

Insulinotropic Effect of Aqueous Ginger Extract and Aqueous Garlic Extract on the Isolated Perfused Pancreas of Streptozotocin Induced Diabetic Rats

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Abstract.- In this study, diabetic condition was induced in rats by intra-peritoneal administration of streptozotocin (STZ) at a dose of 50 mg kg⁻¹. The pancreas was isolated and perfused with 3 mM glucose with or without gliclazide (10 µM), aqueous ginger (10% v/v) or aqueous garlic (10% v/v) extracts. Samples were collected at different time intervals along a 30 minutes perfusion period. Insulin concentrations in the samples were determined using radioimmunoassay technique. Streptozotocin-induced diabetes inhibited the pancreatic response to the stimulatory effect of 16.7 mM glucose particularly during the first phase of insulin secretion. Gliclazide, aqueous ginger and garlic extracts potentiated glucose-induced insulin secretion from the isolated perfused pancreas of STZ-diabetic rats. It could be concluded that ginger and garlic might have an antidiabetic effect that could be mediated at least partially through a direct pancreatic mechanism. This action might be comparable to that of the standard antidiabetic insulinotropic drug, gliclazide.

Key words: Gliclazide, ginger, garlic, streptozotocin-induced diabetic rats.

INTRODUCTION

Diabetes mellitus is a complex, chronic disorder of carbohydrate, fat and protein metabolism that is primarily a result of partial, complete or relative lack of insulin secretion by pancreatic β -cells and/or impairment of insulin action (Anderson *et al.*, 1994). Streptozotocin (STZ), a diabetogenic agent selectively destroys β -cells, presumably, because of the glucose moiety in its structure which enhances its uptake and accumulation into the cells (Gunnarsson *et al.*, 1974; Uchigata *et al.*, 1982). Prior to β -cell destruction, STZ induces a non specific islet inflammation called insulinitis where inflammatory macrophages and pancreatic endothelium are activated and release several inflammatory mediators such as prostaglandin (PGs), leukotrien (LTs) and hydroxylated derivatives hydroxyecosatetraenoic acid (HETEs) (Roselló-Catafau *et al.*, 1994).

Despite remarkable progress in the management of various diseases including diabetes mellitus by synthetic drugs, there has been a renewed interest in indigenous antidiabetic agents

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especially medicinal plants, herbs and spices as reviewed by Tapsell *et al.* (2006). Among these, most noted are ginger and garlic which appear most effective and least toxic (Srinivasan, 2005). Ginger rhizome, having a pleasant aroma and pungency, has been used as a spice and medicine for thousands of years. It contains the pungent principles, gingerols and shogaols, which are phenolic in nature and pharmacologically are the most active components of ginger (Afzal *et al.*, 2001). Garlic bulb contains potentially active chemical constituents including sulfur compounds, enzymes, amino acids, and minerals. Sulfur compounds include alliin (S-allyl cysteine sulfoxide (SACS) and allicin (diallyl disulfide) which are responsible both for garlic's pungent odor and many of its medicinal effects (Murray and Pizzorno, 1999). One of the most biologically active compounds, allicin, does not exist in garlic until it is crushed or cut where injury to the garlic bulb activates the enzyme allinase which metabolizes allin to allicin (Block, 1985).

The aim of the present study was to examine antidiabetic effect of ginger and garlic extracts on the insulin concentration in streptozotocin induced

diabetic rats. In an earlier part of our work it was noted that cytokines increased during diabetes which subsequently resulted in increased hyperglycemia and adhesion of endocelial molecules. It was also reported that ginger and garlic extract have antiinflammatory effect during diabetes (Eid *et al.*, 2005).

MATERIALS AND METHODS

Animals

Male adult albino rats, weighing 200-250 g each obtained from the National Research Center, Giza, Egypt were used in the present study. They received a standard pellet diet (El Nasr Chemical Company, Cairo, Egypt) and were allowed free access to water.

Drugs and chemicals

Streptozotocin, bovine serum albumin fraction V, dextran 60 and urethane were purchased from Sigma Chemical Company (USA). Gliclazide was obtained from Novartis (Egypt), ginger from Mepaco (Egypt), while garlic was purchased from the local market (Egypt). The test reagent kits for the determination of insulin were obtained from Diasorin (Italy). All other chemicals were of analytical grade.

