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Changes in A_1 Adenosine Receptor-Adenylyl Cyclase System of Rat Adipocytes Following Induction of Experimental Diabetes by Streptozotocin Treatment[§]

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ABSTRACT

Adenosine receptors in rat adipose tissues have been reported to be of A_1 subclass, and their stimulation leads to inhibition of adenylyl cyclase, resulting in inhibition of lipolysis. In the present study we investigated changes in A_1 adenosine receptor-adenylyl cyclase system of adipocytes following induction of experimental diabetes in rats.

One week following experimental diabetes were induced by intravenous injection of streptozotocin (50 mg/kg body wt.), adipocytes from rats (170~230 g) fed *ad libitum* were isolated using collagenase. When adipocytes were incubated for 1 h with 1 unit/ml adenosine deaminase and 1 μM isoproterenol, and assayed for glycerol formation, it was found that the inhibition of lipolysis in diabetic adipocytes by (−)-N^6-(R-phenylisopropyl)adenosine (PIA), an A_1 adenosine receptor agonist, was twice that of control adipocytes. In an effort to delineate the mechanism(s), [^{3}H]PIA binding to adipocytic membranes from diabetic and control rats were determined. Neither the affinities nor numbers of A_1 adenosine receptor were significantly different from each other (Best fit parameters for the one-site model are: K_d=0.51±0.09 nM and B_{max}=1.60±0.12 pmoles/mg protein for control membranes; K_d=0.54 ±0.21 nM and B_{max}=1.72±0.31 pmoles/mg protein for diabetic membranes). However, the inhibiton by PIA of the isoproterenol-stimulated adenylyl cyclase activities was found to be 1.9 times higher in adipocytic membranes from diabetic rats than those from controls. These results suggest that the increased sensitivity of inhibition of lipolysis to PIA in adipocytic membranes from diabetic rats is due to changes in signal transduction pathways, rather than alterations of A_1 adenosine receptor molecules themselves.

Key Words: Experimental diabetes, Adipocytes, Adenosine receptor, Adenylyl cyclase

INTRODUCTION

Insulin-dependent (Type 1) diabetes have many associated metabolic disturbances, including increased circulating fatty acid concentrations. The rate of lipolysis depends on the activation of triglyceride lipase by cAMP. The intracellular concentrations of cAMP are in turn regulated by the activity of adenylyl cyclase, which catalyzes the synthesis from ATP and by that of phosphodiesterase, which catalyzes its hydrolysis to 5'-AMP. Both enzyme activities are regulated by insulin (Illiano and Cuatrecasas, 1972; Manganiello and Vaugham, 1973; Kono *et al.*, 1975). It has been

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shown that diabetes induce an increase in cAMP in human adipose tissue as well as in rat adipocytes (Arner *et al.*, 1979; Chiappe de Cingolani, 1983 and 1986).

The adenylyl cyclase system of rat adipocytes contains stimulatory receptors for β-adrenergic agonists, ACTH and glucagon (Birnbaumer and Rodbell, 1969). Opposing the actions of these stimulatory receptors are several inhibitory receptors, including those for adenosine, nicotinic acid and prostaglandins (Londos *et al.*, 1978 and 1981). These receptors exert stimulatory or inhibitory effects on this enzyme depending upon whether they are coupled to stimulatory (G_s) or inhibitory (G_i) guanine nucleotide binding regulatory proteins. In the case of adenosine, the inhibitory effects are mediated *via* A_1 adenosine receptors (Londos *et al.*, 1980). It has been suggested that adenosine is important in tonic inhibition of lipolysis in basal conditions (Kather *et al.*, 1985).

In the present study we examined the effects of streptozotocin-induced diabetes on the adenosine receptor-adenylyl cyclase system in rat adipocytes.

MATERIALS AND METHODS

Chemicals

(−)-N^6-R-[G-^{3}H]phenylisopropyladenosine ([^{3}H] PIA, specific activity 49.9 Ci/mmol) and adenosine 5'-[α-^{32}P]triphosphate ([^{32}P]ATP, specific activity 3,000 Ci/mmol) were purchased from Amersham International plc (Green End Aylesbury, Buckinghamshire, UK); Bovine serum albumin (BSA), fatty acid-free BSA, crude bacterial collagenase, adenosine deaminase from calf intestine, (−)-N^6-(R-phenylisopropyl)adenosine (PIA), HEPES, ATP, cAMP, GTP, creatine phosphate, creatine phosphokinase, Tris-HCl, alumina, Dowex 50, EDTA and Ficoll 400 from Sigma Chemicals (St. Louis, MO, USA); Streptozotocin from Boehringer Mannheim (Mannheim, FRG); Ketostix® and Diastix® from Miles Sankyo Co (Tokyo, Japan); GF/B glass microfiber filter from Whatman International Ltd. (Maidstone, UK). All other chemicals were of analytical grade.

