THE POSSIBLE ANTIDIABETIC ROLE OF SELECTIVE β_3 ADRENOCEPTOR AGONIST IN ANIMAL MODELS OF DIABETES MELLITUS.

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ABSTRACT

Control of blood glucose is a key objective in the management of type I and type II diabetes mellitus (DM) as well. The basis for development of new antidiabetic drug is the proper control of the hyperglycemia and further more could manage accompanying disorders especially dyslipidemia. The aim of this study was to investigate a newly developed β_3 adrenergic agonist (BRL 37344) to confirm its effect on blood glucose in two animal models of D.M. which induced by streptozotocin (STZ) in a dose of 70 or 40 mg/Kg for induction of type I or type II D.M., respectively and try to explore for the possible underlying mechanism of this effect. Oral administration of BRL 37344 for 4 successive days in either STZ type I or STZ type II diabetic rats produced significant reduction in mean serum glucose level and non-significant change in mean serum insulin level in comparison with non treated STZ diabetic rats. BRL treatment significantly increased glucokinase activity and decreased glucose-6-phosphatase activity in hemogenated hepatic tissues which were isolated from both STZ diabetic rat models. In in-vitro study, BRL produced non-significant changes in insulin secretion from isolated pancreatic tissue of STZ type II diabetic rats, while BRL showed significant increase in glucose uptake by isolated soleus muscle from both STZ

diabetic rat models. These results showed that selective stimulation of the β_3-adrenoceptors in diabetic rats, produced significant antihyperglycemic effect. This effect is not through stimulation of pancreatic insulin secretion but it is through inhibition of hepatic glucose output or enhanced glucose utilization by skeletal muscles.

INTRODUCTION

As diabetes mellitus must almost be long life treated disease, the basis for relieving the hyperglycemia should affect the underlying defects of glucose metabolism. The new antidiabetic should be at least as effective as the existing drugs and should preferably have other advantages, for example a novel mode of action, favourable kinetics, and ease of administration or beneficial effects on conditions that are commonly associated with diabetes such as obesity, dyslipidemia, hypertension and other cardiovascular diseases [1].

It had been known for long time that β agonist have to increase blood glucose through hepatic glucose production [2], and at that time no one thought that some β agonist could have antidiabetic effect [3]. This atypical effect is via atypical β_3 receptors that have been cloned [4]. Nonogaki, 2000 stated that abnormality of sympathetic effects including disturbance in β_3 receptor signaling is likely to cause obesity and impaired glucose tolerance in rodents and human [5]. However, molecules with affinity for β_3 adrenoceptors have been tested because of their potential anti-obesity effect. It is known that these agents stimulate adipose tissue thermogenesis through an increase in the mitochondrial uncoupling protein expression in both brown and white adipose tissues. Moreover, β_3 adrenoceptors stimulate lipolysis in white adipose tissue [6]. Several studies have reported that β_3 adrenergic agonists affect plasma glucose, triglycerides and free fatty acids in situations of hyperglycemia and dyslipidaemia [7]. In ob/ob mice, a chronic treatment with β_3 adrenergic agonists resulted in a marked decrease in plasma glucose and triglycerides [8]. In contrast, evaluation the effects of β_3 agonist (BRL 26830A) in obese diabetic women showed reduced levels of insulin with unchanged glucose, along with a

fall in plasma concentration of glycerol and palmitate [9].

Therefore, current work tested the proposal that the antiobesity β_3-adrenergic agonists may have also an antidiabetic effect in streptozotocin (STZ)-induced diabetes model in rats as comparative study with standard oral hypoglycemic drug; gliclazide and parental hypoglycemic drug; insulin. In addition the study tried to explore the possible mechanisms for this potential antidiabetic effect.

MATERIALS AND METHODS

Animals :

230 male Sprague Dawley albino rats (180-200g) were used. Rats were housed under similar conditions to keep them in normal and healthy states. They were allowed free access to food and water

Drugs :

1- Streptozotocin (Sigma Aldrich chemical company) as "streptozotocin powder bottle" containing 1000 mg streptozotocin (STZ).
2- Sodium salt of 4,2 (3-chlorophenyl) 2-hydroxy ethyl aminopropyl phenoxyacetic acid) [BRL 37344] powder "Tacris cookson".
3- Sodium salt of (N-hexahydro cyclopenta (C) pyrrol ٢ ذ(IH) ذYL)-amino) carbonyl)-4-methyl benzene sulfonamide (gliclazide) powder, (Amoun pharmaceutical Co.)
4- Neutral Protamine Hagedorn (N.P.H) insulin (Novo Nordisk pharmaceutical company). Each ml contains 40 U NPH insulin.
5- Soluble insulin; actarapid (Novo Nordisk pharmaceutical company). Each ml contains 20 U soluble insulin.

Experimental design :

Rats were classified into three main classes, 1st class for induction of STZ-type I diabetes mellitus, 2nd class for induction of STZ-type II diabetes mellitus and 3rd class was kept as control non-diabetic groups.

- Induction of STZ-Type I diabetes mellitus in rats :

62 rats were injected intraperitonially (I.P) by a single dose of 70 mg/kg STZ which was dissolved in citrate buffer PH 4.5 before use (10). 30 rats were designed for in vivo study and the rest (32 rats) were for in vitro isolated soleus muscle study.

- Induction of STZ-Type II diabetes mellitus in rats :

94 rats were injected I.P by a single dose of 40 mg/kg STZ (11). 30 rats were designed for in vivo study and the rest were for in vitro isolated pancreas (32 rats) and isolated soleus muscle studies (32 rats).

