Biochemical Pharmacology, Vol. 41, No. 1, pp. 146–148, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

Effects of the anti-AIDS drug dideoxyinosine on hepatic glycolysis in the perfused rat liver: role of intracellular calcium stores

(*Received* 6 *April* 1990; *accepted* 8 *August* 1990)

In the urgent search for a drug against acquired immunodeficiency syndrome (AIDS), several compounds have been shown to stop human immunodeficiency virus (HIV) replication *in vitro* [1]. 3'-Azido-2',3'-dideoxythymidine (AZT), a dideoxyinosine derivative, has been approved in a number of countries, including the United States, for use in patients with severe HIV infection [1]. Unfortunately, the effectiveness of AZT is limited by serious side-effects such as bone marrow and gastrointestinal toxicities [1]. Dideoxyinosine (ddI), another modified nucleoside, is now undergoing clinical trials [1]. Although information on potential effects of this compound on different organs including the liver is scarce, recent reports indicate that ddI causes hepatotoxic effects via unclear mechanisms [2, 3]. Guinzberg *et al.* [4] have reported that adenosine and inosine produce a dose-dependent stimulation of ureagenesis in isolated rat hepatocytes. Furthermore, nucleosides increase glucose production and stimulate hepatic oxygen uptake in the perfused rat liver [5]. Mechanisms responsible for these effects are not clear; however, intracellular calcium and the adenylate cyclase system have been implicated [5, 6]. Since the liver is the major site for the metabolism of nucleosides [7], and since we have found recently that ddI metabolites accumulate in hepatic tissues [8], the purpose of this study was to investigate the effects of ddI on glycolysis in the perfused rat liver, as a measure of its effects on hepatic carbohydrate metabolism.

Materials and Methods

Male Sprague–Dawley rats (200–250 g) were used in this study. Livers were removed surgically and were perfused with normal or calcium-free Krebs–Henseleit bicarbonate buffer (pH 7.4, 37°) saturated with an oxygen–carbon dioxide mixture (95:5) in a noncirculating system as described previously [9]. Briefly, the fluid was pumped into the liver via a cannula placed in the portal vein and the effluent flowed past a Teflon-shielded, Clark-type oxygen electrode for measurement of hepatic oxygen uptake in order to monitor tissue viability and potential microcirculatory effects caused by this nucleoside. Dideoxyinosine was dissolved in Krebs–Henseleit buffer and was infused into the liver, following 15 min of equilibration with buffer alone. In some experiments, 150 μM ATP was infused (three periods of 2 min each, within 15 min) in calcium-free perfusate prior to ddI, in order to deplete intracellular calcium stores [10]. Calcium chloride (1.3 mM) was infused where indicated to replenish intracellular calcium stores [10]. Concentrations of the metabolites in the effluent perfusate were determined by standard enzymatic procedures [11], and rates of production were determined from the effluent concentrations of metabolites, the flow rate and the liver wet weight.

Results and Discussion

The present study shows that therapeutic concentrations of the anti-AIDS drug dideoxyinosine stimulated hepatic glycolysis in the perfused rat liver. Infusion of ddI (10 μg/mL) increased rates of production of lactate plus pyruvate immediately and maximally by 25 ± 6 μmol/g/hr (Figs. 1 and 2), and caused a transient increase in hepatic oxygen uptake which returned to normal values within 4 min (not shown). At the same time, the lactate/pyruvate ratio in the effluent perfusate remained constant (Fig. 2), indicating that the ddI-induced increases in hepatic glycolysis are not due to vasoconstrictive effects on the liver vascular beds.

A calcium-dependent mechanism has been proposed for the effects of these compounds on hepatic carbohydrate metabolism. In our study, the absence of physiological extracellular calcium concentrations delayed, but did not reduce maximal increases in lactate and pyruvate production caused by ddI (Figs. 1 and 2), suggesting that extracellular calcium ions are not essential for the ultimate ddI-mediated effects on carbohydrate metabolism. Concomitant with the gradual increase in lactate plus pyruvate production caused by ddI, the lactate/pyruvate ratio in the effluent perfusate decreased and reached control values within 15–20 min (Fig. 2). At low perfusate calcium concentrations, transient efflux of calcium from the liver was observed in response to infusion of adenine nucleotides [12, 13], and inositol triphosphate, the putative intracellular second messenger believed to act via release of intracellular calcium, also increases following treatment with ATP [14]. These findings suggest involvement of intracellular calcium in the hepatic responses to adenine nucleotides. In this study, repeated infusion of ATP during perfusion with calcium-free medium, which is known to deplete intracellular calcium stores [10], attenuated the ddI-induced effects on lactate and pyruvate production markedly (Fig. 1), whereas brief

