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by both increasing its deposition and inhibiting its removal. It is suggested that insulin thus plays a major role in the pathogenesis of atherosclerosis.

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et al. (1965). In any one experiment animals of the same weight were used. The animals were injected intravenously, using the tail vein, with Gey and Gey buffer (2.5 ml. per 100 g. body-weight) containing 4 PC per ml. of the 14C-labelled substrate alone was injected. Since insulin is known to inhibit tissue-lipase in arterial tissue, excess of circulating insulin is likely to reduce the rate of removal. It is suggested that insulin thus plays a major role in the pathogenesis of atherosclerosis.

Introduction

The association of ischemic vascular disease and diabetes mellitus has been known for many years (Brunnhofer, 1927, Warren et al., 1966), and has been confirmed by the International Atherosclerosis Project (Robertson and Strong 1940). It has recently become apparent that abnormalities of carbohydrate metabolism are present in a large proportion of patients with ischemic heart-disease, without clinical diabetes mellitus. These abnormalities may be summarised as follows:

(a) Many patients with premature coronary-artery disease have hyperglycemia and abnormal glucose-tolerance tests (e.g., Smoov et al., 1962, Cohen and Shaffir 1961, Epstein 1967). That this abnormality is not a result of acute myocardial infarction is shown by its presence long after the acute episode, and also in patients with angiographically proven coronary arteriosclerosis without myocardial infarction (Focht et al., 1966, Heine et al., 1967). Abnormal high levels of fasting-cuing insulin have been found in subjects with similar abnormalities of glucose tolerance (Vale and Basset 1960, Heet et al., 1965).

(b) Abnormally high insulin responses to glucose loading have been found in patients with ischemic heart-disease (Nikula et al., 1965, Peters and Hales 1965, Traupmann et al., 1967).

(c) Increased striolbuim insulin-antagonism was found in 75% of patients with ischemic heart-disease compared with 27% of normal controls (Voluce-Owen and Ashton 1963).

(d) Abnormal plasma-lipid patterns, similar to those found in diabetes, are common in patients with ischemic heart-disease (Adleserberg and Eister 1959, Arltbe et al., 1965). In particular, carbohydrate-induced hypertriglyceridemia is common in these patients (Reaven et al., 1960)—an abnormality which is associated with high plasma-insulin levels (Dawson and Allbringer 1966, Reaven et al., 1967, Eden and Fahnler 1968).

The common factor in these abnormalities is a high level of circulating insulin. It therefore seemed important to investigate the action of insulin on vascular metabolism.

Method

Male Winter rats weighing 90-120 g. fed and maintained, were investigated by a modification of the method of Radford et al. (1965). In any one experiment animals of the same weight were used. The animals were injected intravenously, using the tail vein, with Gey and Gey buffer (2.5 ml. per 100 g. body-weight) containing 4 5C per ml. of the 14C-labelled substrate alone or with 4000 units per ml. of insulin. The substrate was sodium-acetate-1-14C (specific activity 64 mCi per mmole) in the first set of experiments, and 2-glucone-1-14C (2 mCi per mmole) in the second. In each experiment half of the animals received buffer and substrate, and the remainder buffer, substrate, and insulin. 1 hour after injection the animals were killed and the radioactivity in the arterial tissues determined. The whole aorta was removed to obtain a representative sample of the dissected free of adventitious tissue. It was then weighed on a torsion balance and placed in 1 ml. of a 2:1 solution of chloroform/methanol. Total lipids were isolated and purified by a modification of the method of Focht et al. (1967). The aorta was reweighed in chloroform/methanol (2:1) at 70°C for 3 hours (Hun and Reav 1966). The solution was centrifuged and the supernatant washed with calcium chloride (0.5%) to remove water-soluble radioactive compounds. The purified lipids were made up to 2.5 ml. 0.5 per cent. samples were pipetted into 5 ml. of scintillation fluid and counted for 10 minutes in a Packard 3320 liquid scintillation spectrometer. The channel-background was determined by counting 200,000 counts and subtracting the channel-background. The channel-background was used to correct for channel-background. The channel-background was used to correct for channel-background. The channel-background was used to correct for channel-background.
The mean uptake of sodium-acetate-1-14C, in counts per mg. per minute 24.8±5.1, was 0.0039±0.0002 (100%) in 16 controls, and 0.0135±0.0068 (123.9%) in 17 rats which received insulin. The mean uptake of D-glucose-1-14C, in counts per mg. per minute 24.8±5.1, was 0.037±0.010 (100%) in 16 controls, and 0.0214±0.0026 (117.5%) in 20 rats which received insulin. The values obtained in each experiment were similar. Application of Student’s t test shows that the difference between the control and insulin-treated groups in each site is highly significant (P<0.01). These results show that insulin greatly enhances the incorporation of both glucose and acetate into the lipids of the rat aorta.

The 14C-labelled lipid in the aortic wall is probably triglyceride synthesised from fatty acid and D-glucopyrophosphate, both of these having been metabolised from the injected substrates (Chernick et al. 1949). Further work on this is in progress.

Discussion

The results reported here show that insulin stimulates the synthesis of triglyceride and cholesterol in the arterial wall and suggests that this results in the accumulation of important amounts of lipid. It is well known that maturity-onset diabetics, who have high serum-insulin levels, lay down fat in their adipose tissue. It is suggested, that by the same mechanism, they can also accumulate fat in their arterial walls. This process would act for many years, long before the diabetes mellitus became clinically apparent. Indeed, a vascular catastrophe may well precede the appearance of clinical diabetes, and appropriate dietary treatment at this time may prevent it ever appearing.

Mahler (1965) has shown that insulin inhibits tissue-lipase in arterial tissue, and suggests that this results in the accumulation of lipids and hence the formation of atherosclerosis. The present work provides evidence that insulin also stimulates lipogenesis in vascular tissue, an action similar to its effect on adipose tissue (Renold et al. 1965). Excess of circulating insulin would thus cause an accumulation of protein and fat in both insulin-resistant and insulin-sensitive tissue, with its deposition and inhibiting its removal.

The published reports contain no consistent evidence for a direct and rapid action of insulin on vascular tissue. Wyethberger and Ben-Tor (1965) reported that the oxygen consumption and glucose uptake of rat aortas were depressed by alloxan diabetes and increased by insulin in vivo and in vitro. Other workers have confirmed the effect of alloxan diabetes (Foquet and Sipiersten 1964, Urensilu et al. 1963, Yalcin and Winegrad 1963), but were unable to demonstrate any insulin effect in vitro on either normal or diabetic aortas (Molchandani and Winegrad 1967, Urensilu et al. 1963). Treatment of diabetic animals with insulin for more than 18 hours was required to return arterial metabolism towards normal. It seems, therefore, that the insulin effect is unobservable for demonstrating insulin action on vascular tissue. The reason is not clear.

There has been no reported attempt to induce experimental atherosclerosis with insulin. However, the opposite situation has been found to occur. Rabbits rendered insulin-deficient by adenohypophyseal lesions developed less striking vascular lesions on an atherogenic diet than normal controls (Duff and McMillan 1949, McGill and Voldman 1949). This effect was reversed by treatment with insulin (Duff et al. 1954). Stansler et al. (1960) found that administration of insulin inhibited the reduction of atherogenic lesions in chickens when the atherogenic diet was replaced by a normal diet. Insulin also had an inhibitory effect on the atherogen-promoting action of exogenous oestrone in chickens.

The evidence presented here, together with that published recently, supports the hypothesis that insulin plays a major role in the pathogenesis of atherosclerosis (e.g. Voldman et al. 1967). Further work is in progress.

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