Insulin-like effect of pinitol

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Introduction

D-chiroinositol is structurally related to the phosphatidylchino-
ositol phosphates which participate in the insulin signalling
pathways that stimulate glucose transport (Holman & Kasuga,
1997; White, 1997). Reduced urinary excretion of D-
chiroinositol has been observed in rhesus monkeys and human
subjects with impaired glucose tolerance, insulin resistance and
type 2 diabetes mellitus (Kennington et al., 1990; Ortmeeyer
et al., 1993a; Suzuki et al., 1994). Acute administration of D-
chiroinositol reduced plasma glucose concentrations in streptozotocin (STZ)-diabetic rats, and increased glucose
utilization in insulin-resistant monkeys (Ortmeeyer et al.,
1993b; Fonteles et al., 1996). D-chiroinositol also improved
glucose tolerance in normal rats and increased glycosynthesis in
diaphragm (Ortmeeyer et al., 1993b; Huang et al., 1993).
The leaves of Bougainvillea spectabilis are used as a
traditional treatment for diabetes in Asia and the West Indies
(Narayanan et al., 1987). D-pinitol (1D-3-0-methyl-chiroino-
ositol; Figure 1), a 3-methoxy analogue of D-chiroinositol, has
been identified as an active principle, and is reported to reduce
glucose concentrations in alloxan diabetic rats (Narayanan et
al., 1987). Since this observation has not been confirmed or
evaluated from a potential therapeutic perspective, the present
study has investigated the effect of D-pinitol on blood glucose
homeostasis in normal mice, hypoinsulinaemic STZ-diabetic
mice and hyperinsulinaemic ob/ob mice. The study has also examined the effect of D-pinitol on glucose
transport by cultured L6 muscle cells.

Methods

The chemicals and their sources were: D-pinitol from Aldrich,
Gillingham, U.K.; human actrapid insulin from Novo Nordisk,
Crawley, U.K.; 2-deoxy-D-[3H]-glucose (15.0 Ci mmol
-1) from Amersham International, Amersham, U.K.; Hi-safe II
scintillant from Fisons, Loughborough, U.K.; cell culture
reagents from Gibco, Paisley, U.K.; other chemicals from Sigma,
Poole, U.K. and BDH, Poole, U.K. Plastic ware was from
Sarstedt, Leicester, U.K.

Animals

Obese-diabetic ob/ob mice and normal homozygous lean +/+ mice from the Aston colony were used at 12–16 weeks of age. The origin and characteristics of these mice have been described previously (Flatt & Bailey, 1981; Bailey et al., 1982). Mice were housed in an air-conditioned room at
22±2°C with a lighting schedule of 12 h light (0800–2000 h) and 12 h dark. A standard pellet diet (Economy Rodent Breeding Diet, SDS, Witham, Essex, U.K.) and tap water were provided ad libitum. Hypoinsulinaemic diabetes was induced in 5 h fasted lean mice by intraperitoneal (i.p.) injection of STZ 160 mg kg⁻¹ in citrate buffer, pH 4.5. Food was returned 4 h after injection of STZ, and animals were accepted as diabetic if the basal plasma glucose concentration was >12 mmol l⁻¹ after 9 days. For the 62 STZ-diabetic mice used in the present study the plasma glucose and insulin values (mean ± s.e.mean) were 19.8±1.5 mmol l⁻¹ and 160±51 pmol l⁻¹. Values in the control non-diabetic state were 7.2±0.5 mmol l⁻¹ and 283±33 pmol l⁻¹.