- Control non-diabetic groups :

They contain 74 rats. Each rat received I.P injection of 0.2 ml citrate buffer (vehicle in which STZ was dissolved). 10 rats were designed for in vivo study and the rest were designed for isolated pancreas study (n=32) and for isolated soleus muscle study (n=32).

D.M was confirmed by measurement of blood glucose level on the 3rd day following STZ injection. Rats with fasting blood glucose ≥250 mg/dl were considered diabetic (12).

(A) In Vivo Experiment :

70 male rats were divided into 7 groups each of 10 rats. The 1st group served as control non-diabetic rats. The 2nd group served as non-treated STZ type II diabetic rats. The 3rd group served as gliclazide treated type II diabetic rats. The 4th group served as BRL treated type II diabetic rats. The 5th group served as non-treated STZ type I diabetic rats. The 6th group served as insulin treated type I diabetic rats. The 7th group served as BRL treated type I diabetic rats.

In control groups (1, 2 and 5) each rat received 1ml saline orally by gavage. In groups 4 and 7, each rat received BRL 37344 orally in dose of 1mg/kg (7). In group 3, each rat received gliclazide orally in a dose of 10 mg /kg (13). In group 6, each rat received 5 units insulin (NPH) S.C. (14). Treatment for all groups was once daily for 4 successive days.

In the 5th day after an overnight fasting, animals were killed by decapitation and blood samples were obtained, centrifuged and the sera were separated and preserved at -20 for determination of fasting serum glucose by the classical enzymatic method (15) and fasting serum insulin by radioimmunoassy (16). At the same time liver from each rat was gently and quickly removed and frozen in liquid nitrogen then preserved

at -80 [17] till determination of hepatic glucokinase [18] and glucose-6-phosphatase enzyme activities [19].

Determination of hepatic glucokinase enzyme activity :

Hepatic glucokinase activity was measured by spectrophotometrical detection of the NADH produced by the glucose -6- phosphate dehydrogenase (18). NADH formation is proportional to the concentration of glucose-6-phosphate (umol G-6-P/gm/min) and the latter is proportional to glucokinase enzyme activity.

Determination of hepatic glucose-6-phosphatase enzyme activity :

It was determined by measuring the inorganic phosphate (umol inorganic phosphorous (Pi)/mg protein (pr.)/min) released in the dephosphorylation of glucose-6-phosphate by glucose-6-phosphatase [19].

(B) In Vitro Experiments :

This part of the study aim to study the effect of tested drugs on insulin secretion from isolated pancreas and glucose uptake by isolated soleus muscles.

1) Effect of BRL agent on insulin secretion from isolated rat pancreas :

The method adapted in the present study to measure the insulin secretory activity of isolated rat pancreas was described by Weber et al., 1987[20].

After decapitation of rats, pancreas from each rat was gently and quickly removed and minced. The minced fragments of each pancreas were cultured in vitro on millipore membranes suspended at the medium 95% O_2 and 5% CO_2 interface in petri dishes at 37C °. Culture media CMRL-1066 supplement with 10% human serum albumin, penicillin (100 U/dl), streptomycin (1000 ug/dl) non-essential amino acids (1ml/dl) and L glutamine (10 mmol/dl) [20].

Rats were divided into 8 groups (each group of 8 rats). The groups 1 to 4 contained pancreases from control non-diabetic rats and the groups 5 to 8 contained pancreases from STZ induced type II diabetic rats. Glucose concentration in cultured media was 100 mg% in groups 1,2,5,6 and 300 mg % in groups 3,4, 7 and 8.

Groups 1, 3, 5 and 7 were not treated with test drug while groups 2, 4, 6 and 8 were treated with BRL 10-10 M/L for 6 days [21].

Determination of insulin in culture media of isolated pancreases :

After culture for 6 days in CO_2 incubator, media of each sample plate was collected and 200 units of Trasylol in 1ml was added to each sample and stored at -20C° till the time of radioimmunoassay of insulin in different samples [16].

2) Effect of BRL on glucose uptake by isolated soleus muscles :

This experiment was done according to the method of Ivy [22]. 12 groups of rats were used in this study (n=8 rats in all groups).

Rats were killed by quick decapitation and the soleus muscle from each rat was rapidly removed and frozen in liquid nitrogen, then preserved at-80 C° till the time of assay of glucose uptake [23]. The isolated muscles were placed in small flasks with a tight-fitting stopper, containing 3 ml Kreb's ringer bicarbonate solution PH 7.4 and gased with 95% O_2 and 5% CO_2, KCl 0.35, Ca Cl_2 0.28, KH_2PO_4 0.016, $NaHCO_3$ 0.21, Mg SO_4, $7H_2O$ 0.029, glucose 2, and bovine serum albumin 3 [24]. BRL 37344 was added in a dose of 10-10 M/L [25] in the presence or absence of crystalline insulin in a dose 10-7 M/L [26]. Incubation was made in a thermostatically controlled metabolic shaker for one hour with shaking rate 100 rpm [27].

The groups 1,2,3,4 were taken from control non diabetic rats, the groups 5,6,7,8 were taken from STZ type I diabetic rats and the groups 9,10,11,12 were taken from STZ type II diabetic rats. The soleus muscle from rats in the groups 1, 5, and 9 were served as non-treated groups, the groups 2, 6, and 10 were treated with insulin alone 10^{-7} ml/L [26], the groups 3, 7, and 11 were treated with BRL alone 10^{-10} m/L [25] and the groups 4, 8, and 12 were treated with both insulin and BRL together in the same previous doses.

Determination of glucose uptake by isolated soleus muscles

The glucose level was determined in the incubation medium at the end of the incubation period using the en-

zymatic method of Trinder, 1969[15]. Glucose was calculated as ug/mg wet tissue/ hour using the following equation:

Glucose uptake = M x V x 1000/ 100 x N

M= difference in the concentration of glucose/100 ml of medium between the control (without tissue) and the tissue flask.

