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Activation of I_2-imidazoline receptors by agmatine improved insulin sensitivity through two mechanisms in type-2 diabetic rats

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ABSTRACT

In an attempt to clarify the role of endogenous opioid in peripheral I_2-imidazoline receptors activation for improvement of insulin action, bilateral adrenalectomy was carried out in rats with insulin resistance induced by 4-week fructose-rich chow feeding. Single intravenous (i.v.) injection of agmatine (1 mg/kg) for 30 min increased the plasma β-endorphin-like immunoreactivity (BER) in a way parallel to the reduction of plasma glucose in sham-operated fructose chow-fed rats; this action of agmatine was totally abolished by BU224 at sufficient dosage (1 mg/kg, i.v.) to block I_2-imidazoline receptors. The plasma glucose lowering effect of agmatine was markedly reduced but not totally deleted by adrenalectomy in fructose chow-fed rats. A direct effect of agmatine on glucose homeostasis can thus be considered. The hyperinsulinemic-euglycemic clamp technique was performed to evaluate insulin sensitivity. The effect of agmatine on elevation of the average rate of glucose infusion at the glucose clamp steady state in sham-operated fructose chow-fed rats was lessen in adrenalectomized fructose chow-fed rats, but was completely abolished by BU224. The obtained results suggest that the improvement of insulin sensitivity by agmatine is produced by two mechanisms, stimulation of adrenal gland to enhance β-endorphin secretion and a direct activation of peripheral I_2-imidazoline receptor in tissues, for the amelioration of insulin action.

Insulin resistance is a key feature of impaired glucose tolerance in type-2 diabetes that can be characterized by a diminished ability of insulin sensitive tissues and a marked decrease of glucose metabolism in response to insulin [3]. These impairments in glucose metabolism are associated with a high risk of cardiovascular diseases and they may play a role in the development of hypertension, dyslipidemia, and atherosclerosis [15]. Primary treatment goals in diabetes include restoration and maintenance of normoglycemia, avoidance of diabetic complications, and prevention of cardiovascular events [15].

Agmatine is an endogenous amine synthesized from the decarboxylation of L-arginine by arginine decarboxylase and it binds to several targets as an endogenous ligand for imidazoline receptors [8]. Imidazoline receptors are introduced to mediate the antihypertensive action of clonidine and analogues in brain [6]. Cerebral imidazoline receptors in modulation of opioid functions have been indicated [19]. Functionally, peripheral imidazoline receptors are mediated in movement of smooth muscle, increase of insulin release and regulating the renal excretion of sodium, potassium and water [9]. Thus, imidazoline receptors will become a therapeutic target in the treatment of diabetes and others [9].

There are three subtypes of imidazoline receptors, the I_1-imidazoline receptor which mediates the sympatho-inhibitory actions to lower blood pressure, the I_2-imidazoline receptor which is an important allosteric-binding site of monoamine oxidase and the I_3-imidazoline receptor which regulates insulin secretion from pancreatic beta cells [9]. Recent advance indicated that activation of I_2-imidazoline receptors by agmatine produce a decrease of plasma glucose in streptozotocin-induced diabetic (STZ-diabetic) rats, the established type-1 diabetes-like animal model [11,12]. Activation of peripheral I_2-imidazoline receptors by agmatine to recover the insulin action in type-2 diabetic rats has also been indicated [13].

Otherwise, it has been shown that β-endorphin possess the ability to reverse the impaired insulin-stimulated glucose disposal in rats with insulin resistance [16,17]. In addition to release with adrenocorticotrophic hormone from pituitary gland, adrenal gland is another main source of β-endorphin [1]. It has been indicated that activation of I_2-imidazoline receptors in adrenal gland by agmatine enhances the secretion of β-endorphin to stimulate opioid receptors located on peripheral tissues for increase of glucose

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utilization in STZ-diabetic rats [11]. However, less information is available regarding the relationships between endogenous opioid and peripheral I_2-imidazoline receptors in the improvement of insulin action under insulin resistance. Thus, it is of special interesting to clarify this point in the present study using rats with insulin resistance induced by a diet containing 60% fructose [13].