**Procedures with animals**

D-pinitol was dissolved in phosphate buffered saline for oral (p.o.) administration by gavage, and by i.p. injection. The acute effect of D-pinitol p.o. was determined in STZ-diabetic and obese-diabetic ob/ob mice. D-pinitol was administered at doses up to 100 mg kg⁻¹, and controls received vehicle only (5 ml kg⁻¹). Blood samples (20 μl) were taken from the tail tip at intervals up to 6 h. Food was withheld for the duration of all acute tests. An insulin hypoglycaemia test was undertaken in STZ-diabetic mice 6 h after administration of D-pinitol (100 mg kg⁻¹ p.o.). Human actrapid (1 u kg⁻¹) was administered i.p. injection and blood samples were taken at intervals up to 2 h thereafter. The acute effect of D-pinitol (5 and 100 mg kg⁻¹ p.o.) was also studied in normal lean nondiabetic mice, and blood samples were taken up to 4 h. An i.p. glucose challenge was then given (2 g kg⁻¹ glucose in a 40% w v⁻¹ solution) and blood samples were taken up to 90 min thereafter. The acute effect of D-pinitol (100 mg kg⁻¹) administered i.p. was studied over 6 h in STZ-diabetic mice.

The chronic effect of D-pinitol (100 mg kg⁻¹ i.p. twice daily) was determined in STZ-diabetic mice. Mice were studied for 11 days on D-pinitol treatment, and for 10 days after D-pinitol treatment was stopped. A control group received injections of vehicle only. Blood samples were taken for plasma glucose analysis, and body weight and food intake were noted at intervals.

**Analyses**

Plasma glucose was measured by an automated glucose oxidase procedure (Stevens, 1971), and insulin was measured by radioimmunoassay using an Amerlex magnetic separation procedure (Amersham Life Science, Amersham, U.K.) with rat insulin as standard.

**L6 muscle cells**

Cultured L6 muscle cells (Yaffe, 1968) from the European Culture Collection, Porton Down, U.K. were grown as monolayers in Dulbecco’s modified Eagle’s medium (DMEM) containing 1 mM glutamate, 1 mM pyruvate and 25 mM glucose. The medium was supplemented with 5% foetal calf serum (FCS), 100 u ml⁻¹ penicillin G, 100 μg ml⁻¹ streptomycin and 25 μg ml⁻¹ amphotericin B. Cells were maintained at 37°C with humidified 95% air and 5% CO₂. Experiments were undertaken in 24-well plates seeded from pre-confluent flasks with 5×10⁶ cells in 1 ml. The cells (passage 10) were grown to confluence and the medium was changed to DMEM containing 0.5% FCS and 2.5 mM glucose for 24 h to induce differentiation and fusion of myoblasts into myotubes and preclude further division (Poyner et al., 1992). Myotubes were then incubated with test substances (bovine insulin 10⁻⁶ M and/or D-pinitol 10⁻⁶–10⁻³ M and/or LY294002 10⁻⁵ M) for 10 min, 4 h or 24 h.

After exposure to test substances for incubations of 10 min to 24 h, uptake of 2-deoxyglucose (2DG) was determined during an additional incubation period of 10 min. The monolayers of myotubes were washed with glucose-free Krebs Ringer bicarbonate (KRB) buffer at 22°C, then incubated in 1 ml of this buffer supplemented with 0.1 mM 2-deoxy-D-glucose and 2-deoxy-D-[³H]-glucose (0.2 μCi ml⁻¹) for 10 min at 22°C. Buffer was aspirated, and cells were washed twice with ice-cold KRB buffer, lysed with 0.5 ml 1 N NaOH, and ³H was counted in 5 ml Hi-safe II scintillant using a Packard 1900TR liquid scintillation counter. Most experiments were undertaken three times in multiples of six wells (i.e. n = 18). Six control wells and six wells treated with 10⁻⁴ M insulin were included in each 24 well plate. Uptake of 2DG was expressed as the percentage change compared with control (100%).

**Statistical analyses**

Data are presented as mean ± s.e.mean. Treatment groups for animal studies were compared by ANOVA and data at individual time points were compared to time zero by Student’s paired t-test. Comparisons between groups at the same time points, and between tissue culture treatments were made using Student’s unpaired t-test with Bonferroni’s correction. Probability values of P<0.05 were considered to be significant.

