Title: AMINO ACIDS FAIL TO REVERSE HALOTHANE DEPRESSION OF ALBUMIN SYNTHESIS

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Introduction: We have recently shown a) that isolated perfused rat livers (IPRL) exposed to 1.5% halothane in a mixture of $O_2/CO_2$ or to $O_2/CO_2$ alone produced urea, albumin and transferrin at constant rates during a 4.25 hour perfusion and b) that halothane depressed albumin and transferrin synthesis by nearly one-half without disturbing urea production (1). Previous studies showed that a mixture of essential amino acids stimulated albumin synthesis in both the intact rat and the IPRL (2-4). In other experiments, ethanol added to the perfusate profoundly inhibited albumin synthesis by IPRL from normal donors, an effect completely negated by adding amino acids (5). Protection of the liver by amino acids from one of the actions of a generally recognized hepatotoxin, ethanol, has led us to examine the effect of amino acid treatment on albumin synthesis by the IPRL exposed to halothane.

Materials and Methods: Animal use was approved by the Medical Research Committee of the University of Cape Town. Male Long Evans rats (290-304g), housed under controlled conditions of temperature and humidity and allowed access to food and water ad libitum, were used as liver and red cell donors. Male New Zealand White rabbits, maintained under similar conditions, were used as plasma donors. Seventeen livers were perfused ex situ for 4.25 hours with a heterologous mixture of heparinized rabbit plasma and rat red cells. Plasma and red cell proportions were adjusted to an Hct of 25-29%. Perfusate Hct, flow rate, pressure and $O_2$ and $CO_2$ partial pressures, as well as bile production, were monitored continuously.

Five livers were exposed to $O_2/CO_2$ (95/5)(group 1) and twelve to $O_2/CO_2$ and 1.5% halothane (groups 2 and 3). To the perfusate of 6 of the halothane-exposed IPRLs (group 3), a mixture of 10 essential amino acids was added, each at a concentration of approximately 10 times the normal rat plasma level. In this group, donor rats received an amino acid mixture intragastrically 1 hour preoperatively. L isomers of arginine, asparagine, isoleucine, lysine, methionine, phenylalanine, proline, threonine, tryptophan and valine were added together and their relative proportions were derived from the serum amino acid profile.

The concentration of newly synthesized albumin was measured by radial immunodiffusion. The antisera used reacted with rat but not rabbit albumin. Urea production and albumin synthesis rates were estimated by linear regression of accumulated values with rates taken as equal to the slopes of the fitted lines. Results in the 3 groups were compared by one-way analysis of variance and by Duncan's multiple range comparison procedure.

Results: Perfusate flow ranged from 1.9 to 3.6 ml/min per gram liver. Bile flow ranged from 1.3 to 1.9 µl/min per gram liver. No differences were noted between groups for either of these parameters.

Urea production (mean ± SEM) was 8.72 ± 0.98, 9.64 ± 0.91 and 40.0 ± 1.71 mg/hr per 300g rat in groups 1, 2 and 3 respectively. The means of groups 1 and 2 did not differ significantly. The four-fold increase in urea production in group 3 reflects amino acid treatment. A constant rate of urea production over 4.25 hours was a characteristic finding in all experiments. Similarly, a constant rate of albumin production was found throughout the course of each liver perfusion. Albumin synthetic rates (ASR), expressed as mg/hr/300 g rat, are given as mean ± SEM for each experimental group. Albumin synthetic rates did not differ significantly between groups 2 and 3.

| | Group I | Group 2 | Group 3 |
|---|---|---|---|
| ASR | 12.13±1.36 | 6.98±0.92* | 8.68±0.84* |

*differ significantly from control, $p < 0.05$

Discussion: Although amino acids stimulate albumin synthesis in protein depleted rats and block ethanol depression of albumin synthesis, we could show no such protective effect in rat livers exposed to halothane. No significant difference was noted between groups given halothane with amino acids versus those given halothane alone. The mechanism of inhibition of albumin synthesis by either ethanol or halothane is unknown at the present time. Differences in response to amino acid treatment suggest that there may be fundamental differences in these mechanisms.

References:

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