

THE ENZYMATIC BASIS FOR THE METABOLISM AND INHIBITORY ACTION OF VALPROATE (VP): DEHYDROGENATION OF VP-CoA BY 2-METHYL-BRANCHED CHAIN ACYL-CoA DEHYDROGENASE (2-meBCAD). Michinori Ito, Yasuyuki Ikeda, Gaetano Finocchiaro, John Arnez, and Kay Tanaka. Yale Sch. of Med. New Haven, and *CUNY, N.Y. (Spon. by W. Roy Breg).

1015

VP is an eight carbon branched chain fatty acid. It is widely used as an anti-convulsant. Serious toxic side effects have occasionally been observed. VP is considered to be mainly metabolized via β -oxidation and glucuronide formation, and has been shown to inhibit fatty acid oxidation. However, the enzymatic mechanisms are currently unknown. We studied the VP-CoA oxidation by acyl-CoA dehydrogenases (ADs) and its inhibitory action on these enzymes using purified enzyme preparations. ADs catalyze the first reaction of the β -oxidation. Five of them are known. They are short (SCAD), medium (MCAD), long chain acyl-CoA dehydrogenases (LCAD), isovaleryl-CoA dehydrogenase (IVD) and 2-meBCAD. We purified all five of them to homogeneity from rat liver. We also purified SCAD, MCAD and IVD to homogeneity from human liver. Human LCAD was partially purified from placenta mitochondria. AD activities were assayed spectrophotometrically using phenazine methosulfate and dichloroindophenol (DCIP) as electron acceptors. Only 2-meBCAD dehydrogenated VP-CoA. The specific activity was 167 nmol of DCIP reduced/min/mg protein. This value was 7.6 percent of that for S-2-methylbutyryl-CoA, the best substrate for this enzyme. 2-Propyl-2-pentenoyl-CoA was identified as the reaction product by using GC/MS. No other ADs dehydrogenated VP-CoA to any significant degree. Inhibitory effects were tested using human ADs. 0.1 mM VP-CoA slightly inhibited MCAD alone. At 0.3 mM, it moderately inhibited SCAD, MCAD and IVD: the inhibition ranged from 18% for IVD to 25% for MCAD.

EFFECTS OF SUBLETHAL DOSES OF ENDOTOXIN AND ASPIRIN (ASA) ON RAT HEPATIC ENERGY METABOLISM: AN ANIMAL MODEL OF REYE SYNDROME. L. Kilpatrick-Smith, S.D. Douglas, R.A. Polin, B.E. Corkey, University of PA Sch. of Med., Depts. of Peds. & Biochem., Phila., PA

1016

The administration of a sublethal dose of endotoxin (LPS) to fasted rats results in biochemical and histopathological perturbations similar to Reye Syndrome (RS), i.e. hyperammonemia, elevated serum lactate and free fatty acids, and hepatic microvesicular fat deposition and swollen mitochondria in the absence of cellular necrosis (Yoder et al, Infect. Immun., 1985). In this study, hepatic energy metabolism was assessed in freeze clamped liver samples (12 hrs post treatment) obtained from (250-300 gm Sprague-Dawley) rats which had received an intraperitoneal injection of placebo-5% dextrose (C), 0.25mg LPS/kg (LPS), placebo + 1hr later 50mg ASA/kg (ASA) or 0.25mg LPS/kg + 1hr later 50 mg ASA/kg (LPS/ASA). In both LPS and LPS/ASA, ATP/ADP declined by 31% ($p < 0.005$), [total ketones] decreased by 40% ($p < 0.0005$) and [acetyl CoA] declined by 37% ($p < 0.005$) as compared to C and ASA. In LPS/ASA, there were increases in [isovaleryl CoA], [isobutyryl CoA] and [B-methylcrotonyl CoA] as compared with ASA (60%, $p < 0.01$) and LPS (90%, $p < 0.005$). These results suggest inhibition of fatty acid oxidation and abnormal branch chain amino acid (BCAA) oxidation and are analogous to data obtained in patients with RS showing hyperammonemia, compromised FA oxidation and accumulation of intermediates of BCAA oxidation. Accumulation of BCAA intermediates with LPS/ASA is a possible mechanism for the potentiation of RS by ASA. These findings provide further biochemical evidence that a sublethal dose of LPS administered to fasted rats produces an animal model of RS. (Supported by NS 17752)

CHANGES IN LACTATE PRODUCTION IN ENTEROCYTES UNDER AEROBIC AND ANAEROBIC CONDITIONS. Robert E. Kimura, Kris Snow, Marco Ferretti. (Spons. by G. Chan) U of Utah, Salt Lake City, UT.