Animals

Male Sprague-Dawley rats weighing 170 to 230 g, which have been acclimatized in the animal care facilities of the university for more than a week, were used in all experiments. Rats were allowed free access to food and tap water, under a light-dark cycle with the light on from 6 a.m. to 6 p.m..

Induction of experimental diabetes

In all experiments, animals were divided into two groups. Animals received streptozotocin (50 mg/kg body wt., dissolved in the citrate buffer containing 50 mM citric acid and 50 mM sodium citrate, pH 4.5) or an equivalent volume of vehicle under light ether anesthesia into a tail vein (Rerup, 1970). Glucose and ketone body were checked in the urine using Diastix® and Ketostix®, respectively, every 2 or 3 days for a week. The animals were then killed one week following streptozotocin treatment. On the day of sacrifice levels of glucose and ketone body were checked around 10 a.m., and then for the diabetic group, rats whose blood glucose concentrations were over 300 mg/dl and ketone body was negative were selected. The two parameters of control rats were all negative. Under the light ether anesthesia 0.5 ml of blood was collected from the inferior vena cava using a syringe with heparin, and stored in the refrigerator for the determination of glucose by the glucose oxidase method (Raabo and Terkildsen, 1960).

Preparation of isolated fat cells

Isolated fat cells were prepared accoring to the method of Rodbell (1964). Rats were killed by cervical dislocation around 10 a.m. to avoid any circadian variations, and epididymal fat pads were quickly removed and pooled in Buffer A (118 mM NaCl, 4.74 mM KCl, 2.54 mM $CaCl_2$, 1.19 mM $MgSO_4$, 1.19 mM KH_2PO_4, 2.5 mM glucose, 25 mM HEPES, pH 7.4). These fat fads were then incubated with crude collagenase (3 mg/g fat tissue/3 ml Buffer A) at 37℃ for 1 hour in a Dubnoff shaking incubator (110 cycles/min). The isolated fat cells were gently filtered through a fine mesh silk screen. The cell suspension was centrifuged in an IEC clinical centrifuge at 1,000 rpm for 10 sec and the infranatant was removed. After two volumes of Buffer A were added to them, the tubes were centrifuged under the same

conditions. This washing procedure was repeated a total of four times.

Preparation of plasma membrane fractions

Plasma membranes were prepared as described by McKeel and Jarett (1970). Fat cells were homogenized in 4 volumes of ice-cold Buffer B (0.25 M Sucrose, 1 mM EDTA, 10 mM Tris-HCl, pH 7.4) using a Potter-Elvejehm homogenizer. The resulting homogenates were then centrifuged at 15,000 rpm (23,000 xg) for 15 min in a Sorvall RC-5B centrifuge, the supernatant was removed, and the pellets were selected for crude membrane fractions.

For the determination of adenylyl cyclase activitites, these crude membrane fractions were washed twice with Buffer C (50 mM Tris-HCl, pH 7.4 at 4℃ containing 1 mM $MgCl_2$), resuspended to the protein concentration of approx. 2 mg/ml in Buffer C, and stored in 20 μl-aliquots in a −70℃ deep freezer.

For [^{3}H]PIA binding assays, the crude membrane fractions, which had been centrifuged at 15,000 rpm (21,000 xg) for 15 min, were resuspended in Buffer B and centrifuged at 3,000 rpm (800 xg) for 10 min. The supernatants were centrifuged once more at 15,000 rpm (21,000 xg) for 20 min, and the resulting pellets were resuspended in Buffer B. After loading samples into 5 ml of linear Ficoll gradient (5~25 w/v % in 0.25 M sucorse) they were centrifuged at 24,000 rpm (58,000 xg) for 30 min in a HITACHI SCP85H ultracentrifuge. Sharp bands just below the sample interface were removed, diluted 4-fold with Buffer B, and washed by centrifuging at 15,000 rpm (23,000 xg) for 15 min and then by resuspending the resulting pellets in Buffer C. This washing procedure was repeated once more, and the resuspension was stored in 50 μl-aliquots in a −70℃ deep freezer.

