFFECTS OF ADENOSINE ON GLUCOSE AND LIPID METABOLISM AND HEPATIC BLOOD FLOW

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Abstract—The effect of adenosine on carbohydrate and lipid metabolism has been investigated in mice. Adenosine caused hyperglycaemia which developed gradually over 30 min. Adenosine also caused stimulation of fatty acid synthesis in adipose tissue, which could be a result of the hyperglycaemia, as well as of the insulin-like action of adenosine on adipose tissue. There was no significant effect on hepatic synthesis of fatty acids or cholesterol. Adenosine caused vasoconstriction in the perfused rat liver; this effect could cause glycogenolysis, through hypoxia. These results are discussed in the light of the hypothesis that adenosine could be a regulatory "messenger" in tissues.

Evidence has accumulated which suggests that adenosine plays a part in regulating metabolic processes. Thus adenosine has an important role in isolated fat cells, simulating the action of insulin on glucose and lipid metabolism, e.g. in exerting an antilipolytic effect to offset the action of high concentrations of lipolytic hormones [1–3]. Adenosine also has other effects which include inhibition of gluconeogenesis in isolated hepatocytes [4] and inhibition of insulin secretion by B-cells of the pancreas [5]. Studies *in vivo* show that adenosine can affect both carbohydrate and lipid metabolism in rats [6, 7]. Thus adenosine increases the incorporation of U-^{14}C glucose into liver glycogen and lipids of epididymal fat with an associated augmentation of the total liver glycogen turnover rate. Adenosine also produces a moderate hyperglycaemia in rats [6].

With the aim of expanding the above observations, and in view of the possible role of adenosine as a "messenger" in cells, we have studied the action of adenosine in mice. In particular, we have measured the rate of synthesis of fatty acids and cholesterol by the incorporation of [^{3}H] from ^3H_2O, which is the only simple method for measuring the total rate of conversion of acetyl-units to lipid in liver [8, 9]. The rate of incorporation of ^3H_2O into newly synthesized fatty acids of both liver and epididymal fat pads as well as liver cholesterol, has been followed. Also, the time course of the increase in blood glucose has been documented to permit assessment of the relations between this effect and the other metabolic actions of adenosine. Finally, effects of adenosine on glucose release have been tested in the perfused rat liver.

MATERIALS AND METHODS

Reagents. Adenosine, glucose oxidase, and adrenaline bitartrate were obtained from Sigma (London) Chemical Co. Ltd., (Kingston-upon-Thames, U.K.). Peroxidase was obtained from Boehringer Corp., (Uxbridge, U.K.). For liquid scintillation counting, 5-(4-diphenylyl)-2-(4-t-butylphenyl)-1-oxa-3,4-diazole was purchased from Ciba Limited (Basle, Switzerland). ^3H_2O was from the Radio-chemical Centre (Amersham, U.K.). Organic solvents were from BDH Chemicals Ltd. Other reagents were A.R. grade, or the purest commercially available.

Experimental procedure. Male mice, including genetically obese (*ob/ob*) animals, and rats aged about 10 weeks, were bred in the Department of Biochemistry at Imperial College. ^3H_2O (3 m Ci/mouse) and adenosine (0.05–0.2 mg/g mouse) suspended in saline were administered i.p. (simultaneously, in one injection). The same volume of saline was administered to the controls. After 1 or 2 hr heparinized blood samples were obtained rapidly by cardiac puncture within 12 sec of cervical dislocation, and either mixed with equal volume of 6% perchloric acid, or plasma was separated (after cooling to 0°) by centrifugation at 2000 r.p.m. for 15 min. Tissues (liver and epididymal fat pads) were rapidly frozen in liquid N_2 and weighed for lipid extraction.

Livers were perfused with 50 ml bicarbonate-buffered saline containing washed rat erythrocytes, and 2.5% (w/v) bovine serum albumin [10]. Perfused livers of fed rats contained 10 mM glucose, which remained at a steady concentration during perfusion, and perfused livers of 48-hr starved rats contained a mixture of glycerol, lactate and pyruvate, which was infused [10]. After about 1 hr perfusion, adenosine was added (in 0.1–2 ml. of aqueous solution); perfusate flow rate was followed by collecting measured aliquots of the effluent fluid.

