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CHANGED RESPONSIVENESS TO ADENOSINE OF ISOLATED LEFT ATRIA OF DIABETIC RATS

DİABETİK SIÇANLARDAN İZOLE EDİLEN SOL ATRİADA ADENOZİNE İLİŞKİN YANITVERİRLİKTEKİ DEĞİŞMELER

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The sensitivity and responsiveness of direct and indirect (antiadrenergic) effects of adenosine in the isolated left atrial preparations of streptozotocin-induced diabetic rats were examined Diabetes was induced with streptozotocin (45 mg/kg). After 12 weeks atrial responses to direct and indirect effects of adenosine were assessed using an isolated left atrial preparations. Diabetic rats at 12 weeks after the induction of diabetes showed a significant increase to direct and indirect inotropic effect of adenosine. APT-sensitive K+channel blocker glibenclamide prevented only enhanced responses to adenosine induced direct inotropic effect but not indirect inotropic effect.

Bu çalışmada streptozotosin (STZ) ile diabet oluşturulan sıçanlardan alınan isole sol atria preparatlarında, adenosinin direk ve indirek etkilerinin duyarlılığı ve yanıtverirliği araştırılmıştır. Diabetic sıçanlar, diabetin indüksiyonundan sonraki 12. haftada adenosinin direk ve indirek etkisine anlamlı artış gösterdiler. APT-sensitif K^+ kanal blokeri olan glibenklamid, yalnızca adenosinin artan direk etkisini önledi. Artan indirek etkiyi ise önlemedi.

Keywords: *Adenosine, direct, indirect effects, diabetic rats*

Anahtar kelimeler: *Adenosine, direk, indirek etki, diabetik ratlar*

Introduction

Diabetes mellitus is associated with cardiac impairments in humans (1) and experimental animals (2,3). The mechanism(s) involved in this diabetic cardiomyopathy is still unclear. The decreases in myosin APTase activity and in the number of β-adrenoceptors of the myocardium in diabetes mellitus are considered to be results of thyroid hormone deficiency.

Adenosine is a potent extracellular messenger in the heart. In atrial tissue, adenosine produces two effects, a direct effect to decrease contractility in the absence of catecholamine (4,5) an indirect effect to specifically attenuate catecholamine stimulated cAMP accumulation and contractility (6,7). The negative inotropic action of adenosine in atrial myocardium is mediated by the extracellular A_1-respector (8,9).

The purpose of the present study is to clarify whether sensitivity or responsiveness of both direct and indirect effect of adenosine were altered in the 12 weeks diabetic rat heart. In this study we have found to be increased responsivene to adenosine induced direct and indirect inotropic effects in diabetic atria as compared control atria. We therefore investigated whether activated ATP-sensitive channels blocked by glibenclamide induced a negative inotropic response of the atrium and whether glibenclamide antagonized the cardiac responses to receptor-operated K^+ channel opener adenosine.

Methods

Experimental animals and treatment

Diabetes was induced in male albino rats 12 weeks old, with a single injection of streptozotocin (STZ) (45 mg/kg, i.v.). STZ was dissolved in 0.1 M citrate buffer. Age-matched control rats were used in each experiment. Blood samples were collected at the time of sacrifice and were assayed for plasma glucose, T_3 and T_4 levels. Experiments were performed at 12 weeks after the induction of diabetes. All animals were used at 12 weeks after the onset of diabetes.

Blood samples were collected at the time of sacrifice for assay of plasma T_3 and T_4 levels. All animals were provided with food and water ad libitum.

Experimental protocol

The animals were sacrificed at the time described above by a sharp blow to the head and decapitation. Upon decapitation, blood samples were collected and assayed for plasma glucose. The hearts were rapidly removed and placed in oxygenated Krebs-bicarbonate solution (composition, mM in distilled water; NaCl,

118.4; KCl, 4.7; $CaCl_2.2H_2O$, 1.9; $NaHCO_3$, 25; $MgSO_4.7H_2O$, 1.2; KH_2PO_4, 1.2; and glucose, 11.7), gassed with 95% O_2; 5%CO_2, and maintained at 37±0.5°C. After visible blood had been removed from the tissue, left atria were excised and suspended vertically in a chamber containing 25 ml of the above solution. Left atria were secured to bipolar platinum electrodes, and a second cotton through the atrial appendage was passed to the transducer. Tissues were electrically stimulated (Grass Stimulator S44) at a frequency of 1 Hz with rectangular wave pulses of 4 ms duration at a voltage approximately 20% above threshold. The tissues were allowed to equilibrate for 60 min under a resting tension of 1 g.

