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Postreceptor effect of metformin on insulin action in mice

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To investigate the possibility that metformin (dimethylbiguanide) modifies insulin-mediated glucose metabolism by an effect that is independent of insulin receptor binding, glycogenesis and insulin binding were measured in soleus muscles isolated from streptozocin diabetic mice after treatment with 60 mg kg^{-1} metformin daily for 10 weeks. This dose of metformin increased insulin-stimulated glycogenesis but did not affect insulin binding in the soleus muscles of streptozocin diabetic mice. The results suggest that metformin can influence postreceptor sites of insulin action independently of insulin receptor binding.

The antihyperglycaemic agent metformin (dimethylbiguanide) is used in the treatment of type 2 (non-insulin dependent) diabetes mellitus (Hermann 1979; Van der Kuy & Hulst 1981). The antihyperglycaemic action of the drug has been attributed to a reduced rate of intestinal glucose absorption (Lorch 1971), reduced gluconeogenesis (Meyer et al 1967), and increased glucose uptake and metabolism by skeletal muscle (Frayn & Adnitt 1972; Bailey & Puah 1985). Metformin improves glucose tolerance in type 2 diabetic patients without stimulating insulin secretion, but there is evidence that a presence of insulin is required, suggesting that metformin might potentiate insulin action (Hermann 1979; Bailey 1985). This could be achieved in part by increased insulin receptor binding, which has been observed during metformin treatment (Holle et al 1981; Lord et al 1983a). However, metformin can increase the hypoglycaemic response to insulin in streptozocin diabetic mice without a measurable effect on hepatocyte insulin receptor binding (Lord et al 1983b). Thus the drug might influence postreceptor sites of the insulin effector pathway independently of effects at the level of the insulin receptor. To investigate this possibility, the present study examines the effect of metformin on insulin-stimulated glycogenesis in soleus muscles of streptozocin diabetic mice, using a dose of metformin which does not produce a measurable effect on insulin receptor binding.

Materials and methods

Adult, male, Theiller Original, albino mice were used at 30 weeks of age. The mice were maintained as described previously (Lord et al 1983b). Diabetes was induced by an intraperitoneal injection of streptozocin (100 mg kg^{-1} in citrate buffer, pH 4·8) after a 6 h fast. (Streptozotocin, Sigma, Poole, UK).

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Blood samples (30 μl) for plasma glucose determination (Stevens 1971) were taken from the tail tip of conscious mice after 2 weeks, and the mice were divided into two equihyperglycaemic groups. One group received metformin hydrochloride (60 mg kg^{-1} daily for 10 weeks) in the drinking water, and the other group served as an untreated diabetic control. Body weight and fluid intake were monitored, and the concentration of metformin in the drinking water was adjusted accordingly (Lord et al 1983b). A group of mice which was not treated with either streptozocin or metformin served as an untreated normal (non-diabetic) control.

Blood samples for plasma glucose determination were taken on the day of experimentation. Mice were then killed by cervical dislocation, and soleus muscles were isolated (Maizels et al 1969), weighed, washed in saline and their tendons tied across a polythene horseshoe to maintain a slight tension. Individual muscles were preincubated and incubated in Krebs-Ringer bicarbonate buffer supplemented with 20 mg ml^{-1} insulin-free bovine serum albumin, pH 7·4, gassed with 95% O_2 and 5% CO_2 and shaken at 92 cycles min^{-1}. Glucose and other constituents were added as described below.

For determination of glycogenesis, muscles were preincubated at 37 °C for 15 min in 2 ml of buffer containing 5 mM glucose. The muscles were then incubated at 37 °C for 60 min in 3 ml of buffer containing 5 mM glucose, 0·5 μCi ml^{-1} [U-^{14}C]glucose and either no insulin or 6·8 nM insulin. Formation of [^{14}C]glycogen was determined as previously (Puah & Bailey 1985).

Insulin receptor binding was assessed by preincubating muscles at 20 °C for 15 min in 2 ml of buffer containing 2 mM pyruvate. Incubations were at 20 °C for 4 h without shaking in 1·5 ml of identical buffer supplemented with 0·17 nM [^{125}I]insulin (specific activity 250–280 μCi $μg^{-1}$) and varying concentrations of unlabelled insulin (range 0·5–90 nM). Non-specific binding was determined by incubations in the presence of 8 μM insulin. After incubation, muscles were washed 5 times for 5 min in 3 ml of ice-cold saline containing 5 mg ml^{-1} bovine serum albumin. Muscles were then hydrolysed in 0·3 ml of 1 M NaOH, and aliquots were removed for determination of protein (Lowry et al 1951) and ^{125}I-radioactivity.