Determination of glycerols

Lipolysis were evaluated by determining glycerols, the final product of lipolysis, in the incubation media. Isolated fat cells (approx. 10^5 cells in 0.5 ml Buffer A containing 4% fatty acid-free BSA) were incubated at 37℃ for 1 h. Four hundreds microliters of the buffer below floating fat cells were pipetted into Eppendorf tubes containing 20 μl of 60% $HClO_4$ and tubes were vortexed. After centrifugation at 2,000 xg for 2 min, supernatants were transferred into another tubes and neutralized with $KHCO_3$. Precipitated $KClO_4$ was removed by centrifugation at 2,000 xg for 4 min, and glycerols present in the supernatant were determined by the method of Garland and Randle (1962).

[^{3}H]PIA binding assay

Determination of A_1 adenosine receptor binding to rat fat cell membranes was performed using [^{3}H]PIA by the method of Ukena *et al.* (1984). Aliquots of fat cell membranes (20~40 μg protein) were incubated with 1 to 60 nM [^{3}H]PIA and adenosine deaminase (1 unit/ml) in Buffer C at 37℃ for 1 h. The total volume of the reaction mixtures was 500 μl. Bound and free radioligands were separated by rapid filtration of a 400 μl-aliquot of the assay mixtures through a Whatman® GF/B glass microfiber filter (25 mm diameter). The filters were immediately washed with two 5 ml portions of ice-cold 50 mM Tris-HCl buffer, pH 7.4. After transferring the filter into a vial containing 13 ml scintillation cocktail, the radioactivity was determined in a liquid scintillation counter. Specific binding was defined as the amount of the radioligand bound in the absence of competing ligands minus the amount of that bound in the presence of 10 mM PIA.

Determination of adenylyl cyclase activity

Adenylyl cyclase activity was determined by the method of Salomon *et al.* (1974). The total volume of the incubation medium was 50 μl which contained 0.1 mM [^{32}P]ATP, 0.1 mM cAMP, 4 mM $MgCl_2$, 1 mM GTP, 1 mg/ml BSA, 2 mM creatine phosphate, 25 unit/ml creatine phosphokinase, 0.5 unit/ml adenosine deaminase and 30 mM Tris-HCl (pH 7.5) (Londos *et al.* 1978). Reaction was started by adding membrane fractions into the tubes and carried out at 37℃ for 15 min. [^{32}P]cAMP formed was separated from [^{32}P]ATP using alumina and Dowex 50 columns, and the radioactivity from [^{32}P]cAMP was counted in Beckman liquid scintillation counter.

Determination of protein concentrations

Protein concentrations were determined by the method of Bradford using bovine serum albumin

as standard (Bradford, 1976).

Data analysis

Binding assays were analyzed using a nonlinear least-square curve fitting program for complex ligand-receptor system (Munsen and Rodbard, 1980). Comparisons between groups were carried out using the Student *t*-test.

RESULTS

Changes in body weight, weight of fat tissue and plasma glucose concentration

During the one week period after injection of streptozotocin, diabetic rats lost the body weight approx. 40 g in contrast to control rats which gained 10 to 20 g (Table 1). On the day of sacrifice, the plasma glucose concentration of diabetic rats were 3 to 4 times higher than those of controls, and wights of epididymal adipose tissue of diabetics were reduced to 25 to 50 percent of those of control rats.

Inhibition of lipolysis by PIA

In order to compare lipolysis rate in fat cells from diabetic and control rats, the isolated adipocytes were incubated at 37℃ for 1 h and the amount of glycerol released into the media were determined. Cell concentrations were approx. 2×10^5 cells/ml, and the incubation buffer included adenosine deaminase for degradation of endogenous adenosine (Schwabe *et al.*, 1975). As shown in Fig. 1, the amounts of glycerol formed in the diabetic adipocytes which were stimulated by 10^{-6} M isoproterenol were not significantly different from those of controls. Lipolysis in adipocytes from both diabetic and control rats was decreased in a dose-dependent manner by PIA, an adenosine receptor agonist which is not degraded by adenosine deaminase (Fain *et al.*, 1972; Trost and Stock, 1977). However, there was a significant difference in the degree of inhibition, that is, the inhibition of lipolysis by 10^{-4} M PIA in the diabetic adipocytes were two times that of controls.

