Pancreatic Insulin in Diabetes Mellitus

JOHN R. TURTLE, M.D, M.R.A.C.P, Associate Professor in Medicine, University of Sydney, Australia

The nature of the pancreatic defect in diabetes mellitus has not been elucidated, although five decades of active research have been directed towards the problem since the discovery of insulin by Banting and Best in 1921. The disease is characterised by insulin deficiency either relative or absolute, but the metabolic disorder is only partly corrected by treatment with insulin. Until the precise nature of this insulin deficiency has been elucidated diabetes will remain a problem for the clinician and the investigator.

The delivery of insulin into the circulation from the pancreas involves two distinct processes: insulin synthesis and insulin secretion. Although these processes are interdependent to a certain extent, a number of stimuli or inhibitors act only on insulin secretion with no effect on insulin synthesis. Only by a detailed consideration of insulin synthesis and secretion can an attempt be made to understand the disorder in diabetes mellitus.

**Insulin Synthesis**

Insulin is synthesised in the pancreatic islet beta cell via a single chain precursor peptide proinsulin (Steiner et al., 1967). This peptide is cleaved at two positions by a trypsin-like enzyme to release the characteristic double-chain insulin molecule. Proinsulin appears to be an intermediate in the synthesis of insulin, accounting for between 2 and 5 per cent of the total pancreatic insulin content (Chance and Ellis, 1969). Proinsulin has little intrinsic hypoglycaemic activity, and it seems that it must be converted to insulin, with the release of a 32 amino acid ‘connecting peptide’, before any biological activity is manifest (Shaw and Chance, 1968).

Could there be a disorder of proinsulin synthesis, hydrolysis, or abnormal release of proinsulin into the circulation in diabetes mellitus? Although in normal subjects there is approximately 1 to 2 per cent of the total circulating immunoreactive insulin present as proinsulin, this may increase to 5 to 10 per cent in diabetes mellitus (Melani et al., 1970) and in obesity or other conditions where there is rapid insulin turnover. It seems likely that an increase in the concentration of circulating proinsulin reflects the rate of insulin turnover.
per se, rather than indicating the presence of a specific defect in the proinsulin hydrolysis mechanism. Silink and Turtle (1971) have developed a radioimmunoassay for proinsulin that does not cross-react with insulin, and they have used this to evaluate the control of proinsulin secretion in isolated pancreatic islets and in vivo. Proinsulin was secreted from the pancreas after stimulation by glucose, tolbutamide, theophylline, and beta adrenergic stimulation, and reflected purely precursor release under conditions of maximum insulin secretion. Furthermore, proinsulin was secreted into the circulation of obese patients, untreated diabetics, and patients with islet cell tumours of the pancreas in amounts which reflected the rapidity of insulin synthesis and release from the pancreas in these conditions.

Studies of the enzyme that activates the hydrolysis of proinsulin to insulin have not been undertaken, although it seems unlikely that there could be a specific defect of proinsulin hydrolysis in diabetes. It remains to be seen whether the connecting peptide, which may be released into the circulation after proinsulin hydrolysis, has any intrinsic biological activity and whether the measurement of circulating 'connecting peptide' in insulin-treated diabetics could provide an indication of potential pancreatic reserve in a situation in which insulin immunoassay or bioassay are not possible.

INSULIN SECRETION

As the insulin secreted by the pancreas each day is only 25 per cent of the total insulin content, an efficient storage system must be present to prevent massive insulin release and hypoglycaemia (Williams and Ensinck, 1966). During insulin secretion, the storage granule is made soluble and insulin is secreted by emiocytosis. Substrate, usually glucose, is utilised by the pancreatic islet when insulin is secreted. A number of systems are involved in insulin secretion. These probably feed into a final common pathway, either depending on substrate oxidation and the formation of ATP, or the uptake of calcium (Renold, 1970).