After 60 min. equilibration period, adenosine was examined in two ways; First, the direct inotropic effects were examined upon the resting tension by cumulative addition of adenosine (1-1000 μM). Second, the indirect effect was measured on tissues in which the tension was raised by addition of isoproterenol (10^{-7} λM)Concentration response curves were obtained foradenosine in the absence and presence of isoprenaline (10^{-7} M). Inotropic responses to increasing agonist concentrations were obtained by a cumulative increase in the organ chamber. On the other hand, we studied the effects of high dose of glibenclamide (3 μmol) on the negative cardiac responses to adenosine in the control and diabetic atria. Each was atria allowed to remain in contact with the drug for 3 min before each succesive concentration of the agonist was added. When the maximum inotropic effect to the agonist was attained, the atria was washed twice and allowed to equilibrate for 30 min before another concentration response curve was obtained. No more than two concentration response curves were performed in each atria in order to maintain maximum tissue responsiveness.

Drugs

Adenosine, *dl*-isoproterenol hydrochloride, streptozotocin and glibenclamide were purchased from Sigma (U.S.A.). Adenosine and isoproterenol were dissolved in glass distilled water. Glibenclamide was dissolved in dimethylsulfoxide.

Analysis of results

Results are given as the mean s.e. Responses to adenosine are expressed as % of the control value. The unpaired Student's *t* test was used to compare diabetic and age-matched controls. Values of $P<0.05$ and 0.01 were considered statistically significant.

Results

Biological parameters are shown in Table 1. Rats with streptozotocin-induced diabetes weighed significantly less than mondiabetic control rats after 12 weeks of diabetes. The serum glucose levels were elevated significantly in the diabetic groups.

Cumulative addition of adenosine (1-1000μM)

Table 1. Characteristics of controland diabetic rats at 12 weeks (n=6-8)

Treatment	Body weight (g)	Blood glucose (mg/ml)
Control	321±12.4	125±24.2
Diabetic	155±6.5*	587±14.3*

All values given are the mean ± SEM
*p<0.05 compared with untreated diabetic control rats

produced concentration-dependent decreases in developed tension in tissues from both control, and diabetic animals (Table 2). In the present study compared to atria from control animals, atria from 12 weeks diabetic rats demonstrated a hyperresponsiveness to the direct negative inotropic effect of adenosine while exhibiting no change in sensitivity to adenosine.

Table 2. Comparison of potency of direct effect of adenosine in left atrium from control and diabetic rats at 12 weeks.

Treatment	pD_2	Maximum response
Nondiabetic control	4.66± 0.06	57.6 ± 1.3
+Glibenclamide	4.12 ± 0.01*	31.5 ± 1.8*
Diabetic	4.60 ± 0.04	73.0 ± 3.3*
+Glibenclamide	4.22 ± 0.01*	22.1 ± 2.4*

All values given are the mean ± SEM
*p<0.01 compared with untreated diabetic control rats

The effects of diabetes on the adenosine attenuation of β-adrenergic receptor mediated stimulatory mechanisms were investigated by measuring isoproterenol-enhanced atrial contractility in the presence of the adenosine. Adenosine attenuated the response to isoproterenol in a dose-dependent manner in control and diabetic preparations. Atria from diabetic animals exhibited significantly greater pD_2 value and maximum response to the indirect (antiadrenergic) effect of adenosine than corresponding atria from control rats

(Table 3).

When glibenclamide (3 µmol) was added to the isolated organ bath, it did not produce any significant effects on the conrol and diabetic atrial preparations. Glibenclamide prevented enhanced direct response to adenosine, while it did not alter indirect inotropic responses of control and diabetic atria.

Table 3.Indirect effect of adenosine in left atrium from control and diabetic rats

Treatment	pD2	Maximum response
Control	4.12± 0.03	53.3 ± 1.21
+Glibenclamide	4.12 ± 0.01	57.8 ± 0.06
Diabetic	4.70 ± 0.07*	82.1 ± 3.12*
+Glibenclamide	4.61 ± 0.02*	77.5 ± 0.5*

All values given are the mean ± SEM
*p<0.01 compared with untreated diabetic control rats

Discussion

Cardiac dysfunction has become one of the major complications in chronic diabetes. This study examined the ability of left atria from 12 weeks STZ-diabetic It was observed that not only direct inotropic response of adenosine was found to be enhanced, but also indirect negative inotropic response was increased in these pathological conditions.