Groups of data were compared using Student's *t*-test. Differences were considered to be significant for $P < 0·05$.

Results

The dose of streptozocin used (100 mg kg^{-1}) produced a mild to moderate hyperglycaemia (normal 7·3 ± 0·5 mmol $litre^{-1}$, n = 6; untreated streptozocin 17·5 ± 2·4 mmol $litre^{-1}$, n = 8; $P < 0·05$). Body weight in untreated streptozocin diabetic mice (43·8 ± 1·4 g, n = 8) was not different from normal mice (40·5 ± 1·1 g, n = 6), although weight loss may occur in more severely streptozocin diabetic mice (Bailey & Puah 1985). Untreated streptozocin diabetic mice showed similar soleus muscle wet weight (15·4 ± 0·7 mg, n = 16) and soleus muscle protein content (1·10 ± 0·06 mg $muscle^{-1}$, n = 16) to normal mice (14·5 ± 0·8 mg and 1·33 ± 0·22 mg $muscle^{-1}$, n = 12, for wet weight and protein content, respectively). Treatment of streptozocin diabetic mice with metformin (60 mg kg^{-1} day^{-1} for 10 weeks) did not significantly alter plasma glucose concentrations (15·9 ± 2·0 mmol $litre^{-1}$, n = 6), body weight (42·8 ± 0·9 g, n = 6), soleus muscle wet weight (15·5 ± 0·8 mg, n = 12) or soleus muscle protein content (1·09 ± 0·08 mg $muscle^{-1}$, n = 12).

Basal glycogenesis (in the absence of added insulin) was similar in soleus muscles of the three groups of mice studied (Fig. 1). Insulin (6·8 nM) increased glycogenesis in each group. Muscles of untreated streptozocin diabetic mice showed a smaller insulin-stimulated increase in glycogenesis than those of normal mice. Treatment with metformin (60 mg kg^{-1} daily) for 10 weeks improved the insulin-stimulated increase in glycogenesis by muscles of streptozocin diabetic mice.

Specific binding of [^{125}I]insulin was calculated as the difference between total binding and non-specific binding. The latter was approximately 20% of total binding. Fig. 2 shows the percentage of specifically bound insulin plotted as a competition curve against total insulin. At a low concentration of total insulin, specific binding of insulin was increased in soleus muscles of streptozocin diabetic mice. However, the metformin treatment did not significantly alter specific insulin binding in the muscles of streptozocin diabetic mice.

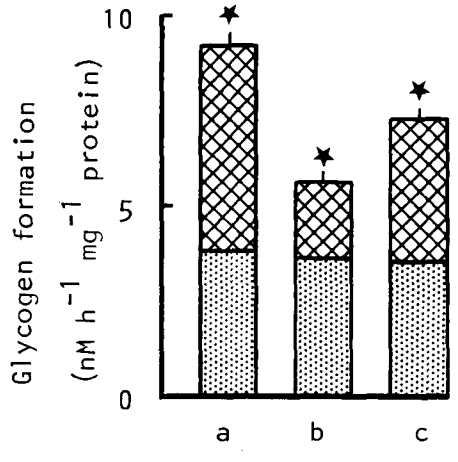

FIG. 1. Basal (dotted bars) and insulin-stimulated (hatched bars) (6·8 nM insulin) increase in glycogenesis of isolated soleus muscles from untreated normal mice (a), untreated streptozocin diabetic mice (b), and streptozocin diabetic mice treated with 60 mg kg^{-1} day^{-1} metformin for 10 weeks (c). Values are mean ± s.e.m. of 5 determinations. *$P <$ 0·05 compared with all other groups.

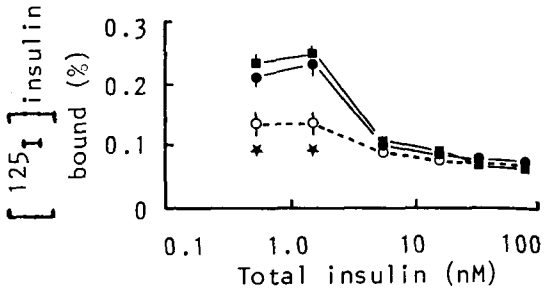

FIG. 2. Specific insulin binding to soleus muscles of untreated normal mice (○), untreated streptozocin diabetic mice (●) and streptozocin diabetic mice treated with 60 mg kg^{-1} day^{-1} metformin for 10 weeks (■). Data are presented as a competition curve showing the percentage of specifically bound [^{125}I]insulin plotted against the total insulin concentrations. Values are mean ± s.e.m. of 6 determinations. *$P < 0·05$ compared with untreated and metformin treated streptozocin diabetic mice.