V= Volume in ml of incubation medium in the flask.

N= wet weight of tissue in ug.

STATISTICAL ANALYSIS

All data were reported as mean ± S.E. and were analyzed by one way ANOVA for in vivo data. The Wilcoxon test has been used when comparing insulin secretion in vitro and glucose uptake by isolated tissues. P value of <0.05 was considered to indicate statistical significance.

RESULTS

A) In vivo study :

As illustrated in Table (1) I.P injection of streptozotocin (STZ) in a dose of 70 mg/kg and 40 mg/kg in albino rats for induction of STZ type I and STZ type II diabetes, respectively, produced significant increase in the mean serum glucose to 639 mg % and 295.7 mg%, respectively, as compared with the mean serum glucose 97.3 mg % in non-diabetic control rats. STZ produced also significant decrease in mean fasting serum insulin in both models to 0.1 and 0.5, respectively ng/ml as compared with the mean serum insulin 0.81 ng/ml in non-diabetic control rats.

Oral administration of BRL(1mg/ kg/day) or S.C injection of 5 units of NPH insulin/day for 4 successive days in STZ type I diabetic rats also produced a significant reduction is mean fasting glucose to 222 or 209 mg %, respectively on comparison with the mean fasting serum in non-treated STZ type I diabetic rats (639 mg%). Oral administration of either BRL 37344 in the same dosage schedule or gliclazide (10 mg/kg/day) for 4 successive days in STZ type II diabetic rats produced a significant reduction in the mean serum glucose to 110 or 101 mg%, respectively on comparison with non-treated STZ type II diabetic rats (295.7 mg%). (table 1).

Oral administration of BRL (in same above mentioned dosage schedule) in either STZ type I or type II diabetic models produced a non significant change in the mean fasting serum insulin (0.11 or 0.41 ng/ml, respectively) on comparison with those of the corresponding serum insulin of either non-treated type I or type II models (0.1 or 0.5 ng/ml, respectively). Insulin treatment in same dosage schedule in STZ type I diabetic rats also produced non significant changes in mean serum insulin (0.1 ng/ml) on comparison with those of the corresponding serum insulin of non-treated type I models (0.1 ng/ml). Meanwhile, gliclazide produced a significant increase in mean fasting serum insulin (0.7 ng/ml) in STZ type II diabetic rats on comparison with non treated corresponding group (table 1).

Table (2) showed that single I.P injection of STZ in a dose of 70 mg/kg or 40 mg/kg produced a significant decreased in mean hepatic glucokinase activity to 1.5 or 1.84 umol G.6.P/gm/min in type I or type II diabetic rats, respectively as compared with glucokinase activity in control non-diabetic rats (4.8 umol G.6.P / gm/min). These doses of STZ also produced a significant increase in mean hepatic glucose-6-phosphatase activity to 0.37 or 0.34 umol Pi/mg pr./ min in both models, respectively as compared with their activity in control non-diabetic rats (0.16 umol Pi/mg pr./min).

Oral administration of BRL or S.C injection of NPH insulin in the same dosage schedules to STZ type I diabetic rats produced significant increase in hepatic glucokinase activity (4.1 or 4.6 umol G-6-P/gm/min, respectively) and significant decrease in hepatic glucose-6-phosphatase activity (0.14 or 0.13 umol Pi / mg pr./ min, respectively) in comparison with those values in non-treated STZ type I diabetic rats (table 2)

Similarly, oral administration of either BRL or gliclazide in the above dosage regimens to STZ induced type II diabetic rats produced significant increase in mean hepatic glucokinase activity (4.3 or 4.7 umol G-6-P/ gm/min, respectively) and significant decrease in mean hepatic glucose-6-phosphatase activity (0.14 or 0.13 umol Pi/mg pr./min, respectively)

compared with the corresponding activity in the non-treated type II diabetic rats (table 2)

B) In vitro study :

1) Effect of BRL on insulin secretion from isolated rat pancreas :

Table 3 showed that there was significant decrease in mean insulin level in culture media of isolated pancreatic tissue from STZ type II diabetic rats (0.42 ng/ml) as compared with the mean insulin level in culture media of isolated pancreatic tissue from non-diabetic rats (0.7 ng/ml).

Increase in glucose concentration in culture media from 100 to 300 gm% increased significantly the mean insulin level in culture media from 0.7 to 1.3 ng/ml or from 0.42 to 0.67 ng/ml in culture of isolated pancreatic tissue from non-diabetic rats or from STZ type II diabetic rats, respectively.

Addition of BRL 37344 in concentration 10^{-10}M/L for 6 days to culture media of pancreatic tissue isolated from either non-diabetic rats or STZ type II diabetic rats and in the presence of either glucose concentration 100 mg% or 300 mg%, produced no significant changes in media insulin levels in the culture of either non-diabetic pancreatic tissues (0.7 or 1.2 ng/ml) or type II diabetic pancreatic tissues (0.4 or 0.7 ng/ml), respectively as compared with media insulin levels in the culture of non-treated pancreatic tissues from either non-diabetic rats (0.7 or 1.3 ng/ml) or type II diabetic rats (0.42 or 0.67 ng/ml), respectively (table 3).

2) Effect of BRL on glucose uptake by isolated soleus muscles :

As regards the glucose uptake by isolated soleus muscle, tables 4,5 showed that there were significant decrease in mean glucose uptake by isolated soleus muscle of STZ type I and STZ type II diabetic rats (0.7 and 0.9 ug/mg wet tissue/hour, respectively) as compared with non-diabetic rats soleus muscles (2.8 ug/mg wet tissue/ hour).