Analytical methods. Glucose was estimated by a glucose oxidase method [11]. The lipids of both liver and epididymal fat pads were isolated by homogenization of the tissues in chloroform–methanol (2:1, v/v) as described by Folch *et al.* [12]. The homogenate was filtered under vacuum using a sintered glass funnel and collected in stoppered

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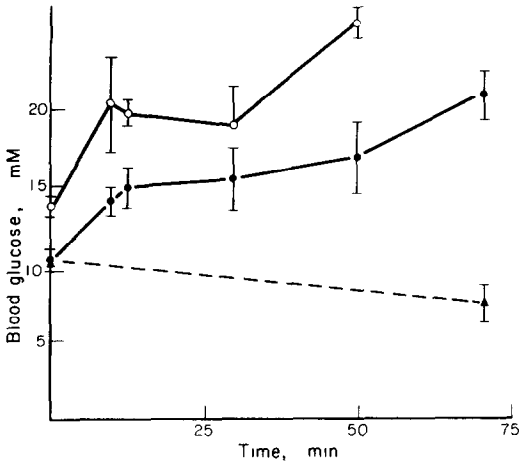

Fig. 1. Time course of increase in blood glucose concentration in response to adenosine

Mice received 0.20 mg/g of adenosine i.p. Results are means ± S.E.M. (bars) of three to five measurements, in lean (●) or genetically obese (*ob/ob*) mice (○). Control injections produced no significant change in blood glucose concentration, 10–75 min after injection (– – ▲ – –).

tubes. The fatty acids of liver and adipose tissue were isolated after saponification (in 5 N NaOH in 30% v/v ethanol for 3 hr at 90°). After removal of the non-saponifiable fraction (a step which was not necessary in case of adipose tissue as there was a negligible amount of radioactivity in this fraction), the alkaline residue was acidified with conc. HCl. The fatty acids released were then extracted with three sequential portions (10 ml.) of petroleum ether (40°–60°). The extract of the fatty acids was evaporated in scintillation vials under N_2 and the amount of [^{3}H] radioactivity in the samples was determined in a Packard Tri-Carb liquid-scintillation spectrometer in 10 ml fluid containing 6 g of 5-(4-diphenylyl)-2-(4-*t*-butyl phenyl)-1-oxa-3,4-diazole/l of toluene. Liver cholesterol was isolated from the non-saponifiable fraction as digitonide with 2 ml of 50% digitonin in 50% ethanol [13]. The digitonide precipitate was washed with 6 ml acetone–ether (1:2) followed by 6 ml of ether. After evaporation of the ether the residue was dissolved in 1 ml 2-methoxyethanol and the amount of [^{3}H] radioactivity in the samples was determined using the above scintillation fluid.

To determine the specific activity of ^3H_2O in plasma 20 μl of plasma were counted in 1 ml of 2-methoxy-ethanol plus 10 ml of the scintillation fluid. The d.p.m. were computed from the c.p.m. by a channels ratio method [14].

Rates of fatty acids and cholesterol synthesis (from all sources of acetyl units) were calculated from [^{3}H] contents of lipids by using the quotient ([^{3}H] in lipid, expreessed in d.p.m.)/(spec. radioactivity of plasma ^3H_2O, expressed as d.p.m. per g atom of H in total H_2O) [8, 9]. From this value, amounts of newly synthesized lipid, expressed in C_2-units, may be obtained for fatty acids by dividing by 1.7 and for cholesterol by dividing by 1.4 [15]. The quantitative aspects of measuring the total rate of *de novo* synthesis of fatty acids and of cholesterol by measuring the incorporation into lipids of [^{3}H] from ^3H_2O have been evaluated in liver and in adipose tissue [8, 9, 13, 15, 16].

