Properties of the hepatocyte lactate transporter: effect of pH and temperature, and susceptibility to inhibition

JOHN P. MONSON, JACQUELINE A. SMITH, ROBERT D. COHEN and RICHARD A. ILES
Unit of Metabolism and Endocrinology, London Hospital Medical College, London E1 1BB, U.K.

We have presented evidence for a specific transport component of lactate entry into isolated rat hepatocytes (the preceding paper (Monson et al., 1981)). The present paper presents experimental work designed to characterize further the transport.

Suspensions of isolated rat hepatocytes were prepared from starved male Sprague–Dawley rats, as previously described (Monson et al., 1981), and incubated with $^{3}H_{2}O$ at $37^\circ C$ under various conditions of temperature and pH and in the presence or absence of potential inhibitors for 15 min before the addition of $L(+)-[U-^{14}C]lactate$ (0.25 $\mu Ci/ml$). Cells were separated from surrounding medium by centrifugation through silicone oil exactly 15 s after the lactate addition and intracellular $^{14}C$ radioactivity was determined (c.p.m./ml of cell water). We have shown that, under these conditions, less than 12% of $^{14}C$ measured in the cells after 15 s is in a form other than lactate.

Lactate-transport mechanisms previously demonstrated in the erythrocyte (Halestrap, 1976) and the Ehrlich ascites-tumour cell (Spencer & Lehninger, 1976) are susceptible to specific inhibition with $\alpha$-cyanocinnamate and some of its derivatives. In the absence of $\alpha$-cyanohydroxycinnamate, the 15 s uptake of $L(+)-[U-^{14}C]lactate$ is inhibited in isolated hepatocytes (Fig. 1). Maximum inhibition occurs at approx. 2 $\mu M$ $\alpha$-cyanohydroxycinnamate and the $K_{i}$ is 0.5 $\mu M$. Inhibition is not complete, however, and we suggest that the remaining uptake is not transporter-mediated but occurs by simple passive diffusion (Monson et al., 1981). A similar degree of inhibition is achieved with the thiol reagent $p$-chloromercuriphenylsulphonate, but not with the general anion-transporter inhibitor 4-acetamido-4'-isothiocyanostilbene-2,2'-disulphonic acid or ouabain. A combination of the uncoupling agent dinitrophenol and the glycolytic inhibitor fluorid in is lactate entry into cells (in addition to increasing the flux through the respiratory chain), suggesting that the transporter may be energy-dependent.

We have attempted to differentiate between transported and diffused $^{14}C$ lactate entry in 15 s by performing parallel incubations in the presence and absence of 10 $\mu M$ $\alpha$-cyanohydroxycinnamate. By thus estimating the transported $^{14}C$ activity at various temperatures (4, 15, 25 and $37^\circ C$), it appears that temperature-dependence of the transport is high between 4 and $25^\circ C$ (apparent activation energy = 56.8 $\times 10^{3}$ J.mol$^{-1}$), but falls sharply at higher temperatures (apparent activation energy = 15.9 $\times 10^{3}$ J.mol$^{-1}$). This pattern is similar to that of other anion-transfer processes, for example chloride (Dalmark, 1976) and the erythrocyte lactate transporter (Deuticke, 1978).

Again, by using $\alpha$-cyanohydroxycinnamate in parallel, we have compared the total $L(+)-[U-^{14}C]lactate$ uptake and that portion due to transport at pH 6.8 and 7.4. The hydrogen-ion ratio (extracellular/intracellular) was determined by using the weak acid 5,5'-dimethylloxazolidine-2,4-dione. In a series of four experiments (individual cell preparations), both the total $^{14}C$ entry and the transported $^{14}C$ entry were significantly higher at lower pH ($P < 0.001$ and $P = 0.03$ respectively), indicating that lactate transport is enhanced by a decrease in extracellular pH. The ratio of total $^{14}C$ entry to extracellular $^{14}C$ radioactivity was higher than the hydrogen-ion ratio in each instance, and thus neither passive nor facilitated diffusion of non-ionized lactic acid can account for all the lactate entering the cell. The findings are consistent with a lactate–hydrogen-ion antiport or lactate–hydrogen-ion symport mechanism, but in order to explain the cellular alkalization known to occur with lactate consumption (Cohen et al., 1971), the lactate–OH$^{-}$ (or H$^{+}$) stoichiometry would have to be greater than unity.


Glucose, insulin release and the control of cyclic AMP efflux and cyclic AMP content of rat islets of Langerhans

IAIN L. CAMPBELL and KEITH W. TAYLOR
Department of Biochemistry, The London Hospital Medical College, Turner Street, London E1 2AD, U.K.

The pattern of glucose-mediated insulin release from the islets of Langerhans can be correlated with changes in the islet content of cyclic AMP. Thus under conditions of increased extracellular glucose concentration both insulin release and intra-islet cyclic AMP concentration are increased (Charles et al., 1973; Grill & Cerasi, 1973; Zawalich et al., 1975; Caterson & Taylor, 1979). In addition, impaired glucose-induced insulin release has been shown to be accompanied by decreased cyclic AMP responses
to glucose in the islets of neonatal rats (Grill et al., 1975) and diabetic Chinese hamsters (Rabinovitch et al., 1976). The effect of glucose on increasing islet cyclic AMP has not only been demonstrated in whole rat islets, but also in broken cell preparations of the islets of mice (Capito & Hedeskov, 1977) and rat (Campbell & Taylor, 1980).

It would thus appear that cyclic AMP has an important function in the molecular events that lead to increased insulin release due to glucose. The purpose of the present report was to investigate further the nature of glucose-induced alterations in rat islet cyclic AMP and to correlate any such alterations with insulin release.