Recently it has been shown that there are two phases of insulin secretion. An acute release of insulin within the first 1 to 3 minutes after exposure to the stimulus is followed by a sustained prolonged increase of secretion over the next 20 to 30 minutes (Grodsky et al., 1970, Porte, 1970). Cerasi and Luft (1967) have shown that the insulin response to glucose is delayed in diabetes mellitus, and the early acute release of insulin does not occur. As there is a definite abnormality of the insulin secretory response pattern in diabetes, a study of the mechanism of action of a number of insulinogenic stimuli has been essential (Turtle, 1969a).
The Second Messenger Hypothesis

Adenosine 3'5' monophosphate (cyclic AMP) has been shown to be an intracellular mediator of the actions of many hormones (Sutherland et al., 1965, 1968). According to the concept of cyclic AMP as a second messenger, the hormones travel in the circulation to their target tissues to alter the concentrate of the cellular messenger, in this case cyclic AMP, usually by activating or inhibiting adenyl cyclase, the enzyme that synthesises cyclic AMP from ATP.

The Role of Cyclic AMP as a Determinant of Insulin Secretion

Turtle et al. (1967) showed that the methyl xanthine, theophylline, an inhibitor of the phosphodiesterase that hydrolyses cyclic AMP to AMP, activated insulin secretion in the rat. A biphasic effect on insulin secretion was noted with theophylline, similar to the effect of glucose. Turtle and Kipnis (1967) then demonstrated that the concentration of pancreatic islet cyclic AMP mediated insulin release from isolated pancreatic islets in response to theophylline, epinephrine, adrenergic blocking agents, and glucagon. Subsequently Hagon and Turtle (1969), McCluskey et al. (1970) and Turtle and Hagon (1970) have shown that a number of hormones that activate or inhibit insulin secretion do so by a direct action on adenyl cyclase, thus changing the intracellular concentration of cyclic AMP. Gastrin, glucagon, secretin, and epinephrine all have a definitive action on insulin secretion in this manner.

The Action of Cyclic AMP on Pancreatic Islet Metabolism

Although a number of hormones modified insulin secretion by acting on cyclic AMP, the mechanism whereby cyclic AMP itself activated insulin secretion has not been defined. Turtle (1969b) showed that inhibitors of phosphorylation, such as mannoseptulose and glucosamine, or inhibitors of glucose-6-phosphate, such as 2-deoxyglucose, all inhibited cyclic AMP-activated pancreatic insulin secretion, indicating that some processes in glycolysis were necessary for insulin secretion to occur after cyclic AMP stimulation. Recently, McCluskey et al. (1970) have used an isolated teleost islet system in an attempt to overcome the difficulties of juxtaposition of endocrine and exocrine tissue in mammalian islets. This system responded to hormones and cyclic AMP with insulin secretion in a manner similar to mammalian islets. In the teleost islet, cyclic AMP activated insulin secretion only if glycogen were present and phosphorylase activation occurred. After prolonged fasting, islet glycogen was depleted and insulin secretion did not occur. If adequate glycogen was present, cyclic AMP caused a 50 per cent fall in glycogen and increased both glucose and ATP. Thus, the actions of those
hormones that depend on the cyclic AMP mechanism in the pancreas are mediated through a final common pathway that provides substrate, in this case glucose, for energy production via glycolysis.

**Could the cyclic AMP System be Disordered in Diabetes Mellitus?**
Whatever is the cause of diabetes, a number of the metabolic consequences of the disease are a direct result of changes in the cyclic AMP in other tissues. In adipose tissue, insulin deficiency leaves the actions of the lipolytic hormones unopposed. Epinephrine activates lipolysis via cyclic AMP in adipose tissue, leading to free fatty acid release—a major factor in the development of ketosis. In liver, insulin deficiency leaves epinephrine and glucagon unopposed, resulting in gluconeogenesis, glycogenolysis, and inhibition of glycogen synthesis, all mediated by cyclic AMP (Sutherland and Robison, 1969). Whether or not there could be a disorder in the pancreatic cyclic AMP system that is responsible for the absence of insulin secretion from the acute pool in diabetes is a subject of great interest to investigators in diabetes research.

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