Tables 4 and 5 showed that in vitro administration of either insulin (10^{-7} M/L) or BRL (10^{-10} M/L) to non-diabetic rat soleus muscles resulted in a significant increase in glucose uptake (4.3 or 4.8 ug/mg wet tissue / hour, respectively) as compared with

the corresponding non-treated non-diabetic rat soleus muscles (2.8 ug/ mg wet tissue /hour, respectively).

Similarly, in vitro administration of either insulin (10^{-7} M/L) or BRL (10^{-10}M/L) to STZ type I and STZ type II diabetic rat soleus muscles produced a significant increase in glucose uptake (2.5± 0.14 or 2.5± 0.12 ug/mg wet tissue /hour and 2.6+ 0.11or 2.6± 0.14 ug/mg wet tissue/ hour, respectively) as compared with their corresponding non-treated diabetic groups (0.7 and 0.9 ug/mg wet tissue /hour, respectively) (tables 4 and 5)

Addition of both BRL and insulin together to isolated soleus muscles of non-diabetic, STZ type I diabetic and STZ type II diabetic rats resulted in significant increase in glucose uptake (7.9, 4.7 and 4.9 ug/mg wet tissue / hour, respectively) as compared with the corresponding soleus muscles which were treated with either BRL or insulin alone (4.3 or 4.8, 2.5± 0.12 or 2.5± 0.14 and 2.6± 0.14 or 2.6± 0.11, respectively).

Table (1) : Effect of BRL 37344 on fasting serum glucose and serum insulin levels in streptozotocin type I and type II diabetic rats

Group	Mean fasting serum glucose±S.E. (mg%)	Mean fasting serum insulin±S.E (ng/ml)
Non-diabetic rats:		
Saline (1 ml oral/day)	97.3±0.47	0.81±0.03
STZ-type I diabetic rats:		
Saline (1 ml oral/day).	639±0.82[a]	0.1±0.003[a]
NPH Insulin (5 units S.C/day)	209±2.3[ab]	0.1±0.007[a]
BRL 37344 (1 mg/kg oral/day)	222±1.6[abc]	0.11±0.803[ac]
STZ-type II diabetic rats:		
Saline (1 ml oral/day)	295.7±0.85[a]	0.5±0.02[a]
Gliclazide (10 mg/kg oral/day)	101.2±0.6[ab]	0.7±0.004[ab]
BRL 37344 (1 mg/kg oral/day)	110±1.3[abd]	0.41±0.002[ae]

- Mean ± S.E.: is the mean value of 10 rats per group ± standard error
- Treatment for 4 successive days

a= significant difference from saline treated non-diabetic rat group (P<0.05).
b= significant difference from saline treated STZ-type I or II diabetic rat groups (P<0.05).
c= non-significant difference between BRL and insulin treated STZ-type I diabetic rat group (P>0.05)
d= non-significant difference between BRL and gliclazide treated STZ-type II diabetic rat group (P>0.05)
e= significant difference between BRL and gliclazide treated STZ-type II diabetic rat group (P<0.05)

Table (2) : Effect of BRL 37344 on hepatic glucokinase and glucose-6-phosphatase enzymes in streptozotocin type I and type II diabetic rats

Group	Mean liver glucokinase activity±S.E. (umol-G-6-P gm/min^{-})	Mean liver glucose-6 phosphatase activity±S.E. (umol-Pi/mg pr./min^{-})
Non-diabetic rats:		
Saline (1 ml oral/day)	4.8±0.1	0.16±0.01
STZ-type I diabetic rats:		
Saline (1 ml oral/day)	1.5±0.01[a]	0.37±0.003[a]
NPH Insulin (5 units S.C/day)	4.6±0.13[b]	0.13±0.008[b]
BRL 37344 (1 mg/kg oral/day)	4.1±0.18[bc]	0.14±0.004[bc]
STZ-type II diabetic rats:		
Saline (1 ml oral/day)	1.84±0.2[a]	0.34±0.003[a]
Gliclazide(10 mg/kg oral/day).	4.7±0.19[b]	0.13±0.009[b]
BRL 37344 (1 mg/kg oral/day).	4.3±0.16[bd]	0.14±0.007[bd]

- Mean ± S.E.: is the mean value of 10 rats per group ± standard error
- Treatment for 4 successive days

a= significant difference from saline treated non-diabetic rat group (P<0.05).
b= significant difference from saline treated STZ-type I or II diabetic rat group (P<0.05).
c= non-significant difference between BRL and insulin treated STZ type I diabetic rat group (P>0.05).
d= non-significant difference between BRL and gliclazide treated STZ type II diabetic rat group (P>0.05).

Table (3) : Effect of BRL 37344 on insulin secretion from pancreatic tissues isolated from streptozotocin –type II diabetic rats

Group of pancreatic tissue	Media glucose concentration	Drug Concentration	Mean media insulin±S.E. (ng/ml)
Non-diabetic rats	100 mg%		0.7±0.004
Non-diabetic rats	100 mg%	BRL(10^{-10}M/L)	0.7±0.003
Non-diabetic rats	300 mg%		1.3±0.004[a]
Non-diabetic rats	300 mg%	BRL(10^{-10}M/L)	1.2±0.007
STZ-type II diabetic rats	100 mg%		0.42±0.004[a]
STZ-type II diabetic rats	100 mg%	BRL(10^{-10}M/L)	0.4±0.005[ad]
STZ-type II diabetic rats	300 mg%		0.67±0.007[bc]
STZ-type II diabetic rats	300 mg%	BRL(10^{-10}M/L)	0.7±0.005[be]

- Mean ± S.E.: is the mean value of 8 rats per group ± standard error
- Drug treatment in vitro was for 6 days

a= significant difference from non-diabetic rat group with media glucose 100 mg% (P<0.05).
b= significant difference from non-diabetic rat group with media glucose 300 mg% (P<0.05).
c= significant difference from non-treated STZ-type II diabetic rat group with media glucose 100 mg% (P<0.05).
d= non-significant difference from non-treated STZ-type II diabetic rat group with media glucose 100 mg% (P>0.05).
e= non-significant difference from non-treated STZ-type II diabetic rat group with media glucose 300 mg% (P>0.05).