RESULTS

Effects of adenosine on blood glucose. Adenosine at a dose of 0.20 mg/g caused an increase in the blood glucose concentration after 30 min, compared to the relatively minor change in mice which received a control injection (Fig. 1). The time course of this effect was investigated; it was found that the extent of the effect on the blood glucose concentration increased gradually during 10–70 min after the administration of adenosine (Fig. 1). Adenosine

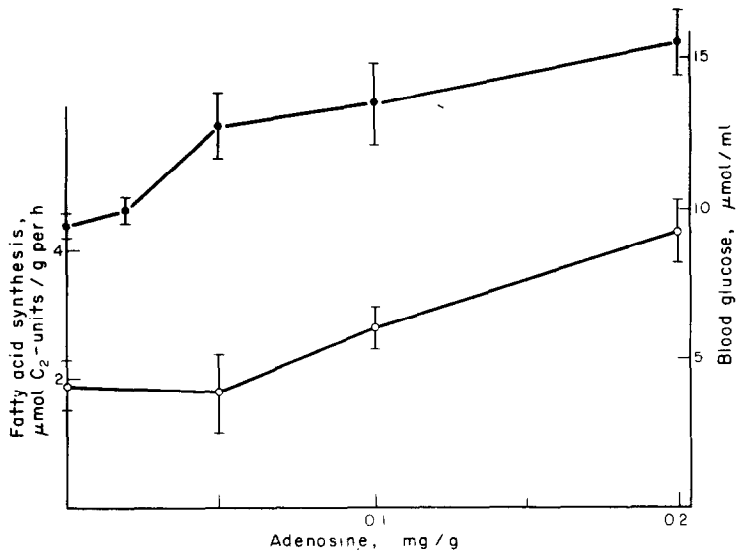

Fig. 2. Concentration-dependence of effects of adenosine

Mice received adenosine i.p. Blood glucose concentration (●) was measured after 30 min. Fatty acid synthesis in epidymal fat (○) was measured with ^3H_2O, over 30 min. Results are means ± S.E.M. (bars) of three measurements.

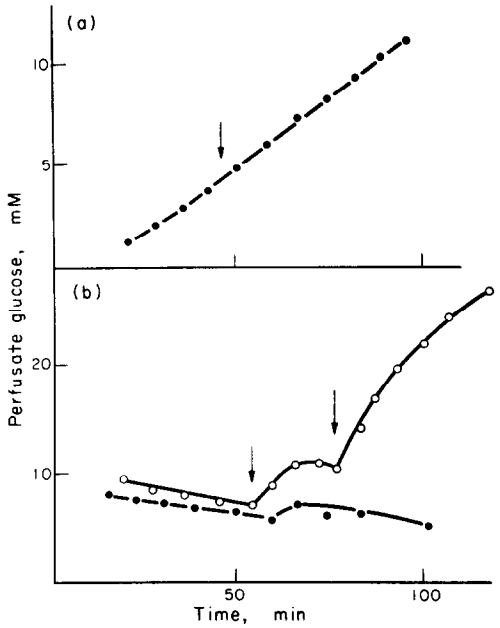

Fig. 3. Effect of adenosine on glucose release by perfused rat liver

Livers of either 48-hr starved (a) or well-fed rats (b) were perfused with 50 ml. Krebs–Ringer–bicarbonate saline containing albumin, rat erythrocytes and either (a) gluconeogenic substrates, or (b) glucose [10]. Adenosine (0.25 mg in (a), 1 mg in (b)) was added as indicated by the first arrow. Glucose concentration was measured in fluid perfusing the liver. Results in (a) are the averages from two perfusions, and in (b) are from two representative experiments: in one perfusion, adenosine was followed by 50 mU vasopressin (second arrow).

caused hyperglycaemia in genetically obese mice, of about the same order as that in lean mice (Fig. 1).

The interest of an effect such as that of adenosine in animals depends partly on its potency. Therefore the dose-dependence of the effect of adenosine on the blood glucose concentration (measured after 30 min) was studied. Adenosine at doses ranging from 0.05 to 0.2 mgm/g caused increasing elevation of blood glucose level (Fig. 2).

Since adenosine can offset hormonal responses, e.g. in adipose tissue in a manner analogous to insulin [1–3], the effect of adenosine on adrenaline-induced hyperglycaemia was tested. Adenosine at a dose of 0.05 mg/g did not alter the effect of adrenaline (0.01 mg/mouse) on blood sugar concentration (results not shown).

In the hope of bringing out effects of adenosine *in vitro*, glucose release was measured in perfused liver experiments. Adenosine produced a small and variable increase in glucose output in livers from fed rats (Fig. 3) probably as a result of vasoconstriction (Fig. 4). As vasopressin can exert a potent glycogenolytic effect in liver [10], the effect of adenosine on this response was tested in the perfused rat liver: there was no modulation of this response (Fig. 3). Also, there was no effect of adenosine on gluconeogenesis from lactate in perfused livers from starved rats (Fig. 3).