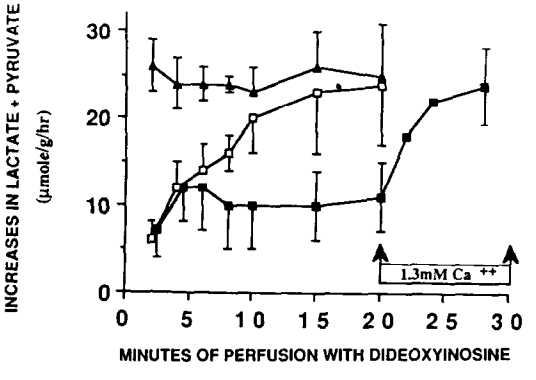

Fig. 1. Stimulation of hepatic glycolysis by dideoxyinosine in the presence and absence of calcium. Rates of lactate plus pyruvate production in the effluent perfusate were determined in the presence of extracellular calcium (▲), in the absence of added perfusate calcium (□) and in the absence of added perfusate calcium and following infusion of ATP (■). Data are means ± SE from four rats per group.

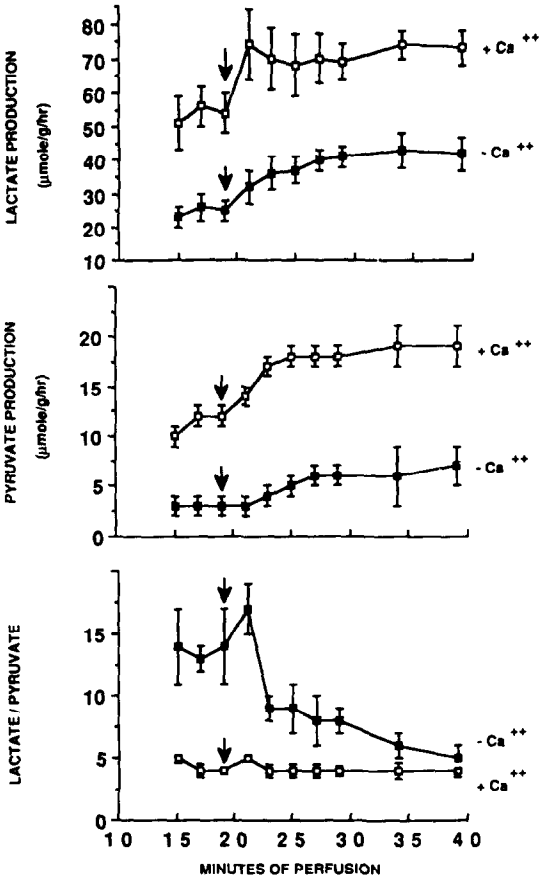

Fig. 2. Effect of dideoxyinosine on carbohydrate production and lactate/pyruvate ratios in the effluent perfusate. Concentrations of lactate and pyruvate in the effluent perfusate were determined periodically and rates of production, as well as ratios, were calculated. Data are means ± SE from four rats per group. Vertical arrows indicate the onset of ddI infusion.

infusion of calcium (1.3 mM), which replenishes the intracellular stores [10], restored hepatic responsiveness to ddI (Fig. 1).

In the presence of physiological concentrations of calcium in perfusate, therapeutic concentrations of the anti-AIDS drug ddI stimulated hepatic glycolysis significantly. Similar maximal increases were observed when ddI was infused in the absence of added perfusate calcium. In contrast, when intracellular calcium stores were depleted, ddI caused only slight stimulation of hepatic glycolysis, which was restored to control values upon infusion of calcium simultaneously with ddI. These findings suggest that ddI stimulates hepatic carbohydrate metabolism by mobilizing calcium from intracellular calcium stores. Stimulation of hepatic glycolysis, in conjunction with the poor nutritional state of AIDS patients, is expected to lead to the depletion of hepatic glycogen stores. The subsequent resultant diminution in glycolytic ATP production is known to exert detrimental effects on the liver and render it highly vulnerable to hepatotoxic insults [15, 16]. Indeed, elevations in serum levels of hepatic transaminases, which indicate hepatotoxic manifestations have been reported in Phase I clinical trials with ddI [2, 3]. These effects resulted in termination of administration of ddI to these patients. Our results suggest that patients receiving ddI should be monitored closely for the earliest signs of symptomatic or asymptomatic hepatotoxic effects for prompt discontinuation of this drug before potential irreversible liver injury occurs.

Acknowledgements—Supported, in part, by ES-04778 and the Weldon Spring Endowment Fund of the University of Missouri-Kansas City.