Table (4) :Effect of BRL 37344 on glucose uptake by isolated soleus muscle from streptozotocin-type I diabetic rats

Group of soleus muscle	Drug	Mean glucose uptake ±S.E. (ug/mg wet tissue/hour) .
Non-diabetic rats		2.8±0.1
Non-diabetic rats	Insulin 10^{-7} M/L	4.8±0.22[a]
Non-diabetic rats	BRL10^{-10} M/L	4.3±0.16[a]
Non-diabetic rats	BRL10^{-10}M/L+insulin10^{-7}M/L	7.9±0.1[ace]
STZ-type I diabetic rats		0.7±0.001[a]
STZ-type I diabetic rats	Insulin 10^{-7} M/L	2.5±0.14[b]
STZ-type I diabetic rats	BRL10^{-10} M/L	2.5±0.12[b]
STZ-type I diabetic rats	BRL10^{-10}M/L+insulin10^{-7}M/L	4.7±0.08[abdf]

Mean ± S.E.: is the mean value of 9 rat soleus muscles.
a= significant difference from non-diabetic rat soleus muscles group (P<0.05).
b= significant difference from STZ-type I diabetic rat soleus muscles group (P<0.05).
c= significant difference from insulin treated non-diabetic rat soleus muscles group (P<0.05).
d= significant difference from insulin treated STZ-type I diabetic rat soleus muscle group (P<0.05).
e= significant difference from BRL treated non-diabetic rat soleus muscles group (P<0.05)
f= significant difference from BRL treated STZ-type I diabetic rat soleus muscles group (P<0.05).

Table (5) : Effect of BRL 37344 on glucose uptake by isolated soleus muscle from streptozotocin-type II diabetic rats

Group of soleus muscle	Drug	Mean glucose uptake ±S.E. (ug/mg wet tissue/hour)
Non-diabetic rats		2.8±0.1
Non-diabetic rats	Insulin 10^{-7} M/L	4.8±0.22[a]
Non-diabetic rats	BRL10^{-10}M/L	4.3±0.16[a]
Non-diabetic rats	BRL10^{-10}/L+insulin 10^{-7}M/L	7.9±0.1[ace]
STZ-type II diabetic rats		0.9±0.01[a]
STZ-type II diabetic rats	Insulin 10^{-7} M/L	2.6±0.11[b]
STZ-type II diabetic rats	BRL10^{-10}M/L	2.6±0.14[b]
STZ-type II diabetic rats	BRL10^{-10}/L+insulin10^{-7}M/L	4.9±0.08[abdf]

Mean ± S.E.: is the mean value of 9 rat soleus muscles.
a= significant difference from non-diabetic rat soleus muscles group (P<0.05).
b= significant difference from non-insulin dependent diabetic rat soleus muscles group (P<0.05)
c= significant difference from insulin treated non-diabetic rat soleus muscles group (P<0.05
d= significant difference from insulin treated STZ-type II diabetic rat soleus muscles group (P<0.05).
e= significant difference from BRL treated non-diabetic rats soleus muscles group (P<0.05)
f= significant difference from BRL treated STZ-type II diabetic rat soleus muscles group (P<0.05).

DISCUSSION

It had been long known that glucose production (glycogenolysis and gluconeogenesis) is increased and not decreased by specific β agonists. There was study in obese patients with type II diabetes mellitus showed that treatment with β_3 agonist (BRL 35135) for 10 days produced plasma glucose reduction associated with increased gluconeogenesis [28]. Moreover, Arch 2002 mentioned that β_3-adrenoceptor agonists are very effective thermogenic anti-obesity and insulin-sensitising agents in rodents. Their main sites of action are white and brown adipose tissue and muscle. They play a role in the regulation of energy balance and glucose homeostasis [29]. This is atypical action of a specific β_3 agonist via atypical effect at least in brown adipose tissue leading to unexpected metabolic effects in form of glucose uptake [30].

The present work is designed to confirm the potential antihyperglycemic effect of one of the relatively new synthesized β_3 agonist BRL 37344 in experimental model of type I and type II diabetes mellitus in albino rats and to search for a possible mode of action of this agent on blood glucose homeostasis.

In the present study, single I.P. injection of STZ in a dose 40 mg/kg or 7o mg/kg significantly increased fasting serum glucose levels and produced significant decrease in fasting serum insulin levels in comparison to fasting serum glucose level and fasting serum insulin level of non-diabetic control group injected with equal volume of saline. This decrease of insulin level following STZ injection is logically expected and could be explained by the known STZ induced destruction of pancreatic B-cell. Again, this pancreatic B-cell destruction by STZ is dose dependent, so high and low dosage of STZ can produce two different grades of diabetic severity that could be comparable to type I and type II diabetes mellitus (31 and 32).

Oral administration of gliclazide (10 mg/kg/day) in STZ- type II diabetic rats and subcutaneous injection of NPH insulin (5 units/day) in STZ-type I rat group for 4 successive days produced a significant decrease in fasting serum glucose, as compared with

non treated corresponding diabetic groups. Similar results were obtained by Jarvinen, 1997 who stated that administration of either insulin or gliclazide in STZ-diabetic rats produce a significant decrease in fasting hyperglycemia due to their actions at hepatic and peripheral levels resulting in decreased hepatic glucose production and increased glucose uptake by tissues as skeletal muscle (33).