Isolation and perfusion of the pancreas

Isolation and perfusion of the pancreas were carried out according to the method described by Grodsky *et al.* (1963) with slight modification. The perfusion medium consisted of Krebs Ringer Bicarbonate Buffer (KRB) (mM: NaCl 118.4, KCl 4.8, CaCl₂.2H₂O 2.5, MgSO₄.7H₂O 1.2, KH₂PO₄ 1.2 and NaHCO₃ 25.0) and was supplemented with 4% dextran, 0.5% bovine serum albumin and glucose [0.54 mg ml⁻¹ (3.0 mM) or 3 mg ml⁻¹ (16.7 mM)] immediately before use. The pH of the final solution was adjusted at 7.35. The temperature was maintained at 37°C and the solution was continuously gassed with carbogen. Perfusion with 3.0 mM glucose/KRB solution for an equilibration period of 15 min was carried out and a sample was collected along the last minute of perfusion to estimate basal insulin release (zero time). This was

followed by perfusion with 16.7 mM glucose/KRB with or without drug for the next 30 min to estimate their effect on insulin release. Samples were collected at 1.0, 1.5, 2.0, 2.5, 3.0, 5.0, 10.0, 20.0, and 30.0 min from the start of perfusion. Insulin content of the samples was then determined using radioimmunoassay technique according to Juhl *et al.* (1991).

Experimental design

Five groups each of 4 albino rats were proceeded as follows: Group 1 received citrate buffer and served as the normal control. The perfusion medium consisted of 3.0 mM followed by 16.7 mM glucose/KRB solution. Group 2 received STZ (50 mg kg⁻¹, i.p.) and served as the diabetic control. The perfusion fluid consisted of 3.0 mM followed by 16.7 mM glucose/KRB solution. Group 3 received STZ (50 mg kg⁻¹, i.p.). The perfusion fluid consisted of 3.0 mM glucose followed by 10 µM gliclazide in 16.7 mM glucose/KRB solution. Group 4 received STZ (50 mg kg⁻¹, i.p.). The perfusion fluid consisted of 3.0 mM glucose followed by 10% (v/v) aqueous ginger extract in 16.7 mM glucose/KRB solution. Group 5 received STZ (50 mg kg⁻¹, i.p.). The perfusion fluid consisted of 3.0 mM glucose followed by 10% (v/v) aqueous garlic extract in 16.7 mM glucose/KRB solution.

Induction of diabetic condition by streptozotocin

Experimental diabetes was induced in non fasted (Tobin *et al.*, 1993) rats by single i.p. injection of STZ in a dose of 50 mg kg⁻¹ (Hounsom *et al.*, 1998). Streptozotocin was dissolved in cold 0.1 M citrate buffer (pH 4.5) immediately before use and was injected within 5 min of its preparation. Streptozotocin-injected rats were provided with a 5% (w/v) glucose drinking solution for the first 24 h to ensure survival through the hyperinsulinemic phase brought about in response to the STZ-induced lysis of β-cells (Hajduch *et al.*, 1998). Animals that showed glycosuria or hyperglycemia (detected by Glukotest strips for urine glucose and haemoglukotest strips for blood glucose) within 24 h (Augusti and Sheela, 1996; Bwititi *et al.*, 2000) were selected. Blood samples were then collected from the retro-orbital sinus of the selected rats and were used for estimation of serum glucose level.

Those with blood glucose level above 200 mg% (Mainzen *et al.*, 2001) were considered diabetic in the present study. Eight days later (Jain and Vyas, 1975), the pancreas of each rat was isolated and perfused with the appropriate solutions. Samples were collected at different time intervals during the perfusion period. Insulin concentration in the effluent from the isolated perfused pancreas was measured using radioimmunoassay kit (Insik-5, P2796, Diasorin-Italy).

Preparation of drugs in perfusion medium

Gliclazide

Gliclazide powder was dissolved in the minimum volume of 10% NaOH solution. It was then added to 3.0 mM or 16.7 mM glucose/KRB solution to make a 10 μ M (Nakayama *et al.*, 1995) gliclazide solution. The pH was then readjusted back to 7.4 using 10% HCl.

Ginger

Aqueous ginger extract was prepared by weighing 55 g of powdered ginger root, mixing them with little bidistilled water (just enough to penetrate and cover the surface of the powder), then keeping at 2-8°C for 24 h. This was then decanted by pressing over a piece of gauze and was centrifuged to yield about 90 ml aqueous extract. The extract was then added to 3.0 mM or 16.7 mM glucose/KRB solution in a concentration of 10% v/v (Srivastava, 1984).