**Results**

**STZ-diabetic mice**

Acute oral administration of D-pinitol (5, 10 and 100 mg kg⁻¹) to STZ-diabetic mice produced a progressive decrease in plasma glucose over 6 h (Figure 2). The greatest effect was observed with a dose of 100 mg kg⁻¹ D-pinitol which significantly decreased plasma glucose by 12% (P<0.05) at 2 h and 22% (P<0.02) at 6 h. The plasma insulin concentration was unaltered by D-pinitol treatment (e.g. 168±34 and 153±17 pmol l⁻¹, mean ± s.e.mean, n = 10, pretreatment and 6 h after 100 mg kg⁻¹ p.o. D-pinitol respectively). Since 100 mg kg⁻¹ D-pinitol produced the greatest glucose-lowering effect over 6 h, this dose was used for subsequent studies. To investigate the effect of D-pinitol on insulin sensitivity in STZ-diabetic mice, D-pinitol (100 mg kg⁻¹ p.o.) was administered as above. After 6 h, when plasma glucose was decreased by 20% (P<0.05; Figure
insulin (1 u kg⁻¹ i.p.) was injected. The rate of glucose disappearance over the 40 min after insulin injection was similar in the D-pinitol (1.1 ± 0.2% per min) and control (1.3 ± 0.2% per min) groups (Figure 3). Acute i.p. administration of D-pinitol (100 mg kg⁻¹) to STZ-diabetic mice decreased plasma glucose at 6 h by 21% (P < 0.05), similar to the decrease produced by oral administration of the same dose of D-pinitol (10⁻³ M D-pinitol increased basal 2DG uptake at each of the three time points studied, but lower concentrations of D-pinitol (10⁻⁴ – 10⁻⁶ M) did not exert a significant effect. D-pinitol (10⁻³ M) produced a larger increase in 2DG uptake at 10 min and 4 h (by 41 and 34% respectively, P < 0.01 versus control) than at 24 h (15% increase, P < 0.05 versus control; P < 0.05 versus 10 min and 4 h). At 10 min the increase in 2DG uptake produced by 10⁻³ M D-pinitol (by 41%) was not statistically significant compared to the control, but it was significantly different from the control at 24 h (P < 0.05).

**Normal mice and obese-diabetic (ob/ob) mice**

Acute oral administration of D-pinitol (100 mg kg⁻¹) to normal non-diabetic mice did not significantly alter basal plasma glucose or insulin concentrations over 4 h, and an i.p. glucose tolerance test at 4 h was also unaffected by the D-pinitol treatment (data not shown). Also, acute oral administration of D-pinitol (100 mg kg⁻¹) did not significantly alter plasma glucose or insulin concentrations over 6 h in ob/ob mice (data not shown).

**L6 muscle cells**

2-deoxy-[³H]-glucose (2DG) uptake by L6 myotubes was stimulated by insulin (24 h incubation) in a concentration-dependent manner with a maximum effect at 10⁻⁷ M, and an ED₅₀ of 2.19 × 10⁻⁸ M (Figure 5). 10⁻⁸ M insulin increased 2DG uptake at 10 min, 4 h and 24 h by 25, 42 and 49% respectively (P < 0.05 versus control; Figure 6). In the absence of added insulin, 10⁻³ M D-pinitol increased basal 2DG uptake at each of the three time points studied, but lower concentrations of D-pinitol (10⁻⁴ – 10⁻⁶ M) did not exert a significant effect. D-pinitol (10⁻³ M) produced a larger increase in 2DG uptake at 10 min and 4 h (by 41 and 34% respectively, P < 0.01 versus control) than at 24 h (15% increase, P < 0.05 versus control; P < 0.05 versus 10 min and 4 h). At 10 min the increase in 2DG uptake produced by 10⁻³ M D-pinitol (by 41%) was not statistically significant compared to the control, but it was significantly different from the control at 24 h (P < 0.05).
Figure 6 2-deoxy-[3H]-glucose (2DG) uptake by L6 myotubes incubated for 10 min, 4 h and 24 h with insulin (10^-8 M) and/or D-pinitol (10^-3 M). Data are expressed as per cent control (no addition of insulin or D-pinitol), mean ± s.e.mean, n = 18. *P < 0.05 versus control at same time interval; †P < 0.05 versus insulin alone.