Oral administration of gliclazide 10 mg/kg/day for 4 days produced a significant increase in serum insulin level in STZ- type II diabetic rats . This result is in agreement with Brady, 1998 who explained that gliclazide binds selectively to the pancreatic B-cell sulfonylurea specific receptor and block the K^+ ATP channels with decrease K^+ conductance resembling the physiological secretagogue glucose. Reduction of K^+ conductance causes membrane depolarization and Ca^{2+} influx through voltage sensitive Ca^{2+} channels resulting finally in insulin release (34).

BRL 37344 (1 mg/kg/day) orally significantly decreased serum glucose level in either types of diabetes in albino rats as compared to non treated corresponding diabetic rats group. Similar results could be obtained with other β_3 adrenoceptor agents that were reported to have antihyperglycemic effect for example Milagro and Martinez, 2000 mentioned that the β_3 agonist "Trecadrine" (diphenyl methylene ethylamine) in alloxan diabetic rats resulted in improvement of hyperglycemia (7). Ferre' et al., 1992 also stated that oral administration of the β_3 agonist "R0 168714" to STZ- diabetic rats resulted in marked reduction of fasting serum glucose to a nearly normal level which was comparable to fasting glucose level of the non diabetic rats (3).

Furthermore, Yoshida, 1992 and Liu and Stock, 1995 stated that BRL37344 administration in a dose 1mg/kg/day for 4 days had decreased fasting serum glucose level in chemically induced diabetes and they suggested that this hypoglycemic effect was owing to enhancement glucose uptake and oxidation by peripheral tissues as skeletal muscle as well as utilization of fatty acids by adipose tissue allowing glucose utilization and

decreased gluconeogenesis in them. Also a decrease in hepatic glucose production associated with changes in insulin resistance leading to improvement of hyperglycemia (35 and 36). Recently, Harada et al., 2005 found that repeated oral administration of new potent rat beta3-AR agonist inhibited body weight gain and significantly decreased glucose, insulin, free fatty acid, and triglyceride concentrations in plasma in mice (37).

On the other hand BRL37344 administration 1mg/kg/day for 4 days produced a non significant change in fasting serum insulin in STZ type II diabetic rats in comparison to non treated corresponding diabetic rats. These negative changes coincide with that reported by Milagro and Martinez, 2000 who stated that the antidiabetic effect of trecadrine (another selective β_3 agonist) was not due to increased insulin release as the drug had no effect on serum insulin level in alloxan diabetic albino rats (7).

In the present study, the results of in vitro study of isolated pancreas go in harmony with the results of the in vivo measurements of serum insulin level, as addition of BRL37344 to pancreatic tissue cultures did not significantly alter the insulin in the culture media denoting that it had no significant effect on insulin release from isolated pancreas. These results are similar to the results of Yoshida, 1992 who demonstrated the absence of stimulatory effect of the β_3 agonist BRL26830A on insulin release of in vitro pancreatic tissue cultures (35). Moreover, Grujic et al., 1997 confirmed this in vitro result, since they reported that the β_3 agonist CL316243 did not produce any significant change in insulin secretion from isolated pancreas and they excluded the role of β_3 receptors itself to produce any direct effect to on β cells of pancreatic islets (21).

In the present study measurement of glucokinase activity in haemogenated hepatic tissue taken from rats following induction of both models of diabetes mellitus by I.P. injection of STZ resulted in a significant decrease of hepatic glucokinase activity in both models. These results were in agreement with Matschinsky, 1996 who stated that insulinopenic diabetes induce a decrease in hepatic glucoki-

nase gene expression [38].

As regard the effect of insulin administered to STZ type I diabetic rats and oral administration of gliclazide to STZ type II diabetic rats, it was found that insulin and gliclazide produced a significant increase in hepatic glucokinase activity, this increase in hepatic glucokinase activity reached almost to the level of hepatic glucokinase of non diabetic control rats and this is possibly owing to upregulation of glucokinase gene expression by gliclazide via a direct action and or via insulin release (39 and 40). Magnusori et al., 1989 presented similar findings and assumed that this effect of insulin is due to an increase of glucokinase gene expression [41].

Oral treatment with BRL 37344 for either types of STZ diabetic albino rats produced significant increase in the activity of hepatic glucokinase enzyme in both STZ diabetic rats models. The enzyme activity of the two diabetic groups treated with BRL37344 were almost equal to those of non diabetic control rats group. These results are in agreement with Ohsaka et al., 1998 and Milagro et al., 1999-A who mentioned that, although insulin seems to be the most important regulator of liver glucokinase gene expression in diabetic rats, glucokinase mRNA is increased in spite of no changes in plasma insulin level after β_3 agonist administration (42 and 17). The present results also confirms that although β_3 agonist BRL 37344 produced marvelous improvement of glucokinase activity yet it had no significant effect on insulin level, so it can be concluded that insulin is not involved in the increase of glucokinase activity observed in β_3 agonist treated rodents. The exact mechanism for a β_3 agonist to increase hepatic glucokinase activity is not completely evident, Milagro et al., 1999-A suggested that this significant increase of glucokinase may be secondary to normalization of glycemia after the administration of the β_3 agonist trecadrine resulted in phosphorylation of glucose to store it preferentially as glycogen [17].

In the present work, measurement of glucose-6-phosphatase enzyme activity in hepatic tissue haemogenate revealed that glucose-6-phosphatase was significantly in-

creased in STZ diabetic rats. In accordance with these findings Nordlie, 1976 and Milagro et al., 1999-A demonstrated that glucose-6-phosphatase activity was increased in STZ induced diabetes and they assumed that the increase of glucose-6-phosphatase is associated with increased hepatic glucose output and participated in development of STZ induced hyperglycemia (43 and 17).