Table 1. Comparison of body weight gains, plasma glucose levels and weights of fat tissue

Groups	Control rats	Diabetic rats
Number of animals	17	21
Body weight gains (g)	12.3±3.3	−42.6±4.6*
Plasma glucose levels (mg/dl)	123.9±6.6	410.7±39.4*
Weights of fat tissue (g)	2.09±0.13	0.53±0.10*

*stands for $p<0.05$

[^{3}H]PIA binding

Saturation experiments were performed using plasma membrane fractions obtained from diabetic and control rats. [^{3}H]PIA binding to adipocytic membranes showed the typical saturation process in both the diabetic and control rats, where the nonspecific binding was increased as the concentration of [^{3}H]PIA was increased (Ukena *et al.*, 1984; Schwabe *et al.*, 1985). When the data were analyzed using the curve-fitting computer program LIGAND, both the diabetic and control membranes were fit to one-site model (Munsen and Rodbard, 1980). The binding experiments

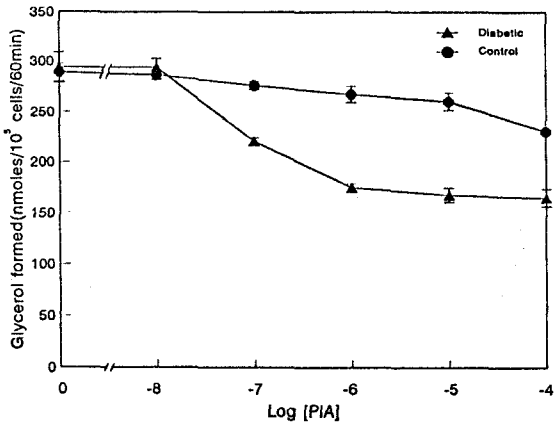

Fig. 1. PIA inhibition of glycerol formation stimulated by isoproterenol and adenosine deaminase in adipocytes from diabetic and control rats. Adipocytes (10^5 cells in 0.5 ml Buffer A) were incubated with 1 unit/ml adenosine deaminase, 1 μM isoproterenol and the indicated concentrations of PIA for 1 h. Values are given as means±standard errors of triplicate determinations.

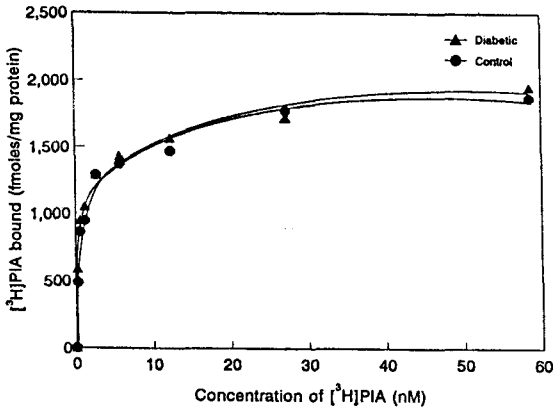

Fig. 2. [³H]PIA binding in adipocytic membranes from diabetic and control rats. Adipocytic membranes were incubated with the indicated concentrations of [³H]PIA for 1 h. Nonspecific binding was determined by adding 10 mM cold PIA into the reaction mixtures. Best fit parameters for the one-site model are: K_d=0.51±0.09 nM and B_{max}=1.60±0.12 pmoles/mg protein for control membranes; K_d=0.54±0.21 nM and B_{max} =1.72±0.31 pmoles/mg protein for diabetic membranes.

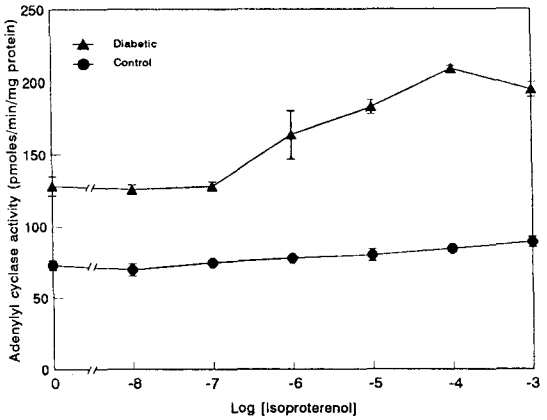

Fig. 3. Isoproterenol-stimulated adenylyl cyclase activities in adipocytic membranes from diabetic and control rats. Adipocytic membranes were incubated with the indicated concentrations of isoproterenol under the "adenylyl cyclase assay" conditions for 15 min. Values are given as means±standard errors of triplicate determinations.