Male Wistar rats weighing 200–250 g were obtained from the Animal Center of National Cheng Kung University Medical College. All animals were used at 6 weeks of age. They were maintained in a temperature-controlled room (25 ± 1 °C) and kept on a 12:12 light-dark cycle (light on at 06:00 h). After 2 weeks on standard chow (Purina Mills, LLC, St. Louis, MO), rats were received the fructose-rich chow (Teklad, Madison, WI) containing 60% fructose for 4 additional weeks to induce insulin resistance [13]. Bilateral adrenalectomy was performed in 4-week fructose chow-fed rats as described previously [5]. Sham-operated animals served as controls. Animals were allowed to recover for 2 weeks after the operation. The animals appeared alert and in good health. The rats were maintained on a fructose chow diet during the post-operation period. Food and water were available *ad libitum* throughout the experiment. All animal procedures were performed according to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, as well as the guidelines of the Animal Welfare Act.

After a 2-week recovery period, blood samples were collected from the tail vein of rats to evaluate plasma levels of glucose, insulin and β-endorphin when the rats were deeply anaesthesized by sodium pentobarbital (30 mg/kg, i.p.). Plasma glucose concentration was measured by glucose oxidase method using commercially available kit purchased from BioSystem (Costa Brava, Barcelona, Spain; Cat. #COD12503). The enzyme-linked immunosorbent assay (ELISA) technique was employed to quantify the plasma levels of insulin and β-endorphin-like immunoreactivity (BER). Rat insulin ELISA kit was obtained from LINCO Research, Inc. (St. Charles, MO, USA; Cat. #EZRMI-13K). The ELSA kit for BER was obtained from Peninsula Laboratories Inc. (Belmont, CA, USA; Cat. #T-4040). All samples were analyzed in triplicate.

Data were expressed as the mean ± SE for the number of animals in the group as indicated in the tables and figures. Statistical differences among groups were determined by using two-way repeated-measures ANOVA. The Dunnett range post hoc comparisons were used to determine the source of significant differences where appropriate. The obtained *P*-value less 0.05 was considered statistically significant.

Two weeks later of adrenalectomy, no difference of the basal plasma glucose and BER levels can be observed between adrenalectomized fructose chow-fed rats and sham-operated control (Table 1). Also, the basal plasma insulin level in fructose chow-fed rats receiving bilateral adrenalectomy was not different from that in sham-operated littermates (Table 1). A 30-min intravenous (i.v.) injection of agmatine (1 mg/kg) was found to enhance insulin action in fructose chow-fed rats, but not due to the change of insulin secretion [13]. In an attempt to determine whether the ameliorating effect of agmatine on insulin-stimulated glucose disposal is mediated by β-endorphin from adrenal gland, fasted fructose chow-fed rats received bilateral adrenalectomy or sham-operation were used to receive an i.v. injection of 1 mg/kg agmatine sulfate (Sigma–Aldrich, Inc., Saint Louis, Missouri, USA; Cat. #M6760) into the tail vein. Rats receiving same injection of vehicle solution (saline) at equivalent volume were grouped as control. After 30-min treatment, blood samples were collected from the tail vein of rats anaesthetized by sodium pentobarbital to evaluate the plasma levels of glucose, insulin and β-endorphin.

In consistent with our previous study [13], agmatine treatment failed to modify the hyperinsulinemia in fructose chow-fed rats with bilateral adrenalectomy or sham-operation (Table 1).

Table 1
Effects of agmatine on the plasma levels of glucose, beta-endorphin (BER) and insulin in fructose chow-fed rats receiving bilateral adrenalectomy or sham-operation.