1017

The aerobic production of lactate from glucose by the intestine has been proposed as an important source of carbon substrate for liver glycogen synthesis. In order to determine the significance of aerobic and anaerobic lactate production by jejunal villus cells, we determined lactate production and energy charge (EC), an indicator of intracellular oxygen availability and oxidative phosphorylation activity, in jejunal villus cells under aerobic and anaerobic conditions. Adult rat villus cells were incubated in a polarographic chamber containing Krebs Ringer Phosphate Buffer, 5 mM glutamine, 5 mM glucose and 5 mg/ml BSA. The rate of O_2 consumption was determined using a Clark electrode. Aliquots of assay mixture were removed and analyzed for lactate, ATP, ADP and AMP conc. after 5 periods (5 min (aerobic), 4 min (anaerobic- O_2 set 3%), 4 min anaerobic, 4 min recovery in which the cells were placed in an aerobic medium and a 4 min aerobic period). Lactate production was calculated by subtracting lactate concentration of successive samples and dividing by the time interval. $EC = (ATP + 0.5 \cdot ADP) / (ATP + ADP + AMP)$. The results are means \pm SD ($n=4$). The rate of O_2 consumption, initially 0.66 ± 0.05 (μ mol/mg/min) was not affected by 8 min of hypoxia (0.7 ± 0.23). The EC during the aerobic period was 0.60 ± 0.07 . The EC decreased to 0.52 ± 0.08 and 0.46 ± 0.05 ($p < 0.03$) at the end of 4 and 8 min of hypoxia, indicating that the hypoxic conditions altered the enterocyte oxidative phosphorylation activity. After the recovery and subsequent aerobic period, the EC increased to 0.67 ± 0.07 and 0.65 ± 0.09 respectively, indicating aerobic metabolism of undamaged enterocytes. Lactate production remained constant during the initial aerobic and anaerobic periods (45.8 ± 2.0 nmol/mg/min, 30.8 ± 9 and 37.0 ± 0). During the recovery period when the EC was initially .46, lactate production increased to 77.8 ± 20.1 ($p < 0.01$). In the subsequent aerobic period, lactate production decreased to its original rate (42.3 ± 13.1). In summary, under aerobic conditions with a high EC, enterocytes can metabolize glucose to lactate and is a possible source of portal venous lactate. Furthermore, enterocyte lactate production can increase two fold during hypoxic conditions.

1018

THE NEONATAL PHENOTYPE OF GALACTOSEMIA

Mark Korson, Mira Irons, Harvey L. Levy. Harvard Med Sch, Children's Hosp, Depts of Pediatrics and Neurology, State Lab Inst, Boston, MA.

Galactosemia (GAL), due to Gal-1- PO_4 uridyltransferase deficiency, is an inborn error of galactose metabolism causing neonatal morbidity and mortality. The general features of GAL are not specific, and the particular characteristics that may better define GAL in the neonate have not been systematically studied. We have analyzed the clinical and biochemical findings prior to treatment in all 23 neonates with GAL identified by routine screening during the past 21 years. At the time of newborn screening identification, only one infant had been diagnosed clinically as having GAL, although 18 were symptomatic; 3/5 asymptomatic infants were biochemically variant. Among all affected neonates 6 (26%) had bacterial infections, 5 with bacteremia and 1 with urinary tract infection; 5/6 infections were due to E. coli. Hyperbilirubinemia during the first week of life was almost exclusively unconjugated, while the conjugated fraction tended to rise only during the second week. Serum transaminase elevations were associated with conjugated hyperbilirubinemia. Coagulopathy was the most distinguishing feature of liver dysfunction, and was disproportionate to the severity of other biochemical indications of liver disease. Of 11 infants studied by slit-lamp ophthalmologic examination, 7 (64%) had "water-droplet cataracts".

GAL produces severe physiologic disturbances which are often unsuspected from a deceptively milder clinical presentation. These disturbances may be related to the long-term sequelae now recognized in early-treated galactosemics.