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Biochemical Pharmacology, Vol. 41, No. 1, pp. 148–151, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

Structure–activity relationships for protein binding of a series of basic non-steroidal anti-inflammatory drugs

(*Received* 26 *March* 1990; *accepted* 18 *August* 1990)

Most non-steroidal anti-inflammatory drugs (NSAID)* are acidic compounds which are highly bound in plasma. They bind mainly to one of two specific drug-binding sites on human serum albumin (HSA), namely sites I and II [1, 2]. Basic drugs bind primarily to α_1-acid glycoprotein or to lipoproteins in plasma [3]. However, we have shown recently that GP53,633, a basic NSAID, can bind to site I providing it is present in the unionised form [4]. The present communication reports studies on the structure–activity relationships for binding of a series of basic NSAID structurally related to GP53,633 (Fig. 1).

Materials and Methods

GP53,633 and its analogues studied were donated by Ciba–Geiby Limited (Australia), who also supplied ^{14}C-labelled GP53,633. ^{14}C-Labelled warfarin was purchased from the Radiochemical Centre (Amersham, U.K.). ^{14}C-Labelled ibuprofen and flurbiprofen were gifts from the Boots Co. Ltd. (Australia). The radiochemical purities of these compounds as stated by the suppliers were greater than 99%. In each case, the radiochemical purity of free drug (on the buffer side after equilibrium dialysis against human serum) was better than 98% [4].

HSA, essentially fatty acid free (Lot No. 110F-9350), 5-dimethylaminonaphthalene-1-sulfonamide (DNSA) and dansylsarcosine were purchased from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). The essentially fatty acid free HSA contained 0.04 mol fatty acid/mol of albumin, as determined by the method of Duncombe [5]. The *Mr* of albumin was taken as 66,500 [6]. Serum was obtained from Ortho Diagnostic Inc. (Lot No. 5S-225) and had a fatty acid content of 0.9 mol fatty acid/mol HSA.

All experiments were performed using 0.1 M sodium phosphate buffer, pH 7.4, containing 0.9% (w/v) NaCl, unless otherwise stated. Fluorescent probe techniques were used to study the displacement of marker probes (DNSA, warfarin and dansylsarcosine) binding to specific binding sites on HSA as previously described [7]. Fluorescence measurements were made at room temperature (22°) using a Perkin–Elmer model MP-3000 spectrofluorometer. Binding was performed by equilibrium dialysis using a Dianorm® apparatus at 37° as described earlier [4]. The concentrations of GP53,633 analogues after dialysis were analysed by HPLC. The mobile phase consisted of acetonitrile and 10 mM trisodium citrate, pH 3.0 (30:70, v/v). The flow rate was 2 mL/min. Absorbance was monitored at a wavelength of 254 nm. Sample (0.2 mL) was mixed with 0.1 mL of acetonitrile and centrifuged for 2 min. An aliquot of 0.07 mL of clear supernatant was injected onto the HPLC column (Waters μ-Bondapak C-18 reversed-phase). The concentration of compound was determined by comparison of the peak height with a calibration curve for each compound.

The pK_a of GP53,633 and its analogues was measured by the UV titration method at a wavelength of 200 nm [4]. The change in UV absorbance with pH indicates the ionisation of a titratable chromophore, probably the imidazole moiety. The *n*-octanol/water partition coefficients of GP53,633 and its analogues were determined by a flask-shaking method [8], using 0.1 M NaOH. After partitioning and centrifugation, concentrations of the compounds were analysed by the HPLC method described earlier.

Results and Discussion

Binding of GP53,633 analogues to HSA and diluted serum. Eight compounds having chemical structures similar to GP53,633 were studied (Fig. 1). Compounds **1** and **2** are GP53,633 and its known metabolite CGP8716 respectively. Apparent pK_a values for **2**, **3**, **4** and **7** were determined by UV absorption and were all found to be very similar to that for GP53,633 (pH 6.4). The apparent pK_a for **9**, however, was higher (pK_a of 7.1).

Figure 1 illustrates the binding of GP53,633 analogues to pure HSA and to diluted serum at the same albumin concentration of 100 μM. Binding of phenylbutazone is also shown for comparison. The binding to HSA of GP53,633 and most of its analogues was generally greater than that of phenylbutazone. This suggests that the analogues would be highly protein bound in plasma with albumin as the major binding protein. The binding of all compounds was higher with serum than with fatty acid free HSA and the HSA to serum free fraction ratios (HSA/Serum) ranged from 3.78 (**7**) to 1.04 (**9**). This suggests that, as with GP53,633 [4], the binding of the analogues is increased by the presence of fatty acids.

Structure–activity relationships for binding of GP53,633 analogues. The binding of GP53,633 and most of its

* Abbreviations: NSAID, non-steroidal anti-inflammatory drugs; HSA, human serum albumin; GP53,633, 2-*tert*,butyl-4(5)phenyl-5(4)-(3-pyridyl)-imidazole (Ciba–Geigy); and DNSA, 5-dimethylaminoaphthalene-1-sulfonamide.