Islets of Langerhans were isolated from fed male Sprague-Dawley rats by the collagenase digestion procedure. Groups of 50 islets were incubated in tubes containing 400μl of a gassed (O₂/CO₂, 19:1) bicarbonate-buffered balanced-salts medium (Gey & Gey, 1936) containing bovine serum albumin (1 mg/ml) and other additions as stated, at 37°C for 30 min. After incubation, the tubes were chilled and centrifuged at 2000g for 1 min at 0–4°C. For the determination of insulin release and cyclic AMP efflux, the medium from duplicate groups of islets was combined and a 50μl portion was removed and stored at −20°C pending assay of insulin by radioimmunoassay. The remaining pooled medium was boiled for 5 min and then centrifuged at 2000g for 15 min. The supernatant was collected and stored at −20°C for cyclic AMP assay. For the determination of intracellular cyclic AMP content, 0.25 ml of ice-cold 20mM-Tris/HCl buffer, pH 7.6, and stored at −20°C, 0.25 ml of 50mM-sodium acetate buffer, pH 6.2, and stored at −20°C, and 0.25 ml of 50mM-sodium acetic acid buffer, pH 6.2, and stored at −20°C, was added to the islet pellet immediately after removal of the incubation medium. The tubes were then boiled for 5 min. After boiling, the islets were disrupted by ultrasonication at a power output setting of 3, for 15 s. In the present study cyclic AMP was assayed by a radioimmunoassay technique (Steiner et al., 1969).

Rat islets of Langerhans incubated with 20mM-glucose showed a total increase in cyclic AMP concentration (as did insulin release) compared with islets incubated with 2mM-glucose. The dynamics of glucose-induced insulin release were closely paralleled by cyclic AMP efflux from the islets. Intracellular accumulation of cyclic AMP showed less similarity to insulin release. Both the time course and glucose-dose–response curves for cyclic AMP efflux were mirrored by the insulin release from the islets. The effect of glucose on insulin release, cyclic AMP efflux and intracellular cyclic AMP was inhibited by mannoheptulose and menadione. Nicotinamide (10mm) augmented the effect of 20mM-glucose on insulin release, cyclic AMP efflux and intracellular cyclic AMP. Probenecid (1mm) greatly decreased cyclic AMP efflux due to 20mM-glucose, but did not affect insulin release or intracellular cyclic AMP concentration. Papaverine (0.1mm), on the other hand, inhibited both insulin release and cyclic AMP efflux due to 20mM-glucose, while enhancing intracellular cyclic AMP content.

As has frequently been observed, glucose-stimulated insulin release is associated with increased cyclic AMP content of the islets and, furthermore, increased cyclic AMP efflux. The observation that mannoheptulose blocked the glucose effect suggests a metabolic involvement in the control of islet cyclic AMP. This is further supported by the fact that menadione, which causes a decrease in the ratio [NAD(P)H]/[NAD(P)+] (Malaissé et al., 1978), inhibited glucose-mediated increases in insulin release, cyclic AMP efflux and intracellular cyclic AMP.

Additional support for this concept comes from the results seen with nicotinamide, an agent that promotes increased NAD+ formation and potentiates glucose-induced insulin release (Deery & Taylor, 1973). Nicotinamide enhanced both glucose-induced cyclic AMP efflux and intracellular cyclic AMP content of the rat islets. It thus appears that glucose extracts its effect on islet cyclic AMP via metabolism, and this may be modulated through alterations in the redox state of the NAD(P)/NAD(P)+ couple.

Finally, the process of cyclic AMP efflux correlates better with insulin release than with fluctuations in the intracellular compartment of the nucleotide. Cyclic AMP efflux can be dissociated from insulin release, as seen here with probenecid. In addition, 3-isobutyl-1-methylxanthine greatly elevates cyclic AMP efflux without altering insulin release above basal values. It is therefore suggested that cyclic AMP efflux, at least in part, is independent of insulin secretory-granule extrusion. Since cyclic AMP efflux is inhibited by probenecid and papaverine (which are known to inhibit anion transport systems), it may be that cyclic AMP is transported out of the B-cell in association with an anion-transport system.


The effects of 4-hydroxypyrazole and L-methionine on hepatic L-histidine catabolism

JONATHAN S. COOK and CHRISTOPHER I. POGSON
Department of Biochemistry, University of Manchester, Manchester M13 9PL, U.K.

Catabolism of L-histidine in liver involves conversion into formiminoglutamate. The formimino group is then transferred to tetrahydrofolate, with production of N⁵-formimino-N¹⁰-formyltetrahydrofolate (Miller & Waelsch, 1957). This N⁵-formimino group may be oxidized to CO₂ via a tetrahydrofolate-dependent pathway (Krebs et al., 1976), or be transferred to metabolic intermediates (see Stokstad & Koch, 1967).

Formate may be oxidized by two pathways, the first involving formation of N⁵-formyltetrahydrofolate (Greenberg et al., 1955) and oxidative decarboxylation of this to CO₂ (Kutzbach & Stokstad, 1968), the second involving direct oxidation mediated by catalase (EC 1.11.1.6) (Chance, 1949, 1950). Krebs et al. (1976) reported that gluconeogenesis from histidine was accelerated when L-methionine was present. This stimulation by L-methionine was associated with increased activity of the tetrahydrofolate-dependent enzyme sequence because of the greater availability of tetrahydrofolate (which, in the absence of L-methionine, is irreversibly 'trapped' as N⁵-methyltetrahydrofolate).

During investigations into the inhibition of tryptophan 2,3-dioxygenase (EC 1.13.11.11) by 4-hydroxypyrazole, a potent irreversible catalase inhibitor (Deis et al., 1977;