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INVOLVEMENT OF NITRIC OXIDE (NO) IN HYPOGLYCAEMIC ACTIVITY OF TOLBUTAMIDE

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Abstract : The study was conducted to find the involvement of Nitric Oxide (NO) using L–arginine, a NO precursor and N^G–methyl L–arginine a nitric oxide synthase inhibitor on tolbutamide activity in normal rabbits. L–arginine (25–300 mg/kg, body weight, oral) produced transient and dose dependent hypoglycaemia. When combined with tolbutamide (40 mg/kg, oral) it produced early and prolonged action. The effect of tolbutamide was blocked by N^G–methyl L–arginine (5 mg/kg, body weight, oral). The results confirm the involvement of NO in tolbutamide activity and the possibility of using L–arginine as a supplement to antidiabetic drugs in blood glucose control.

Key words : L–arginine, N^G–methyl L–arginine, nitric oxide, tolbutamide, blood glucose, rabbits

INTRODUCTION

Type I diabetes is treated with daily injections of insulin and type II is treated with oral antidiabetic drugs like sulphonylureas and/or biguanides coupled with diet control and exercise. However, insulin resistance is seen in chronic cases resulting in poor control of blood glucose and various new approaches like nitric oxide related mechanisms are currently under investigation for the management of insulin resistance. Some literature reports indicate that nitric oxide is diabetogenic (1–4) while others indicate that it is useful in insulin secretion (5, 6). Hence there is need to study the exact role of nitric oxide in diabetes and its influence on antidiabetic therapy.

The objectives of the study were to assess the involvement of nitric oxide in tolbutamide action. The effect of L–arginine, a nitric oxide precursor, was also determined on blood glucose and also on the action of tolbutamide, in normal rabbits

METHODS

L–arginine (Loba chemic, Bombay), N^G–methyl L–arginine (Sigma Chemicals, USA) and Tolbutamide (Hoechst India Ltd. Bombay) were used in the study.

Rabbits of either sex weighing 1.2-2.0 kg were used. They were fasted for 18 h but were allowed water ad libitum before

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the administration of the drugs. During the experiments, both food and water was with held. Diferent doses of L–arginine 25,300 and 500 mg/kg body weight were administered orally to rabbits as aqueous solution at intervals of one week. Blood samples were collected before administration of drugs and also at 0.5, 1, 2, 4, 6 and 8 h after administration. After one week of washout period, rabbits were administered tolbutamide 40 mg/kg body weight in 5% gum acacia suspension, orally and its effect on blood glucose was noted (control). The effect of the same dose of tolbutamide was also studied in the presence of oral doses of 25 mg/kg and 300 mg/kg body weight L–arginine and 2 mg/kg and 5 mg/kg body weight N^G-methyl L-arginine on the same rabbits keeping one week interval in between the experiments.

The blood samples were collected by puncturing the marginal ear vein. Collection was done in vials containing anticoagulant mixture sodium fluoride and potassium oxalate 1:3 and stored in refrigerator at 4°C for blood glucose analysis by Nelson–Somogyi's method (7).

The percent blood glucose reduction was calculated using the formula $(X_0 - X_t)/X_0 X_{100}$ where X_0 is the blood glucose concentration before administration of drug(s) and X_t is the blood glucose concentration at time 't' after administration. Student's paired 't' test was applied to determine significance and $P<0.05$ was considered significant. The data are expressed as mean ± SEM.

RESULTS

Effect of L-arginine: L-arginine produced dose dependent hypoglycaemic effect in normal rabbits. A dose of 300 mg/kg body weight produced about 30% blood glucose reduction for a transient period at 2 h which did not increase further on increasing the dose to 500 mg/kg. The percent blood glucose reduction obtained at different intervals are given in Table I.

TABLE I : Effect of L-arginine and N^G-methyl-L-arginine on tolbutamide hypoglycaemic activity in normal rabbits.

