measured in perchloric acid extracts of medium by the hexokinase/glucose-6-phosphate dehydrogenase method (Bergmeyer et al., 1974) and protein in the cell pellets by the method of Lowry et al. (1951). Figures show mean values ± S.E.M. compared with controls. Glucose production in the absence of pyruvate was negligible in both groups. The magnitude of the stimulation of gluconeogenesis is very similar to the reported increases in activities of hepatic gluconeogenic enzymes in CdCl₂-pretreated rats, although the dose, chronic dosing schedule, and route of administration varied between different groups of workers (see Singhal et al., 1974; Chaputwala et al., 1980). Fig. 1b shows that the addition of CdCl₂ to incubations of normal hepatocytes in vitro produced a dose-related inhibitory response on gluconeogenesis from pyruvate. In the case of incubations with 1 or 10 mM CdCl₂ a high concentration of glucose was found both in the presence and absence of pyruvate, suggesting that at these concentrations cytotoxicity and cell death were occurring (viability <40%). These results are similar to the more limited data obtained by others with rat kidney cortex slices (Rutman et al., 1965; Seiller et al., 1979). The effects of cadmium in the in vitro experiments is considered to be a non-specific aspect of generalized hepatic toxicity. Interference with energy metabolism at the mitochondrial level is probably a major factor in the hepatotoxic response, but inhibitory effects of cadmium on a wide variety of enzymes containing divalent-metal ions as cofactors or essential thiol groups may also contribute.

Chronic administration of CdCl₂ to rats results in a stimulation of hepatic gluconeogenic flux which will contribute to the hyperglycaemic action of this agent in vivo. A direct effect of CdCl₂ on the liver in vivo to account for this stimulation is considered unlikely since, even at low (micromolar) concentrations, gluconeogenesis is inhibited in incubations with hepatocytes in vitro. A possible explanation for the stimulation of gluconeogenesis after chronic dosing is its indirect effect on the sensitivity of the pancreatic β-cells to cadmium and the observed inhibition of insulin secretion (Ghafghazi & Mennen, 1975; Ithakisios et al., 1975). The stimulation of adenylate cyclase and increased hepatic cyclic AMP concentration in rats exposed to cadmium (Merali & Singhal, 1975; Merali et al., 1975) is not inconsistent with this hypothesis, and could therefore indirectly be responsible for the stimulation of gluconeogenesis.


Use of liver snips for studies on gluconeogenesis

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It is well recognized that the liver slice preparation is a deficient in vitro system for studies of gluconeogenesis. The requirement for a simple, rapid, inexpensive preparation remains, however, for certain routine studies. Recently Pollard & Dutton (1982) introduced a novel liver preparation involving liver ‘snips’, which they proposed as an intact cellular preparation capable of performing synthetic metabolic functions. The authors demonstrated its utility for studying glucoronidation reactions. We examined this preparation for its capacity to sustain a more complex area of biosynthetic metabolism, namely gluconeogenesis. Gluconeogenesis is a more stringent test of metabolic integrity than glucuronidation because it requires the integrity of the cell membrane and surface receptors in order to respond to glucoregulatory hormones; it requires co-operation between various metabolic compartments (cytosol, mitochondria, endoplasmic reticulum), and it is critically dependent on energy supply. For this purpose the rate of gluconeogenesis from lactate + pyruvate was compared in ‘snip’ and slice preparations and the ability of the two systems to respond to hormonal stimulation by glucagon was measured. In some experiments, the gluconeogenic rate in isolated hepatocytes was also measured, since this preparation is presently in widespread use and gives rates of gluconeogenesis comparable with those seen with the perfused liver preparation.

Fig. 1 shows the time-courses for glucose production from the lactate + pyruvate mixture for the three in vitro preparations. Glucose production was approximately linear with time over 60 min for all preparations and the time period from 30 to 60 min was used for calculating the rates of gluconeogenesis. The rate of gluconeogenesis with isolated hepatocytes was the greatest, but that from the liver snips (75% of isolated hepatocytes) was comparable taking into account the contribution of non-parenchymal, non-gluconeogenic cells to this preparation. Both were clearly superior to the slice preparation (rates of 34% of that with hepatocytes and 46% of that with snips). Glucagon (10–8 M) stimulated gluconeogenesis from lactate + pyruvate in the snips by 46% (P<0.05), whereas slices were insensitive to hormonal stimulation. The lack of response of gluconeogenesis to glucagon in slices has been observed previously (Ross et al., 1967).