Effect of adenosine on blood flow rate in the liver. Adenosine caused a slowing of flow in the perfused liver, which lasted 1–5 min. The concentration-dependence of this constrictor effect is shown in Fig. 4.

Effects of adenosine on lipid metabolism. At a dose of 0.2 mg/g adenosine produced a mild but insignificant stimulation in the rate of incorporation of [^{3}H] into fatty acid synthesised in the mouse liver (Table 1). But adenosine produced a significant elevation of the rate of incorporation of [^{3}H] into newly synthesized fatty acids of the epididymal fat pads at doses of 0.1 and 0.2 mg/g (Table 1, Fig. 2). However, adenosine at various dose levels (0.1 and 0.2 mg/g) did not alter the rate of incorporation of ^3H_2O into liver cholesterol (Table 1). Thus the effect of adenosine on hepatic cholesterol synthesis from acetate *in vivo* [17] does not appear to be associated with any change in the total rate of synthesis (of which ^3H_2O provides a measure).

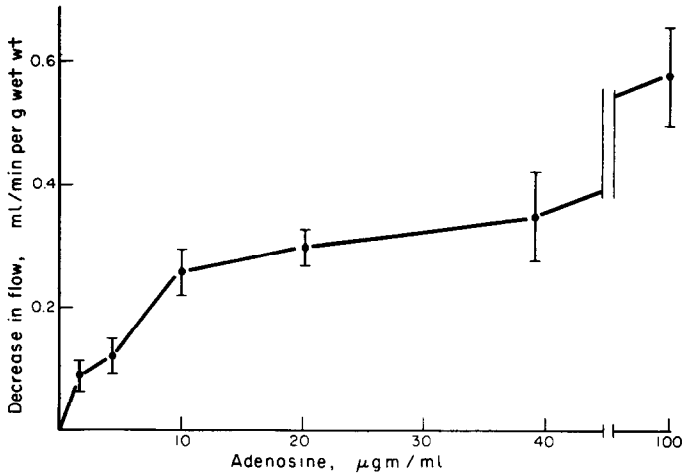

Fig. 4. Concentration-dependence of effect of adenosine on liver perfusate flow rate

Livers of fed rats were perfused as described in Fig. 3. Flow rate was measured by collecting dripping effluent fluid. Adenosine was added to perfusate and changes in flow rate followed. Values are from individual measurements, taken at the time of the peak drop in flow rate, which was 1–2 min after adenosine.

Table 1. Effect of adenosine on synthesis of lipids

Dose of adenosine (mg/g)	Duration of experiment (hr)	Synthesis of fatty acids (μmol C_2-units/g)		Synthesis of cholesterol (% of control value)
		Liver	Adipose tissue	
Control	1	18 ± 2 (4)	3.3 ± 0.7 (13)	100 (3)
100	1	24 ± 1 (4)	5.2 ± 0.5 (4)	90 ± 13 (3)
200	1	—	—	86 ± 48 (3)
Control	2	35 ± 4 (6)	1.6 ± 0.2 (6)*	—
200	2	49 ± 13 (4)	8.1 ± 2.9 (5)*	—

* $P < 0.05$.

Fed mice were injected with ^3H_2O between 10.00 and 12.00 hr; after 1 hr liver lipids or adipose tissue fatty acids were analyzed for [^{3}H]. Results are means ± S.E.M. of the number of observations in parenthesis.

DISCUSSION

Adenosine at doses ranging from 0.05–0.2 mg/g produced a clearcut elevation of the blood sugar level in mice. This finding confirms the moderate hyperglycaemia observed in rats when adenosine is injected at a dose of 0.2 mg/g i.p., and expands this observation in establishing the slow onset of this particular effect of adenosine [6]. The gradual increase in blood glucose concentration is more likely to be the cause of the increase in liver glycogen turnover rate after adenosine injection than to be a result of immediate hepatic glycogenolysis [7]. When injected agents such as glucagon and vasopressin act directly on liver to cause glycogenolysis *in vivo*, the onset of hyperglycaemia is much faster [18].