were carried out three times. Best fit parameters for the one-site model were found to be: K_d=0.51 ±0.09 nM and B_{max}=1.60±0.12 pmoles/mg protein for control membranes; K_d=0.54±0.21 nM and B_{max} =1.72±0.31 pmoles/mg protein for diabetic membranes. The values for K_D and B_{max} of [³H]PIA binding to adipocytic membranes from both groups were not significantly different from each other (Fig. 2).

Inhibition of adenylyl cyclase activity by PIA

In an effort to explain for the difference in the degree of inhibition of lipolysis by PIA, changes in signal transduction pathways through A_1 adenosine receptors were investigated by determining adenylyl cyclase activities of adipocytic membranes from diabetic and control animals. When adenylyl cyclase activities were compared in the presence of 0 to 10^{-3} M isoproterenol, the activities of the plasma membranes from both diabetic and control rats were increased in a dose-dependent manner (The concentration of GTP was 10^{-7} M). The isoproterenol-stimulated as well as the basal activities were higher in diabetic rats than those of control rats. The data shown in Fig. 3 are representative of such experiments. As shown in the figure, the 10^{-4} M isoproterenol-stimulated adenylyl cyclase activity was increased 1.6 times in diabetic rats and 1.2 times in control rats higher than their respective basal activities.

Next, the inhibition of isoproterenol-stimulated adenylyl cyclase activity by PIA was studied by determining the enzyme activity at the varied concentrations of PIA (The concentration of isoproterenol was 10^{-4} M and that of GTP, 10^{-5} M) (Fig. 4). Adenylyl cyclase activity was decreased by PIA in a dose-dependent manner in both diabetic and control rats. However, there was a significant difference in the degree of inhibition, that is, the decrease by 10^{-5} M PIA in the diabetic rats was 1.9 times that of control rats. At all the concentrations of PIA used, the adenylyl cyclase activities of membranes from the diabetic rats were 1.8 to 2.1 times those of control rats.

Adenylyl cyclase activities of membranes from these two groups were compared at the varied concentrations of GTP in the presence of isopro-

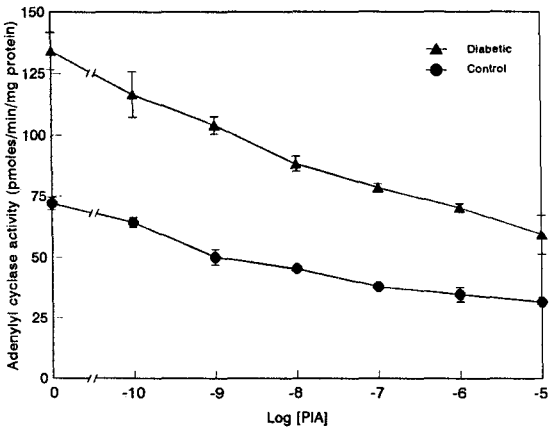

Fig. 4. PIA inhibition of isoproterenol-stimulated adenylyl cyclase activities in adipocytic membranes from diabetic and control rats. Adipocytic membranes were incubated with 100 μM isoproterenol and the indicated concentrations of PIA under the "adenylyl cyclase assay" conditions for 15 min. Values are given as means±standard errors of triplicate determinations.

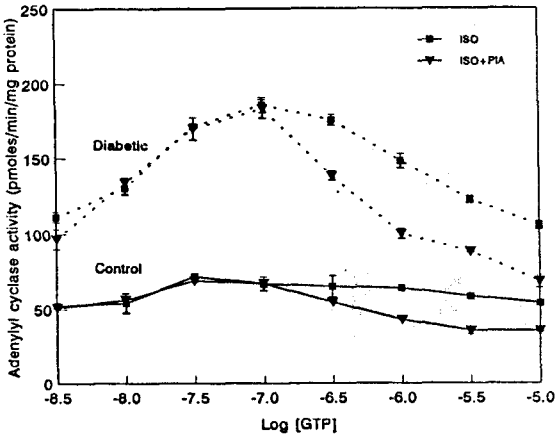

Fig. 5. GTP effects on PIA inhibition of isoproterenol-stimulated adenylyl cyclase activities in adipocytic membranes from diabetic and control rats. Adipocytic membranes were incubated with 100 μM isoproterenol, 10 μM PIA and the indicated concentrations of GTP under the "adenylyl cyclase assay" conditions for 15 min. Values are given as means±standard errors of triplicate determinations.