Garlic

Aqueous extract of garlic was obtained as described by Srivastava (1984) as follows: 35 g fresh garlic were peeled off, crushed and homogenized into a paste, mixed with water, kept for 24 h at 2-8 °C, then decanted. The decanted material was then centrifuged to yield about 200 ml aqueous extract. The latter was added to 3.0 mM or 16.7 mM glucose/KRB solution so that the final concentration was 10% v/v.

Statistical analysis

Data were expressed as Mean \pm SEM. Student's 't' test for paired data was applied to analyze the data within the same group. Student's 't' test for unpaired data was used to compare the

results between two groups (Tallarida and Murray, 1981). Differences with $P < 0.05$ were considered to be statistically significant.

RESULTS

Table I shows that basal insulin release from the isolated perfused pancreas of STZ- diabetic rats was significantly lower than the corresponding normal control value (1.03 ± 0.55 vs. 4.61 ± 0.45 , respectively). In addition, there was a significant reduction in the first phase of glucose (16.7 mM)-induced insulin release that started from the 1st min and lasted till the 5th min of perfusion. The effect was maximal after 3 min of perfusion reaching about 17% of the corresponding normal control value. Perfusion of the isolated diabetic rat pancreas with 10 μ M gliclazide in 16.7 mM glucose/KRB solution showed a significant rise in insulin release as compared to the corresponding diabetic control values. This effect was observed at 1.0, 2.0, 2.5, 3.0 and 5.0 min of perfusion. The drug potentiated glucose-induced insulin release to values reaching a maximum of 285% of the corresponding diabetic control (Table I).

Perfusion of the isolated diabetic rat pancreas with aqueous ginger extract in 16.7 mM glucose/KRB solution significantly increased insulin release at 1.0, 2.5, 3.0 and 5.0 min of perfusion to values reaching about 200, 189, 237 and 195 % of the diabetic control values, respectively (Table I).

A significant increase in insulin release was observed after 1.0 and 1.5 min of the start of the perfusion of the isolated diabetic rat pancreas with aqueous garlic extract in 16.7 mM glucose/KRB solution (10% v/v). The increase was about 312 and 214% respectively of the corresponding diabetic control values (Table I).

DISCUSSION

In the present study, gliclazide improved the early phase of glucose-induced insulin release which is in line with previous data obtained from Goto-Kakizaki rats (Dachicourt *et al.*, 1998), neonatal STZ-diabetic rats (Serradas *et al.*, 1989) as well as Type II diabetic patients (Juhl *et al.*, 2001). Gliclazide acts through a β -cell surface glycoprotein (SU) receptor (Ashcroft and Ashcroft, 1992). It

possibly lowers the glycemic threshold required for a given β -cell secretory response (Pfeifer *et al.*, 1981). The SU receptor on the β -cell is coupled to

Table I.- Effect of glucose, gliclazide, garlic and ginger extracts on serum insulin concentration from the isolated perfused pancreas of STZ-diabetic rats