THE GLYCOGEN SYNTHESIS GLUCOSE "PARADOX" IN NEWBORN RATS. C Kunst, R Kliegman, C Trindade. Case Western Reserve Univ., Dept. Peds., Cleveland, OH

1019

In adults glucose (glu) is paradoxically incorporated into liver glycogen (glyc) indirectly via recycling from lactate. To investigate the pathway of neonatal glyc synthesis double labeled glu as 6- 3H (non-recycle), U- ^{14}C (recycle) at 1 g/kg glu or saline (Sal) was injected (IP) to fasted 6hr old or 1 day fasted 5-7 d old rats (N=8-15). Glu, glyc and dpm were determined at 15', 30', 60', 120' and 180'. After IP Sal, glu increased spontaneously in 6 hr pups (5.5 to 10.1mM) while 5-7 day pups had no glycemic response to Sal. After glu IP blood glu increased further to 15.5mM in 6 hr pups while in 5-7 day pups glu increased to 8.5mM at 15' and then declined. Between 15'-180' glyc declined spontaneously in 6 hr pups after Sal (218 ± 25 to 107 ± 12 μ g/g) while glyc was unchanged in fasted 5-7 day pups (13 \pm 2). After IP glu in 6 hr pups glyc was increased compared to Sal IP only at 180' (143 ± 9 v 107 ± 12 , $p < 0.05$). In contrast, in response to IP glu in 5-7 day pups glyc increased over fasting values and was elevated at 60', 120' and 180'. The ratio of $^3H:^{14}C$ in glyc relative to the injectate was 0.65 ± 0.03 at 15' and did not change at 180' in 6 hr pups suggesting no recycling. In contrast in 5-7 day pups the $^3H:^{14}C$ ratio was 0.69 ± 0.12 and declined to 0.35 ± 0.03 , ($p < 0.01$) at times of net hepatic glyc synthesis (120', 180'). We conclude that the indirect pathway of glyc synthesis through recycling was of greater importance than direct incorporation of glu to glyc in 5-7 day pups. The absence of such an effect in the 6 hr old rats could be due to the limited activity of gluconeogenesis at this time period.

THE GALACTOSE-GLUCOSE "PARADOX" IN NEONATAL MURINE HEPATIC GLYCOGEN SYNTHESIS. C Kunst, R Kliegman, C Trindade. Case West Res Univ, Dept Peds, Cleve, OH

1020

In adults glucose (glu) incorporation to glycogen (glyc) is indirect following recycling from lactate. In neonates galactose (gal) entry to glyc exceeds that for glu but the pathway is unknown. The path of hexose incorporation to glyc was studied in 5-7 day old rats and 6 hr old rats injected (IP) with either double labeled [6- 3H (nonrecycle) U- ^{14}C (recycle)]-glu or (6- 3H)-glu and (U- ^{14}C)-gal in saline (S), N=8-15. In another group of rats lg/kg of glu or gal was used to determine glycemia and glyc synthesis at 15' to 180'. Glu increased from 3.4 ± 0.4 to 8.5 ± 1.5 mM in 5-7 day pups in response to IP glu; there was no glycemic response to gal, although gal levels increased from 0.5 to 6.3 mM at 15'. Glyc increased after IP glu from 14 ± 2 at 15' to 30 ± 3 at 120', ($p < 0.01$) while after IP gal glyc was 44 ± 6 μ mol/g at 120', $p < 0.05$. After IP glu, 3H and ^{14}C dpm in glyc increased slowly with ^{14}C exceeding 3H at 120' and 180'. In contrast IP ^{14}C gal resulted in a large peak of ^{14}C incorporation to glyc which was 5 fold greater than that after ^{14}C glu. The ratio of $^3H:^{14}C$ in glyc relative to the injectate after IP glu decreased from 0.69 ± 0.12 to 0.36 ± 0.03 , $p < 0.01$ between 15' to 180' while the ratio after gal was 0.06 ± 0.007 to 0.15 ± 0.02 . 6 hr old pups also demonstrated augmented incorporation of ^{14}C gal in glyc relative to $^3H^{14}C$ glu. In contrast to 5-7 day pups there was no evidence of glu recycling in 6 hr. pups. In conclusion, gal entry into glyc exceeds that for glu and is not dependent on recycling. Direct incorporation of gal exceeds that for direct incorporation from 3H glu suggesting a preferential diversion of gal for neonatal glyc synthesis.