terenol or both isoproterenol and PIA (The concetration of isoproterenol was 10^{-4} M and that of PIA, 10^{-4} M) (Fig. 5). Biphasic curves were obtained from both diabetic and control rats, where only at the concentrations above $10^{-6.5}$ M GTP the inhibition of adenylyl cyclase activity by PIA was observed in both groups (Cooper *et al.*, 1979). In diabetic membranes the isoproterenol-stimulated activity was decreased 43% at 10^{-5} M GTP, whereas the activity was decreased 19% in control rats. And, in diabetic membranes the inhibition of the isoproterenol-stimulated activity was 63% at 10^{-5} M GTP, whereas the activity was decreased 47% in control rats.

In order to correlate the lipolysis data with adenylyl cyclase activity data, the amounts of proteins present in the plasma membrances of 10^5 adipocytes from both the groups were determined. The protein concentrations were $13.7\pm1.8\,\mu g/10^5$ cells for the diabetic group and $12.6\pm1.6\,\mu g/10^5$ cells for the controls, and the difference between the two groups was not significant statistically.

DISCUSSION

The present study was undertaken to examine the effects of streptozotocin-induced diabetes on the A_1 adenosine receptor-adenylyl cyclase system in rat adipocytes. The data in Fig. 1 demonstrate that there is an increase in sensitivity of inhibition of lipolysis to PIA in adipocytes from diabetic rats in comparison with those from controls. Adenosine is an important regulator of lipolysis in adipose tissue (Kather *et al.*, 1985; Schwabe *et al.*, 1975). It has been suggested that rat adipocytes are subject to tonic inhibition by endogenously produced adenosine, since lipolysis can be activated by removal of endogenously produced adenosine by adenosine deaminase (Schwabe *et al.*, 1975). Thus, it is thought that the increased circulating fatty acid concentrations found in diabetes should be due to some other factors such as the enhanced ability of lipolytic hormones to

stimulate lipolysis, rather than the increased sensitivity of inhibition of lipolysis by PIA.

To investigate the mechanism(s) involved, we determined [^{3}H]PIA binding to plasma membranes from diabetic and control rats. The values for K_d and B_{max} of membranes from control rats were in good agreement with those reported in other studies (Ukena *et al.*, 1984). As shown in Fig. 2, membranes from diabetic and control rats did not differ in their ability to bind to [^{3}H]PIA, suggesting that the A_1 adenosine receptor is not altered in membranes from diabetic rats, and that the increased sensitivity of inhibition of lipolysis to PIA must be due to post-receptor alteration(s).

We, then, examined changes in the isoproterenol-stimulated adenylyl cyclase activity and its inhibition by PIA in adipocytes from diabetic animals. It was found that the ability of isoproterenol to stimulate adenylyl cyclase activity in membranes from diabetic animals was enhanced in comparison with that found in membranes from control rats (Fig. 3) (Zumstein *et al.*, 1980; Chiappe de Cingolani; 1986). The increased response in the diabetic membranes was probably not due to any increase in the amount of stimulatory G-protein, G_s, as the induction of diabetes did not affect the amount of G_s (Strassheim *et al.*, 1990). Instead, as Strassheim *et al.* suggested, it is believed that inactivation of the GTP-stimulatory functioning of G_i in diabetic states removes a tonic inhibitory effect on G_s-mediated activation of adenylyl cyclase, leading to enhanced stimulatory receptor responses.

The induction of diabetes increased the inhibition by PIA of isoproterenol-stimulated adenylyl cyclase activity (Fig. 4). The more pronounced inhibitory potency of PIA in membranes from diabetic animals is probably due to elevated α-G_{i3} levels (Strassheim *et al.*, 1990). As the adenosine receptor binding in the diabetic membranes is normal, this finding could provide an explanation for the increased sensitivity of lipolysis to PIA of adipocytes from diabetic animals. In addition, the GTP dependence of the inhibitory action of PIA was clearly observed in membranes from diabetic membranes as well as control membranes (Fig. 5).