In the present study, gliclazide or insulin administration in rats with type II or type I STZ-diabetes mellitus, respectively lead to significant decrease in hepatic glucose-6-phosphatase activity in both types of diabetes. In accordance with the present results, Nordlie et al., 1965 reported that hepatic glucose-6-phosphatase activity is significantly depressed after insulin treatment in diabetic rats [44]. The action of insulin is either directly or through the release of a low molecular weight insulin mediator [45]. Hepatic glucose production may be suppressed by insulin either in absence or presence of hyperglycemia. In euglycemia, insulin induced a marked decrease in glucose-6-phosphatase concentration because of its strong stimulating effect on glycolysis, in contrast in hyperglycemia, hepatic glucose production is decreased through an increment of glucokinase activity and an inhibition of glucose-6-phosphatase enzyme activity [46].

Jackson and Bressler, 1981 and Muller, 2000 assumed that, gliclazide administration in chemically induced diabetes mellitus in rats lead to decrease hepatic glucose output through inhibition of hepatic glucose-6-phosphatase activity partially via release of endogenous insulin and also via direct inhibition of glucose-6-phosphatase in the diabetic rat liver through insulinomimetic like action (47 and 48).

Oral BRL37344 treatment of either types of diabetic rats for 4 days resulted in significant decrease in hepatic glucose-6-phosphatase activity versus in the non treated type II and I diabetic rats. BRL37344 reduction of the hepatic glucose-6-phosphatase reached to the value which were not significantly different from that of non diabetic control group i.e. it produces almost complete normalization of enzyme activity. Similar findings were

obtained by Milagro et al., 1999-A following treatment with the β_3 agonist "trecadrine" for 4 days to alloxan diabetic rats, it reduced hepatic glucose output via the decrease in glucose-6-phosphatase enzyme activity and they assumed that the decrease in G-6-Phophatase could be explained through the following reasons; firstly a direct effect of the treatment with trecadrine, secondly, the improvement in glucose sensitivity in the hepatocytes, as a consequence of a reduction in blood glucose level that resulted in stimulating the hepatocytes to replenish their glycogen reserve and this is supported by data from hepatic glucokinase activity which is increased after trecadrine administration to phosphorylate glucose for glycogenesis [17].

Liu and Stock, 1995 and D'allaire et al., 1996 reported that administration of a β_3 adrenoreceptor agonist in different animal models of diabetes mellitus had decreased hepatic glucose production and enhanced glucose uptake in peripheral tissues including skeletal muscle and adipose tissue (36 and 49).

The worthy point of great interest presented in the present study as a possible mechanism for the correction of hyperglycemia by the β_3 agonist BRL37344 in STZ induced diabetes mellitus in rats, was the enhancement of glucose utilization in skeletal muscle. In vitro incubation of isolated soleus muscles revealed significant reduction of glucose uptake by incubated soleus muscles isolated either from STZ -type II or I diabetic rats in comparison to soleus muscles isolated from non diabetic control rats. These results could be confirmed and explained by the study of Carvey et al., 1989 who stated that a mature skeletal muscle expresses two glucose transporters GLUT1 and GLUT4 and the latter is decreased in STZ-diabetic rats which leads to decrease in media glucose uptake by soleus muscles isolated from STZ- diabetic rats [50].

In the present study, when insulin alone was added to the incubated soleus muscles obtained from non-diabetic, STZ-type II or STZ type I diabetic rats, the drug stimulated significantly the glucose uptake in those muscles on comparson to their corre-

sponding groups of soleus muscles without insulin administration. Tanishita et al., 1997 mentioned that incubation of L6 myocytes with insulin increased GLUT4 content in the plasma membrane [51]. Furthermore, Kruszynska et al., 2001 stated that the increase in glucose uptake may be owing to the significant increased GLUT4 recruitment in muscles following insulin administration [52].

Addition of BRL37344 alone to the previous mentioned three rat muscle groups increased significantly the glucose uptake by those muscles. There was no significant differences between the effects of addition of either insulin alone or BRL alone on glucose uptake by incubated soleus muscles isolated from either non diabetic rats, STZ-type II or STZ- type I diabetic rats. BRL37344 was found in this study to be a direct potent stimulant of glucose uptake by isolated soleus muscles, since the in vitro isolation of soleus muscle liberates the muscle from any physiological influences that could affect glucose uptake by muscle tissue, including changes of blood flow and the humoral response to the effect of β_3 agonist, thus any drug induced change in this isolated in vitro situation could be assumed to be a direct drug effect [24]. However, presence of other factors that may enter play to affect skeletal muscle glucose uptake could not be denied on in vivo administration of this β_3 agonist (24). In vitro results that were reported by Liu et al., 1996 stated that, β_3 receptor agonist BRL37344 stimulate glucose uptake and phosphorylation and glycogen synthesis in rat soleus muscle preparation [53]. Moreover, Tanishita et al., 1997 mentioned that in the absence of insulin BRL37344 enhanced glucose transport into L6 myocytes through a signaling pathway different from that of insulin and that the mechanism does not involve the translocation of GLUT4, but may be due to an increase in the intrinsic activity of GULT4 present in the plasma membrane [51]. Further support to the present results was presented by Milagro et al., 1999-B who reported that, in vitro β_3 agonist treated diabetic rats induced an increase in the gene expression ratio GLUT1/GLUT4 in rat gastrocnemius muscle. Thus β_3 receptor agonist stimulates GLUT1 gene expression as compared to GLUT4 (25). Lastly, Board et al.,

2000 stated that BRL37344 at 10^{-10} M stimulated fuel utilisation by isolated mouse soleus muscle through increase uptake and phosphorylation of 2-D-glucose by 40% (54).