Figure 7 2-deoxy-[3H]-glucose (2DG) uptake by L6 myotubes incubated for 4 h with insulin (10^-8 M), D-pinitol (10^-3 M) and/or LY294002 (10^-5 M). C, control; I, insulin; P, D-pinitol; L, LY294002. Data are expressed as per cent control (no addition of insulin or D-pinitol), mean ± s.e.mean, n = 6. *P < 0.05 versus control; †P < 0.05 versus D-pinitol.

Discussion

In this study D-pinitol, the 3-methoxy analogue of D-chiroinositol, exerted an acute and chronically-sustained antihyperglycaemic effect in the murine STZ-induced model of hypoinsulinaemic diabetes. D-pinitol also acutely increased basal (but not insulin-stimulated) 2DG transport by L6 muscle cells. The ability of D-pinitol to reduce the hyperglycaemia of STZ-diabetic mice to near-normal glucose concentrations cannot be attributed to increased insulin concentrations. A change in food consumption can also be excluded, since food was withheld during acute studies and was not significantly altered during chronic D-pinitol treatment. Augmentation of insulin action is also unlikely, because D-pinitol did not significantly increase insulin-stimulated glucose disappearance in STZ-diabetic mice or insulin-stimulated glucose uptake in cultured L6 muscle cells. However, a direct effect of D-pinitol on muscle glucose metabolism could contribute to the antihyperglycaemic effect, since D-pinitol increased basal glucose uptake by cultured L6 muscle cells. The concentration of D-pinitol (10^-3 M) required to significantly increase glucose uptake by the L6 cells was not inconsistent with the most effective dosage used in vivo (100 mg kg^-1) for a lipophilic agent with a large volume of distribution.

Thus D-pinitol might exert an insulin-like effect on glucose transport that is independent of insulin. This does not preclude the possibility that D-pinitol could interact with a pathway of insulin signalling. By analogy with D-chiroinositol (Ortmeyer et al., 1993b; Huang et al., 1993), there is a structural similarity of D-pinitol with inositol phosphates involved in the signalling of insulin via PI3K and protein kinase B (PKB) (Holman & Kasuga, 1997; White, 1997). Indeed the effect of D-pinitol was prevented by LY294002, an inhibitor of PI3K. If D-pinitol provided a substrate or surrogate signal for this pathway it might increase glucose transport without increasing the effect of insulin. However, D-pinitol slightly reduced the effectiveness of insulin in L6 muscle cells by 24 h. Hence it is conceivable that an acute interaction of D-pinitol with the signalling pathways of insulin to glucose transport could limit the extent to which these pathways can be stimulated subsequently by insulin.

Consistent with this view there was no acute effect of D-pinitol on glucose or insulin concentrations in normal non-diabetic mice.

D-pinitol, like insulin (Bailey & Flatt, 1997), was not effective in severely insulin resistant ob/ob mice. These mice probably incur multiple lesions within the signalling pathways between the insulin receptor and glucose transport (Bailey & Flatt, 1997), which could involve rate-limiting defects distal to the site of interaction with D-pinitol.

It is concluded that D-pinitol exerts an acute and chronic insulin-like antihyperglycaemic effect in STZ-diabetic mice. The mechanism of action of D-pinitol does not augment the effect of insulin but might involve an interaction with part of a cellular signalling pathway that links insulin with glucose transport.

References


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