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J. Pharm. Pharmacol. 1989, 41: 345–346
Communicated April 27, 1988

L-Ascorbic acid produces hypoglycaemia and hyperinsulinaemia in anaesthetized rats

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Abstract—L-Ascorbic acid (Vitamin C) produced a marked reduction in the blood glucose concentration following intravenous injection (20-100 mg kg^{-1}) to anaesthetized rats. This hypoglycaemic effect was accompanied by an increase in plasma insulin concentration. D-ascorbic acid produced a similar hypoglycaemic effect.

A relationship between scurvy and diabetes mellitus was proposed many years ago (Owens et al 1941). L-Ascorbic acid was found to lower the blood glucose concentration in animals (Losert et al 1980), in healthy human volunteers (Cheng & Yang 1983) and in diabetic patients (Dice & Daniel 1973). However, the mechanism of this hypoglycaemic effect remains obscure. The present study examined the effects of ascorbic acid on blood glucose and plasma insulin concentrations in the rat. To rule out the pharmacokinetic factors, the effect of L-ascorbic acid has been evaluated by intravenous administration.

Materials and Methods

Sprague-Dawley rats (200-280 g) of either sex were housed singly in suspended wire-mesh cages with free access to food and water in a colony room maintained at 23 ± 1°C under standard lighting conditions (05:00-19:00 h). After a 24 h fast, all the rats were anaesthetized with pentobarbitone (35 mg kg^{-1} i.p.). Before the experiment, their blood glucose content was measured every 30 min. Only the rats with a variation of blood glucose less than 5 mg dL^{-1} between two determinations were needed.

A solution of either L- or D- ascorbic acid, in distilled water was administered by intravenous injection into the femoral vein. Control animals received the distilled water, adjusted to the same pH.

Blood samples (2 mL) were collected from the carotid artery. Blood glucose was measured by the glucose oxidase method (Ames, USA) and plasma insulin was determined by radioimmunoassay (Amersham RIA-kits, UK) using rat insulin (Novo Research Institute, Copenhagen, Denmark) as standard. The insulin samples from the paired study were processed in the same assay, running in duplicate. The limit of detection was 0·8 ng mL^{-1} and the plasma dilution curves were parallel to the standard curves. Intra- and interassay coefficients of variation were 7·4 and 11·3%, respectively.

Addition of L-ascorbic acid (100 mg mL^{-1}) to the glucose standards or to fresh blood did not significantly modify the measured concentration of glucose ($P > 0·05$). Similarly, L-

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ascorbic acid (50 mg mL^{-1}) did not interfere with the insulin radioimmunoassay.

The hypoglycaemic activity of ascorbic acid was calculated according to the formula: (A-B)/A X 100%, where A is the blood glucose concentration (mg dL^{-1}) from control (same pH-treated rats) and B is the value from the ascorbic acid-treated rats. Plasma insulin responses during L-ascorbic acid injection represent the mean increment in plasma insulin concentration above baseline. The basal insulin concentration was derived from the mean of three determinations from −30 to 0 min at 15-min intervals.

Data are presented as the mean ± s.e.m. For statistical analysis, the Student's *t*-test for paired or non-paired data was employed, and *P* values less than 0·05 were considered to be significant.

Results

A single intravenous injection of L-ascorbic acid produced a marked lowering of blood glucose; the maximal effect occurring 5 min after treatment (Fig. 1). The hypoglycaemic action of

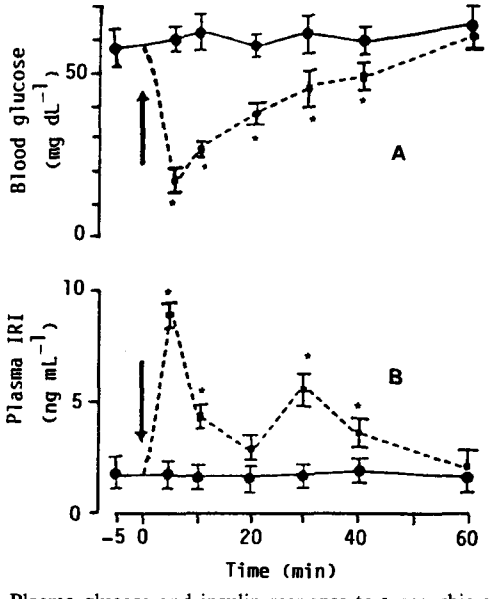

FIG. 1. Plasma glucose and insulin response to L-ascorbic acid in anaesthetized rats. A solution of L-ascorbic acid (65 mg kg^{-1}) was administered at 0 min by single intravenous injection. Change of blood glucose concentration (mg dL^{-1}) is seen in A, while the plasma insulin level (ng mL^{-1}) is seen in B. The broken line represents treated animals, the solid line the control group: vertical bars indicate the s.e.m. from 6 rats. $^{*}P < 0{\cdot}005$ from the control value.

L-ascorbic acid disappeared within 60 min but could be reproduced with another injection 2 h later. The effect of L-ascorbic acid initiated in a dose-dependent manner ranged from 20 to 100 mg kg^{-1} and the approximate ED50 was 65 mg kg^{-1} (n = 8).

Moreover, L-ascorbic acid also produced an increase in plasma insulin concentrations, which coincided with the lowering of the blood glucose level (Fig. 1A). The stimulated secretion of insulin occurred in two peaks; the initial marked one and a later peak (Fig. 1B). The effect of L-ascorbic acid on insulin secretion was also produced in a dose-dependent fashion paralleled to the hypoglycaemic effect ($r = 0{\cdot}97$, $P < 0{\cdot}01$).

A single injection of L-ascorbic acid at 65 mg kg^{-1} into anaesthetized rats produced hypoglycaemia to 66·5 ± 8·1% (n = 6) below control values. The same dose of D-ascorbic acid injected into anaesthetized rats induced hypoglycaemia to 60·9 ± 7·3% (n = 6) below control values. In addition, the increase of plasma insulin concentration (8·9 ± 2·1 vs 7·8 ± 2·2 ng mL^{-1}) was also similar in the rats injected with L- or D-forms of ascorbic acid. There was no statistical difference in the activity between the two stereoisomers through the non-paired Student's *t*-test ($P > 0{\cdot}05$).

Discussion

In the present study, we found that L-ascorbic acid produced a dose-dependent and marked hypoglycaemic effect after intravenous injection in rats. The maximal hypoglycaemic effect occurred at 5 min after injection and coincided with an increase in the plasma insulin concentration (Fig. 1). The doses of L-ascorbic acid required were higher than those required for its action as a vitamin (Ginter & Chorváthová 1983).

Ascorbic acid (either by intravenous injection or orally) was also found to produce a hypoglycaemic effect in the guinea-pig (Losert et al 1980) and in diabetic patients (Dice & Daniel 1973).

From the increase in plasma insulin concentrations, it may be suggested that L-ascorbic acid produces the hypoglycaemia by stimulating the secretion of insulin. A stimulatory effect of L-ascorbic acid on the secretion of insulin was also observed in rat isolated islets (Laycock 1981); this stimulation of insulin secretion was produced in parallel with the increase of cyclic GMP.

D-Ascorbic acid produced similar hypoglycaemic and hyperinsulinaemic effects to those produced by L-isomer. This indicates that ascorbic acid produced its effect through a mechanism without stereoselectivity.

From these results, we suggest that L-ascorbic acid possesses the ability to lower the blood glucose level in anaesthetized rats through stimulation of insulin secretion.

We wish to thank Prof. Hal Malmud for reading this manuscript. Thanks are also due to Miss Shan Wang for her skilled technical assistance.

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