Fructose chow-fed rats	Plasma levels		
	Glucose (mg/dl)	BER (pg/ml)	Insulin (μU/ml)
Sham-operated group			
Basal	132.2 ± 3.2	108.6 ± 9.4	178.3 ± 8.1
Vehicle	131.8 ± 4.3	107.8 ± 10.2	176.4 ± 9.2
Agmatine	114.6 ± 3.1**	171.4 ± 8.4**	175.4 ± 7.3
Agmatine+BU224	130.6 ± 4.6	109.3 ± 9.6	177.8 ± 10.2
Adrenalectomized group			
Basal	133.5 ± 3.9	112.3 ± 10.1	177.9 ± 11.2
Vehicle	132.8 ± 5.1	111.9 ± 9.8	178.4 ± 9.7
Agmatine	125.5 ± 3.7*	112.4 ± 9.2	176.3 ± 10.5

Blood samples from rats receiving an intravenous (i.v.) injection of agmatine (1 mg/kg) for 30 min were used for determination. BU224 (1 mg/kg) was i.v. injected 30 min prior to administration of agmatine. The vehicle (saline) used to dissolve the testing drugs was given at the same volume. Data from animals that did not receive any treatment was taken as basal value. Values (mean ± SE) were obtained from each group of 7 animals. *$P < 0.05$ and **$P < 0.01$ versus data from vehicle-treated sham-operated group, respectively.

Agmatine was found to increase the plasma BER in a way parallel to the lowering of plasma glucose in fructose chow-fed rats received sham-operation (Table 1). Also, agmatine failed to modify the plasma level of BER in fructose chow-fed rats with adrenalectomy (Table 1) showing the mediation of endogenous β-endorphin from adrenal gland in the action of agmatine. However, the plasma glucose lowering action of agmatine was markedly reduced but not totally abolished in fructose chow-fed rats with bilateral adrenalectomy (Table 1).

Subsequently, the role of I_2-imidazoline receptors in the improvement of glucose homeostasis under insulin resistance was identified using BU224, as specific antagonist [10]. The i.v. injection of 1 mg/kg BU224 hydrochloride (Tocris Cookson, Bristol, UK; Cat. #0725) was given 30 min before the i.v. injection of agmatine (1 mg/kg) into the fructose chow-fed rats. Plasma levels of glucose, insulin and β-endorphin in rats were then measured as describe above. In the presence of BU224, the effects of agmatine on plasma glucose accompanying with the elevation of plasma BER in sham-operated rats with insulin resistance were fully eliminated (Table 1). Activation of I_2-imidazoline receptors by agmatine is responsible for the improvement of glucose homeostasis in fructose chow-fed rats. Also, the plasma glucose modified action of agmatine in the absence of adrenal gland seems related to the direct activation of I_2-imidazoline receptors located in peripheral tissues.

Clinically, the initial therapy for type-2 diabetes has been shifting from secretagogues and alpha-glucosidase inhibitors to agents for improvement of insulin sensitivity, known as insulin sensitizer including thiazolidinediones and others [2]. The direct method for assessing insulin sensitivity, the reciprocal of insulin resistance, is the hyperinsulinemic-euglycemic clamp [7]. Thus, hyperinsulinemic-euglycemic clamp was performed 30 min after an i.v. injection of 1 mg/kg agmatine into fructose chow-fed rats. At the start of the glucose clamp (time zero), insulin (40 U/ml; pork insulin, Novo Biolabs, Copenhagen, Denmark) dissolved in saline containing 0.2% bovine serum albumin (Sigma–Aldrich, St. Louis, MO, USA) was appropriately diluted to deliver 40 mU/kg/min intravenously through a catheter inserted in the femoral vein, together with D-glucose (10%, w/v, in saline). Euglycemia was maintained by a variable rate of glucose infusion that was adjusted according to the determination of plasma glucose at 10-min intervals throughout the 2-h clamp. Direct assessment of insulin sensitivity was performed by calculating glucose infusion rate during the last 20 min of the clamp when steady state was reached. A total of 2.5 ml of blood was withdrawn during the clamp; the RBCs were reinfused after each sample in a 1:1 dilution with saline.