The relatively minor effect of adenosine on glucose output in the perfused liver of fed rats also suggests that adenosine is unlikely to cause prolonged hyperglycaemia *in vivo* through a direct stimulatory effect on glycogenolysis in hepatocytes.

Vasoconstriction was observed in the perfused liver, in response to adenosine, and this could lead to activation of glycogenolysis through ischaemia and hypoxia. Thus there was no effect on glucose release in livers from starved rats, which lack glycogen. Also, the concentration of adenosine which produced the moderate glucose release in perfused livers from fed rats was sufficient to cause significant vasoconstriction. Such an effect on blood glucose concentration, via vasoconstriction, might be more exaggerated in the intact rat than was revealed in our perfusion experiments, as the blood volume is smaller *in vivo*. However, the constrictor effect was short-lived (1–3 min) which would weigh against any major role in prolonged adenosine-induced hyperglycaemia. Adenosine is a vasodilator in muscle, so the constrictor action in liver implies that circulating adenosine would direct blood from the portal bed to muscle.

The question arises whether any other effects *in vivo* could contribute to the hyperglycaemic effect of adenosine. A stimulatory effect on gluconeogenesis cannot be countenanced, as adenosine inhibits this process in hepatocytes [4] and does not affect it in perfused liver (present work).

One contributory factor in the hyperglycaemic effect of adenosine could be inhibition of insulin secretion [5]. This provides a second explanation (in addition to hepatic vasoconstriction) for adenosine-induced hyperglycaemia, which is supported by experiments *in vitro*.

Suppression of lipolysis by adenosine in adipose tissue would not be expected to cause hyperglycaemia. However, the finding that adenosine at a very low concentration (0.1 μM) was a potent inhibitor of epinephrine-induced lipolysis and cyclic AMP accumulation in isolated rat fat cells [1–3, 19], prompted us to investigate the effect of adenosine on epinephrine-induced hyperglycaemia in mice. Adenosine did not alter the effect of epinephrine on blood sugar level. Neither did it prevent the stimulation of hepatic glycogenolysis by vasopressin. In other experiments (unpublished) we have found that adenosine does not inhibit or potentiate the stimulation by glucagon or adrenaline of glycogenolysis in the perfused liver of fed rats. Thus no interaction of adenosine with other hormones in the control of glucose metabolism has yet been elucidated, which could explain its hyperglycaemic effect.

Adenosine caused significant stimulation of fatty acid synthesis in adipose tissue, but not in liver. Hence the present work, in which total hepatic lipogenesis was measured by the most practicable method which is available, i.e. with ^3H_2O [8, 9, 13, 15,16], does not lend support to the conclusion drawn previously regarding the effect of adenosine on fatty acid synthesis in liver [6]. The small stimulation of lipogenesis in liver in the present work could be explained by the finding that adenosine produced an increase in total liver glycogen turnover [7], probably as a secondary consequence of the hyperglycaemia. Both glycogen and blood glucose can promote synthesis of fatty acid *de novo* in the liver [9].

The stimulation of adipose tissue lipogenesis by adenosine supports a previous finding that adenosine increased lipogenesis in rat epididymal fat pads *in vivo* from U-^{14}C glucose [6]. Our observation is also in agreement with the notion that adenosine mimics the action of insulin of glucose and lipid metabolism in adipose tissue [1–3]. The effect of adenosine on lipogenesis in adipose tissue could be due to the increase in blood glucose, which is of course a source of fatty acids in this tissue. Adeno-

sine did not affect the rate of incorporation of [^{3}H] from ^3H_2O into liver cholesterol which shows that the effects on this process demonstrated with ^{14}C-labelled precursors [17], do not reflect an increase in the total rate of hepatic cholesterogenesis, but merely adjustments in the relative role of individual blood and tissue precursors in this process.

Taken together, these experiments do not suggest that adenosine exerts major direct effects on metabolism in liver parenchymal cells. The only putative "messenger" effect yet documented *in vitro* is inhibition of gluconeogenesis [4], which could not be relevant to the hyperglycaemia *in vivo*. However, adenosine-induced hepatic vasoconstriction could contribute to the hyperglycaemic effect of adenosine *in vivo*, and this effect is likely also to involve inhibition of insulin secretion through direct action on the pancreatic B-cell [5].

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