it may be concluded that a single species of binding site is predominant on transcortin both at 37° C and at 10° C.

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## Regulation of Insulin Secretion by Short Chain Fatty Acids

EVIDENCE is accumulating that in some mammalian species there may be a direct effect of short chain fatty acids on the secretion of insulin from the pancreas. Thus it has been shown that octanoate causes a marked release of insulin from slices of rat pancreas[1]. Furthermore, butyrate was found to evoke a rapid and sustained elevation of serum insulin when infused into adult sheep[2]. These results contrast with the lack of effect on insulin secretion when longer chain fatty acids, such as palmitate, are incubated with pancreas slices[3].

With the development of methods for the isolation of islets of Langerhans in large numbers[4,5] it has become possible to study the mechanism of insulin secretion by the measurement of concentrations of metabolic intermediates. We have measured intracellular concentrations of glucose-6-phosphate following incubation of rat islets with butyrate, octanoate and citrate. The results suggest that short chain fatty acids are important regulators of glucose-6-phosphate levels in pancreatic islet cells, and in this way they may influence insulin secretion.

Islets of Langerhans were isolated from pancreas by collagenase digestion of tissue taken from male albino Wistar rats which had been fasted overnight. The separated islets were divided into groups of ten and incubated in bicarbonate-buffered medium containing albumin together with either glucose, octanoate, butyrate or citrate. In each experiment, the effects of low and high glucose were compared with those of low glucose plus organic acid. After 30 min of incubation at 37° C the medium was sampled for determination of its insulin content by the immunoassay of Hales and Randle[6]. Rat insulin provided standards for the assay. The islets from each group of incubation flasks were then pooled in pointed centrifuge tubes, the medium removed and the islets quickly frozen to −76° C by immersion of the tube in a bath of solid carbon dioxide and ethyl alcohol. They were then subjected to sonication for 20 s, after the addition of perchloric acid (0·1 ml./ten islets). The supernatants from the perchloric extracts were then neutralized with potassium carbonate, before the estimation of glucose-6-phosphate by the enzymatic cycling procedure described by Lowry *et al.*[7]. This gave us reproducible results in good agreement with those already reported by Matchinsky[8] for rat islets. Similar concentrations of glucose-6-phosphate have been reported by Idahl and Hellman[9] for islets taken from obese hyperglycaemic mice, in which β-cells constitute more than 90 per cent of the islet tissue.

The results obtained are shown in Table 1, which shows that butyrate, octanoate and citrate all stimulate the release of insulin from isolated rat islets of Langerhans, and that paralleling the secretion there is an increase in the intracellular concentrations of glucose-6-phosphate in the islets.

These results can be explained as an effect of short chain fatty acids on the intracellular accumulation of citrate which may then affect phosphofructokinase, resulting in an inhibition of glycolysis and accumulation of glucose-6-phosphate, as has been suggested by Randle *et al.* for other tissues[10]. Furthermore, the results do not support the idea that the release of insulin brought about by sugars is mediated by glycolysis, for, in the presence of fatty acids, glycolysis is inhibited, but rather they suggest that glucose has to be metabolized by some other pathway in order to stimulate insulin secretion. We have already indicated that the pentose phosphate pathway may be important in this respect[11].

Table 1. SECRETION OF INSULIN AND THE INTRACELLULAR CONCENTRATIONS OF GLUCOSE-6-PHOSPHATE IN ISOLATED RAT ISLETS OF LANGERHANS INCUBATED IN THE PRESENCE OF OCTANOATE, BUTYRATE AND CITRATE

| Addition to medium | Insulin secretion ($\mu$ units/10 islets/min; mean ± *S.D.*) | Glucose-6-phosphate levels ($\mu\mu$moles/40 islets; mean ± *S.D.*) |
|---|---|---|
| 2·5 mM glucose | 18 ± 4 (20) | 25·5 ± 7·0 (8) |
| 20·0 mM glucose | 39 ± 6 (20) | 59·5 ± 10·0 (8) |
| 2·5 mM glucose + 5·0 mM sodium citrate | 37 ± 5 (20) | 49·0 ± 12·0 (8) |
| 2·5 mM glucose | 18 ± 4 (20) | 26·5 ± 5·0 (8) |
| 20·0 mM glucose | 38 ± 5 (20) | 65·5 ± 11·0 (8) |
| 2·5 mM glucose + 5·0 mM sodium butyrate | 33 ± 4 (20) | 47·5 ± 10·0 (8) |
| 2·5 mM glucose | 19 ± 3 (20) | 22·5 ± 5·0 (4) |
| 20·0 mM glucose | 40 ± 5 (20) | 51·0 ± 5·0 (4) |
| 2·5 mM glucose + 5·0 mM sodium octanoate | 38 ± 4 (20) | 48·5 ± 7·0 (4) |

It is not known whether longer chain fatty acids can also exercise a regulatory effect by this means, because so far it has been difficult to obtain effects of albumin-bound long chain fatty acids on insulin secretion. In addition, ketone bodies have also not been shown to be effective in releasing insulin in most species, whether used *in vitro*[3,12] or *in vivo*[13]. Thus we found that 5 mM β-hydroxybutyrate did not alter rates of secretion when incubated with isolated rat islets. Nevertheless, ketone bodies and long chain fatty acids may be important in modifying insulin release in certain other species, for Madison *et al.* have obtained effects of these agents in inducing insulin secretion in the dog[14,15]. By contrast, a much more consistent effect of short chain fatty acids on release of insulin has been obtained. Our results indicate that the "glucose fatty acid cycle"[10] may be operative as a means of metabolic regulation in pancreatic islet cells.

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