Gawler *et al.* (1989) have shown that streptozotocin-induced diabetes lead to the loss of expression of G_i in rat liver. However, there is a contradicting report that the amounts of all three-species of G_i in livers from streptozotocin-induced diabetic rats are normal, but there were increased levels of G_s (Lynch *et al.*, 1989). Green and Johnson (1991) have reported that immuno-assayable quantities of G_{i1}, G_{i2}, G_{i3} and G_s were all normal in adipocytes from diabetic rats, but Gpp(NH)p was a much less potent inhibitor of (−)-[^{125}I] N^6-hydroxyphenylisopropyladenosine ([^{125}I]HPIA) binding to adipocytic membranes from diabetic rats than those from control rats. Thus, they suggested that there was less functional G_i in the diabetic rats, probably due to a decrease in the ability of the G protein to interact with receptors. In contrast, Strassheim *et al.* (1990) have shown that receptor-mediated inhibitory responses of G_i effected by PIA, prostaglandin E_2 or nicotinate were either unchanged or even apparently more effective in adipocytic membranes from diabetic rats, which is consistent our observations. They also showed that induction of diabetes did not cause any changes in adipocytic membrane levels of the α, GTP-binding subunits of either G_{i1} or G_{i2} or G_s, but elicited an increase in the level of α-G_{i3}. The reasons for these contradicting results are not obvious at the present time, and further studies are needed to clarify the situations.

In summary, we showed the increased sensitivity of inhibition of lipolysis of PIA, an A_1 adenosine recetor agonist, in adipocytes from streptozotocin-induced diabetic rats. In adipocytic membranes from diabetic animals [^{3}H] PIA binding was not altered, but inhibition by PIA of isoproterenol-stimulated adenylyl cyclase activity was increased, suggesting that the changes are in signal transduction pathways, rather than A_1 adenosine receptors.

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= 국문초록 =

Streptozotocin으로 당뇨병을 유발시킨 쥐의 지방세포에 나타나는 A_1 Adenosine Receptor-Adenylyl Cyclase System의 변화

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흰쥐의 지방세포에 존재하는 아데노신 수용체는 A_1 subclass로 알려져 있다. 따라서 효현제(Agonist)에 의해 자극되면 adenylyl cyclase가 억제되고 결과적으로 지방분해가 억제된다. 본 연구에서는 당뇨병으로 인하여 생기는 쥐의 지방세포의 A_1 아데노신 수용체-adenylyl cyclase 시스템의 변화를 규명하고자 하였다.

흰쥐에 streptozotocin을 투여하여 당뇨병을 유발시킨 후 1주일이 되는 날, 굶기지 않은 쥐에서 collagenase를 사용하여 지방세포를 분리하였다. 분리한 지방세포에 1 unit/ml adenosine deaminase와 1 μM isoproterenol을 가한 후 37℃에서 1시간 동안 incubation하여 생성되는 glycerol을 측정하였을 때, 당뇨병군에서는 대조군에 비해 A_1 아데노신 수용체의 효현제인 (−)-N^6-(R-phenylisopropyl) adenosine (PIA)에 의해 지방분해가 약 두배 더 억제되었다. 그 기전을 밝히기 위하여 지방세포막의 [^{3}H]PIA binding을 측정하였는데, 친화력이나 수용체의 수는 당뇨병군과 대조군 사이에 통계적으로 유의한 차이가 없었다 (대조군의 K_d는 0.51±0.09 nM이었고 B_{max}는 1.60±0.12 pmoles/mg protein이었다. 당뇨병군의 K_d는 0.54±0.21 nM이었고 B_{max}는 1.72±0.31 pmoles/mg protein이었다). 그러나 100 μM isoproterenol 자극에 의한 adenylyl cyclase activity의 PIA에 의한 억제를 비교하여 보았을 때 당뇨병군에서의 억제는 대조군의 1.9배로 나타났다. 이상의 결과로 보아 당뇨병군의 지방세포에 나타나는 변화, 즉 지방분해가 PIA와 같은 A_1 아데노신 수용체의 효현제에 의해 더욱 억제되는 것은 지방세포막의 수용체 자체의 변화때문이 아니라 수용체 이후의 신호전달 체계의 변화때문인 것으로 생각된다.