	0.0 Basal Insulin Release	Time (min)								
		1.0	1.5	2.0	2.5	3.0	*5.0	10.0	20.0	30.0
Effect of glucose on insulin release										
Normal-control	4.61± 0.45	37.15± 9.02*	57.87± 9.22*	56.43± 10.10*	108.73± 9.55*	176.15± 22.31*	114.55± 21.81*	74.76± 22.71*	42.38± 5.59*	123.47± 34.07*
Diabetic	1.03± 0.55 ^a	10.58± 2.16* ^a	23.15± 4.83* ^a	18.81± 5.55* ^a	29.88± 6.44* ^a	30.23± 7.49* ^a	25.46± 7.67* ^a	37.38± 10.29*	59.73± 16.44*	72.31± 14.30*
% of normal control	22.34	28.48	40.00	33.33	27.48	17.16	22.23	50.00	140.94	58.56
Effect of 10 μM gliclazide on glucose -induced insulin release										
Diabetic-control	1.03± 0.55	10.58± 2.16*	23.15± 4.82*	18.81± 5.55*	29.88± 6.44*	30.23± 7.49*	25.46± 7.67*	37.38± 10.29*	59.73± 16.44*	72.31± 14.30*
Gliclazide-treated	2.50± 0.47	30.10± 7.49* ^b	39.27± 9.05*	43.22± 6.09* ^b	67.91± 12.46* ^b	79.63± 15.06* ^b	70.27± 7.31* ^b	56.66± 12.65*	63.78± 15.47*	109.99± 15.66*
% of diabetic control	242.72	284.50	169.63	229.77	227.28	263.41	*276.00	151.58	106.78	152.11
Effect of aqueous ginger extract (10% v/v) on glucose -induced insulin release										
Diabetic-control	1.03± 0.55	10.58± 2.16*	23.15± 4.82*	18.81± 5.55*	29.88± 6.44*	30.23± 7.49*	25.46± 7.67*	37.38± 10.29*	59.73± 16.44*	72.31± 14.30*
Ginger-treated	0.71± 0.21	21.13± 2.75* ^b	26.27± 3.92*	31.31± 3.26*	56.44± 6.39* ^b	71.69± 8.15* ^b	49.57± 5.45* ^b	34.76± 9.25*	32.27± 7.50*	100.91± 10.87*
% of diabetic control	68.93	199.72	113.48	166.45	188.89	237.15	194.70	92.99	54.03	139.55
Effect of aqueous garlic extract (10% v/v) on glucose -induced insulin release										
Diabetic-control	1.03± 0.55	10.58± 2.16*	23.15± 4.82*	18.81± 5.55*	29.88± 6.44*	30.23± 7.49*	25.46± 7.67*	37.38± 10.29*	59.73± 16.44*	72.31± 14.30*
Garlic-treated	1.97± 0.94	32.97± 7.00* ^b	49.50± 6.91* ^b	27.20± 3.32*	16.61± 3.34*	26.00± 4.58*	25.86± 4.14*	30.49± 6.30*	32.69± 6.52*	42.81± 10.45*
% of diabetic control	191.26	311.63	213.82	144.60	55.59	86.01	101.57	81.57	54.73	59.20

*Glucose concentration in each perfusion fluid was 16.7mM.

^aSignificantly different from basal insulin release value at P<0.05.

^bSignificantly different from the corresponding diabetic control value at P<0.05.

an ATP-sensitive K⁺ channel (Cook *et al.*, 1988). It was suggested that the receptor for SUs is the ATP-sensitive K⁺ channel itself (Boyd, 1988). When SU

receptors bind to their receptors, K⁺ efflux through the ATP-sensitive K⁺ channel decreases leading to membrane depolarization and subsequent opening

of voltage-dependent Ca^{++} channel thus permitting Ca^{++} influx which in turn triggers insulin exocytosis (Nelson *et al.*, 1987).

In the present work, aqueous ginger extract produced a significant increase in glucose-induced insulin release from the isolated perfused pancreas of STZ-diabetic rats. The mechanism of the stimulatory effect of ginger on insulin release is probably mediated via ginger's anti-inflammatory effect previously reported by Srivastava (1984) where it has been reported that an influx of pancreatic cyclooxygenase (COX) and lipoxygenase metabolites is involved in the insulinitis associated with STZ-induced diabetes (Roselló-Catafau *et al.*, 1994).

In the present study, aqueous garlic extract produced a brief potentiation of glucose-induced insulin release from the isolated perfused pancreas of STZ-diabetic rats. This is in accordance with the work done by Augusti and Sheela (1996) where allin, a polar compound from garlic, significantly stimulated basal as well as glucose-induced *in vitro* insulin secretion from β -cells of rats. The enhancing effect of garlic on glucose-induced insulin release could be attributed to the possible increase in islet nitric oxide (NO) content by garlic. This is based on earlier reports stating that garlic activates the synthesis of NO (Das *et al.*, 1995) which, in turn, is suggested to participate in the signal transduction pathway mediating the early phase of glucose-stimulated insulin secretion (Spinas *et al.*, 1998). Since PGs inhibit insulin secretion and are responsible for the biphasic pattern of insulin release (Giugliano *et al.*, 1983), it might be proposed that the inhibitory effect of garlic on PGs production (Srivastava, 1984) could be responsible for the stimulatory effect of garlic on insulin release and its lack of the biphasic response.

In summary, STZ-induced diabetes inhibited the early phase of glucose (16.7 mM)-induced insulin release from the isolated perfused pancreas of rats, an effect which was antagonized by gliclazide, aqueous ginger and garlic extracts. This indicates that ginger and garlic might have an antidiabetic effect that could be mediated at least partially through a direct pancreatic mechanism. This action might be comparable to that of the standard antidiabetic insulinotropic drug, gliclazide.

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