In vivo data presented by Abe et al., 1993 stated that BRL 37344 could possibly stimulate non insulin mediated glucose utilization through β_3 receptors in the soleus muscle directly and independently of changes in plasma insulin levels (24). Other in vivo study of Shimazu, 1994 had reported that increased sympathetic activity following electrical stimulation of the ventromedial hypothalamus stimulates glucose transport in skeletal muscle and other peripheral tissues through increased intrinsic activity of the GLUT4 in the plasma membrane (55). Similarly, Liu and Stock, 1995 had reported that the possible direct β_3 receptor role on glucose uptake by rat tissue is via stimulation of GLUT1 mainly which is independent of changes in plasma insulin level too. In addition, Liu and Stock assumed that insulin dependent mechanism may be involved via the stimulatory effect of BRL 37344 on pancreatic B-cell insulin secretion, and in turn, insulin increases glucose extraction by skeletal muscle through its coordinated effects on both the recruitment of GLUT4 and enzymes responsible for glucose utilization by the skeletal muscle (36). These results were further supported by Liu et al., 1998, as they stated that the skeletal muscle expresses two uncoupling proteins which are linked to β_3 receptors facilitating glucose uptake directly (56). Moreover, insulin suppresses plasma non-esterified fatty acids (NEFA) thus enhance glucose oxidation, also it increases muscle blood flow through endothelial dependent vasodilator action (57 and 52). However, the direct action of BRL 37344 on skeletal muscle glucose uptake can't be denied and seems to have the upper hand in enhancing glucose uptake since in the present study BRL 37344 stimulated glucose uptake in isolated skeletal muscle tissue.

In the present study the significant increase in glucose uptake in isolated soleus muscles induced by either insulin or BRL alone was more increased when both insulin and BRL were added together to those muscles. These data was supported by

Tanishita et al., 1997 who stated that the effects of insulin and BRL37344 were completely additive, suggesting that these two agents enhance glucose uptake by L6 myocytes through different mechanisms (51).

In conclusion, this study confirmed that a β_3 agonist BRL37344 has a significant antihyperglycemic effect in insulinopenic STZ induced diabetes mellitus in rats. It has a regulatory role on the liver enzymes which are responsible for hepatic glucose output by facilitating glucose uptake directly and indirectly in skeletal muscle that represent the main anatomic site of glucose utilization in human and in laboratory animals as well.

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الدور المحتمل لأحد منبهات مستقبلات بيتا -٣ الإختيارية لعلاج مرض البول السكرى فى فئران التجارب البيضاء

الهدف من البحث :

هو دراسة دور أحد المنبهات الاختيارية التى تعمل على مستقبلات بيتا-٣ (ب ر ل ٣٧٣٤٤) على مستوى السكر فى الدم فى نموذجين لمرض البول السكرى فى فئران التجارب البيضاء ومقارنة هذا الدور بدوائى الجليكلازيد والإنسولين وقد تمت الدراسة على قسمين :

١- دراسة على الفئران الأحياء لقياس مستوى السكر فى الدم وقياس نشاط إنزيم الجلوكوكينيز وإنزيم الجلوكوز -٦- فوسفاتيز فى كبد الفئران المصابة بالمرض.

٢- دراسة زجاجية لدور هذا المنبه على إفراز الإنسولين من مزارع نسيج البنكرياس من الفئران السليمة والمصابة بالمرض وكذلك دراسة دور هذه الأدوية على إستهلاك الجلوكوز بواسطة العضلات الإرادية (العضلة الأخمصية) المستخلصة من الفئران المصابة بالمرض.

نتائج هذا البحث :

أن إعطاء أدوية الجليكلازيد والإنسولين ودواء ب ر ل (٣٧٣٤٤) لمدة ٤ أيام فى دراسة حية على الفئران البيضاء السليمة والمصابة بمرض البول السكرى بنوعية قد أدى إلى نقص ذو دلالة إحصائية فى مستوى الجلوكوز بالدم ولم يحدث أى تغيير ذو دلالة إحصائية على مستوى الإنسولين فى الدم بعد إعطاء كلاً من الإنسولين ودواء ب ر ل ، بينما إعطاء دوار الجليكلازيد فقط أدى إلى زيادة مستوى الإنسولين فى الدم وذلك بالمقارنة بالفئران المصابة بالمرض ولم تعالج بهذه الأدوية..

كما وجد أن الأدوية الثلاثة تحدث نقص ذو دلالة إحصائية فى نشاط إنزيم الجلوكوكينيز وزيادة ذو دلالة إحصائية فى نشاط إنزيم الجلوكوز-٦- فوسفاتيز بالمقارنة بالفئران المصابة بالمرض ولم تعالج بهذه الأدوية .

كما أن نتائج الدراسة الزجاجية قد أظهرت عدم وجود تأثير مباشر لدواء ب ر ل على إفراز الإنسولين من بنكرياس الفئران السليمة أو المصابة بمرض البول السكرى (النوع الثانى) مع إيجاد تأثير مباشر لدواء ب ر ل على زيادة إستهلاك الجلوكوز بواسطة العضلة الأخمصية لفئران سليمة أو مصابة بمرض البول السكرى بنوعية وهذه الزيادة ذات دلالة إحصائية واضحة ومكافئة لتأثير الإنسولين على العضلة أيضاً، كما أن هذه الزيادة فى إستهلاك الجلوكوز بواسطة العضلة تزيد بإطراد عند إعطاء دواء ب ر ل مع الإنسولين فى وقت واحد .