Discussion

The present study supports previous evidence that specific insulin binding to soleus muscles and hepatocytes is increased in streptozocin diabetic mice (Le Marchand-Brustel & Freychet 1979; Lord et al 1983b). Since streptozocin destroys pancreatic B-cells and reduces insulin concentrations (Cooperstein & Watkins 1981), increased insulin binding may reflect, at least in part, the lifting of a down-regulatory effect of normal insulin concentrations (Bailey et al 1984).

At the dose used (60 mg kg^{-1} daily) metformin did not significantly alter plasma glucose concentrations or insulin binding to soleus muscles of streptozocin diabetic mice. Although larger doses of the drug (e.g. 250 mg kg^{-1} daily) exert little effect on plasma glucose concentrations in mice with long-standing severe streptozocin diabetes, such doses considerably reduce the development of streptozocin-induced hyperglycaemia in mice, associated with a small increase in the binding of insulin to soleus muscles (Bailey & Puah 1985).

While treatment with 60 mg kg^{-1} daily metformin did not measurably affect specific insulin binding, insulin-stimulated glycogenesis was increased in soleus muscles of streptozocin diabetic mice. The concentration of insulin (6·8 nM) added to the muscle preparation to stimulate glycogenesis considerably exceeded the normal range of circulating insulin concentrations. However, degradation of insulin during the 60 min

incubation period may bring this concentration into relevance for the pathophysiological hyperinsulinaemia in some syndromes of type 2 diabetes. Thus in the presence of a high concentration of insulin, it appears that metformin can enhance glycogenesis by an isolated soleus muscle preparation independently of a change in insulin receptor binding. This suggests that the drug may act at postreceptor sites of the insulin effector pathway.

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A study on the aetiology of reserpine ulceration and the antiulcer action of solcoseryl in rat stomach

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The aetiology of reserpine-induced gastric ulcer formation and the antiulcer effects of solcoseryl were studied in rats. Intraperitoneal injection of reserpine produced severe ulceration, as well as mast cell and histamine depletion, in the gastric glandular mucosa. Mepyramine and cimetidine markedly antagonized the gastric lesions, but did not influence the reduced mast cell count; atropine pretreatment significantly inhibited both parameters. Intramuscular injection of solcoseryl lessened ulcer severity and prevented the decreased mast cell counts and histamine levels in reserpine-treated rats. However, the same dose of solcoseryl injected intraperitoneally was ineffective. Solcoseryl, irrespective of the route of administration, did not influence the gastric secretory activities of reserpine. It is concluded that resperine ulceration is both cholinergic- and histamine-mediated, and that the antiulcer effects of solcoseryl appear to be due to prevention of histamine depletion in the gastric mucosa.

Cholinergic activation and consequent mast cell degranulation in gastric glandular mucosae have been shown to contribute largely to ulceration (Ogle & Cho 1977a, 1978, 1979). However, the ulcerogenic mechanisms due to mast cell degranulation are not clear. A similar sequence of events has been observed in stressed animals where significant falls in stomach wall mast cell counts are causally related to increases in gastric histamine release (Cho & Ogle 1978, 1979; Ogle & Cho 1977b). Solcoseryl, a non-protein extract from calf serum has been shown to hasten the healing of skin ulcers (Barre & Alechinsky 1963) and to prevent stress gastric ulceration (Barre & Alechinsky 1963; Debray et al 1972; Jaeger et al 1979). It is, therefore, conceivable that solcoseryl may antagonize ulceration and prevent changes in gastric glandular mucosal histamine levels caused by reserpine. In this study, the aetiology of reserpine ulceration is further investigated by direct measurement of the gastric mucosal histamine content in an attempt to relate any changes to mast cell degranulation in the same region of the stomach. The effects of histamine H_1- and H_2-receptor antagonists are also examined in order to determine the importance of the amine in the pathogenesis of reserpine ulceration. The influence of first pass inactivation of solcoseryl through the liver is also examined by comparing the effects of intramuscular and intraperitoneal routes of its administration.

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