The advantages of the snip preparation over the slice preparation are clear. As to the advantages of snips over isolated hepatocytes, these will depend on the particular aspect of gluconeogenesis under investigation. The isolated hepatocyte suspension is a fairly homogenous preparation of parenchymal cells which, however, lack the normal cellular heterogeneity and architectural arrangement found...
Regulation of cyclic AMP production in rat Leydig cells by LHRH agonist under basal and LH-stimulated conditions

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LHRH agonists have been shown to have short-term (<12h) stimulatory effects on basal and LH-stimulated steroidogenesis in the rat Leydig cell (Hunter et al., 1982; Sharpe & Cooper, 1982). Continuous incubation with LHRH agonists for longer periods (24h) inhibited LH-stimulated testosterone production (Hunter et al., 1982). Recent work (Sullivan & Cooke, 1984) has also indicated a short-term inhibitory effect by LHRH agonist (ICI 118630) on LH-stimulated cyclic AMP production. We have, therefore, investigated the nature of this inhibition.

Initial LH-stimulated cyclic AMP production was linear (Fig. 1a) over the first 10 min of stimulation, and continued at a lower rate for the next 110 min. A 2h preincubation with LHRH agonist (10-6 M) decreased the LH-stimulated cyclic AMP production at all times of incubation (2-120 min) by approximately 40%. The phosphodiesterase inhibitor 1-methylisobutylxanthine (MIX, 0.5 mM) negated the LHRH agonist effect, indicating that increased phosphodiesterase activity, rather than decreased adenylate cyclase activity, caused the lower cyclic AMP levels. Similar results were obtained with cycloheximide; preincubation with or without LHRH agonist in the presence of cycloheximide (10-4 M), followed by a 15 min stimulation with LH gave the following results: LH 101.65 ± 7.6; LH + cycloheximide 118.1 ± 6.4; LH + LHRH agonist 56.5 ± 5.6; LH + LHRH agonist + cycloheximide 99.1 ± 6.0 (pmol of cyclic AMP/106 cells, means ± S.E.M., n = 3). These results indicate that phosphodiesterase synthesis is increased, rather than the enzyme being activated, by LHRH agonist.

This effect is also time dependent, because a preincubation of 2h was required before any effect of LHRH agonist was found (data not shown). This agrees with the time course of increased cytochrome P-450 cholesterol side-chain cleavage enzyme, which also required a 2h preincubation (Hunter et al., 1982; Sullivan & Cooke, 1983).

We have not been able to demonstrate a detectable effect of LHRH agonist alone on cyclic AMP levels, although testosterone production was increased 3-4-fold (Fig. 1b) during a 4h incubation. LHRH agonist enhanced the rate of LH-stimulated steroidogenesis during the 2nd incubation period. MIX slightly decreased steroidogenesis during the second hour of the incubation period, but this was significant at only two points, as shown. Decreased cyclic AMP levels are not, therefore, obligatory for the increased rate of steroidogenesis. The increase in rate of the linear in the liver in vivo. They are time-consuming and expensive to prepare, require considerable surgical skill, and involve pre-treatment of animals with anaesthetic. The snip, although retaining normal hepatocellular architecture, is prepared from the periphery of the liver lobule and, in view of known metabolic zonation across the liver lobule with respect to gluconeogenesis, would not be representative of the 'average' cellular capacity for gluconeogenesis. It is, however, an inexpensive and relatively rapid preparation requiring a minimum of surgical expertise. The amount of suitable material available from a single liver for the preparation of snips is another disadvantage. The superiority of snips to slices is difficult to define but may be related to their differing histological origin and to the fact that a snip has only a single 'cut' face.

Liver snips would appear to constitute a realistic alternative to isolated hepatocytes in studying certain aspects of gluconeogenesis. For example, they may be particularly valuable for screening large numbers of pharmacological or toxicological agents for inhibitory or stimulatory effects on gluconeogenesis. They might also be useful in studying gluconeogenesis in small fragile livers where collagenase perfusion for cell isolation is impracticable (e.g. neonatal rat or mouse liver).

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