Time	*Percent blood glucose reduction*							
(h)	*L-Arginine 25 mg/kg (n=6)*	*L-Arginine 300 mg/kg (n=6)*	*L-Arginine 500 mg/kg (n=6)*	*Tolbutamide 40 mg/kg (n=6)*	*Tolbutamide 40 mg/kg + L-Arginine 25 mg/kg (n=6)*	*Tolbutamide 40 mg/kg + L-Arginine 300 mg/kg (n=6)*	*Tolbutamide 40 mg/kg +N^G-methyl -L-arginine 2 mg/kg (n=3)*	*Tolbutamide 40 mg/kg +N^G-methyl-L-arginine 5 mg/kg (n=3)*
0.5	3.7±0.3	19.6±2.5	17.9±1.6	8.7±1.7	16.1±0.5*	16.1± 3.2*	–3.4±0.6**	–15.0±4.5**
1	8.3±0.4	26.04±1.4	26.4±1.2	17.7±1.5	25.3±1.4*	28.3± 2.6*	–3.2±0.8**	–7.5±3.1**
2	13.2±0.6	30.44±0.3	31.2±3.7	26.3±1.3	30.4±1.1*	31.5± 2.1*	2.8±0.9**	–8.4±4.5**
4	11.8±1.5	11.2±2.05	9.4±1.6	30.6±0.6	32.8±0.9	32.7± 1.4	5.5±2.3**	–5.7±1.8**
6	2.4±0.5	7.3±5.3	5.2±0.4	25.5±2.1	34.5±1.1*	33.1± 1.9*	2.6±0.4**	–2.2±2.2**
8	–3.7±1.6	5.1±2.9	4.7±3.4	12.2±1.6	26.9±0.8*	24.3± 1.2*	1.3±0.02**	–13.4±4.9**

* $P<0.05$, ** $P<0.001$ (Compared with tolbutamide controls 40 mg/kg).

Effect of tolbutamide and the influence of L-arginine on tolbutamide response: A dose of 40 mg/kg body weight which was found to produce about 30% reduction in blood glucose was used in the present study. The presence of L-arginine (300 mg/kg) produced early and prolonged hypoglycaemic action of tolbutamide compared to tolbutamide control. Even lowest dose of L-arginine (25 mg/kg) produced effect equivalent to 300 mg/kg of L-arginine in prolonging tolbutamide action. The above combinations are significant at $P<0.05$ upto 8 h (Table I). There was no significant difference between the effect produced by 25 mg/kg and 300 mg/kg body weight of L-arginine on tolbutamide activity.

Effect of N^G-methyl L-arginine on tolbutamide hypoglycaemic activity: A dose of N^G-methyl L-arginine (2 mg/kg, oral) reduced tolbutamide response. Increased dose (5 mg/kg, oral) totally blocked the tolbutamide hypoglycaemic response (Table I).

DISCUSSION

Various approaches like the search on the use of vanadium compounds (8), thiazolidinediones (9–13), β_3-adrenergic receptor agonists (14, 15), acarabose (16), Vitamin D and creatinine (17), are currently under investigation for the management of insulin resistance in diabetes. Recent studies indicate the involvement of NO in insulin secretion (5, 6). Hence, it is felt that further studies are needed to find the influence of amino acids that can be converted to NO *in vivo* if given as supplements along with antidiabetic drugs. The present study is conducted with L-arginine which is converted by nitric oxide synthase (NOS) to NO and L-citrulline by Ca^{++}/calmodulin dependent mechanism (18).

L-arginine, a precursor of NO is found to lower blood glucose in normal rabbits with maximum effect at 2 h. It is possible that it releases insulin after getting converted to NO and thereby lowered blood glucose (Table I). However, its influence on insulin action and tissue uptake of glucose at cellular level is not known.

Sine L-arginine lowered blood glucose level on its own, its influence on tolbutamide hypoglycaemic action was studied in normal rabbits and it was found that the combination produced early onset of hypoglycaemia which was significant upto 8 h (during the period of study) compared to tolbutamide controls indicating the involvement of NO in tolbutamide activity (Table I).

Since tolbutamide hypoglycaemic activity is blocked in the presence of N^G-methyl L-arginine, an inhibitor of NOS, the involvement of NO in tolbutamide activity is further substantiated (Table I). Tolbutamide is known to produce hypoglycaemic action by release of insulin (pancreatic) and by increase in tissue uptake of glucose (extrapancreatic) (19–21). Since L-arginine, a nitric oxide precursor, improved its activity and NOS inhibitor N^G-methyl L-arginine blocked its response, it appears that NO may be involved not only in tolbutamide induced insulin secretion from pancreas but also in tissue uptake of glucose. Further studies on the influence of L-arginine on the uptake of glucose by isolated rat diaphragm in Tyrode solution are required to confirm its extrapancreatic action and such studies are in progress.

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