10 weeks after the injection of STZ, we have observed that isolated atria from diabetic rats demonstrated increases in direct inotropy in response to adenosine indicating hyperresponsiveness of the adenosine A_1 receptor in the isolated atria from diabetic rats. In the study of Li (10), it has been observed that the chronotropic response of isolated right atria obtained from rats made diabetic 14-15 weeks was significantly enhanced. The supersensitivity of negative chronotropic effect of adenosine on diabetic atria were abolished following pretreatment wiht higher doses of pertussis toxin and it has been suggested that supersensitivity involved in G-proteins. It is known that adenosine has both potassium channel agonistic and calcium antagonistic effect in atrial cells (11,12).

Results presented herein also indicated that the indirect negative inotropic effect of adenosine in left atrium was significantly enhanced in preparations obtained from diabetic rats. Results obtained from diabetic animals are in disagreement with the findings reported earlier reports (13,14).The reasons underlying these differences between studies are unclear at present.

In the present study, glibenclamide at high dose of 3 µmol inhibited the direct inotropic responses to adenosine but did not affect the indirect inotropic responses to adenosine. These results demonstrate that glibenclamide inhibits the cardiac initropic responses to K+ channel opener adenosine. Adenosine induces negative cardiac responses and increases the K+ permeability of the cell membrane (11).

Adenosine is released to a greater extent under pathophsiologic conditions, e.g. hypoxia and ischemia (15). These disturbances in the direct and indirect effects of adenosine may contribute to diabetes-induced cardiac abnormalities. Our results further suggest that hypoxia and ischemia can develop during this diabetic conditions.

References

1. Kannel WB, McGee DL. Diabetes and cardiovascular risk factors: The Framingam study. Circulation 59:8-13 (1979)
2. Garber DW, Neely JR. Decreased myocardial function and myosin APTase in hearts from diabetic rats. Am J Physiol 1983;244:H586-91
3. Penpargkul S, Schiable T, Ypintsoi T, Scheuer J. The effect of diabetes on performance and metabolism of rat hearts. Circ Res 47:911-921 (1980).
4. Burnstock G, Meghji P. The effect of adenyl compounds on the rat heart. Br. J.Pharmacol. 79:211-18, 1983
5. Böhm, M., Bruckner, R., Neuman, J., Schmitz, W., Scholz, H., Starbatty, J. Role of guanine nucleotide- binding protein in the regulation by adenosine of cardiac potassium conductance and force of contraction. Evaluation with pertussis toxin. Naunyn-Schmiedeberg's Arch Pharmacol 332:405-3(1986)
6. Hughes, S. Stone, T. (1983). Inhibition by purines of the inotropic action of isoprenaline in rat atria. Br J Pharmacol 80:149-53, 1983
7. Böhm M, Brückner R, Hackbart I, Haubitz B, Linhart R, Meyer W, Schmitz B, Schmitz W, Scholz H. Adenosine inhibition of catecholamine-induced increase in force of contraction in guinea pig at-

dent effect. J Pharmacol Exp Ther 230:483-92, (1984)
8. Evans DB, Schenden JA. Adenosine receptors mediating cardiac depression. Life Sci. 31:2425-32 (1984).
9. Lohse MJ, Ukena D, Schwabe U. Demonstration of Ri-type adenosine receptors in bovine myocardium by radioligand binding Naunyn-Schmiedebergs Arch Pharmacol 328:279-84 (1985)
10. LI XJ. Supersensitivity of isolated atria from diabetic rats to adenosine and methacholine: modulation by pertussis toxin. J Pharm Pharmacol 44:142-44 (1992)
11. Belardinelli L, Isenberg G. Isolated atrial myocytes: Adenosine and acetylcholine increase potassium conductance. Am J Physiol 244:H734-37 (1983)
12. Jochem G, Nawrath H. Adenosine activates a potassium conductance in guinea pig atrial heart muscle. Experientia 39:1347-49 (1983)
13. Warner EL, Gallasso F, Thompson Cl, Belloni FL. Vasodilative and anti-adrenergic effects of adenosine in diabetic rat hearts. Can J Physiol Pharmacol 70:13-19 (1992)
14. Romano FD, Kopp SJ, Daar JT, Smith CA. The antiadrenergic effect of cyclopentyladenosine on myocardial contractility is reduced in vivo in diabetic rats. Can J Physiol Pharmacol 72:1245-55 (1994)
15. Olsson RA. Changes in content of purine nucleoside in canine myocardium during coronary occlusion. Circ Res 26:301-306, (1970)

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