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Effects of Diabetes and Hypothyroidism on Fast, Slow and Total Contractile Responses to Phenylephrine in Rat Aorta

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Summary *: Peripheral vascular disease is a common complication in diabetes. Hypothyroidism is associated with diabetes mellitus and it plays an important role in the development of diabetes-induced complications. It has been indicated that diabetes induces enhanced vasoconstriction to catecholamines in different vascular preparations. In this study we have investigated the fast and slow components of the mechanical response to 1 μM phenylephrine (PE) contracted in aortic rings isolated from insulin dependent diabetic and noninsulin dependent diabetic and hypothyroid rats. We conclude that insulin dependent and non-insulin dependent diabetes mellitus in the chronic stage increase the fast , slow and total contractile response of PE. This enhanced responses to PE is not due to hypothyroid state.*

Key words: *Diabetes mellitus, hypothyroidism, phenylephrine, aorta*

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Diabet ve Hipotiroidizmin Fenilefrinin Sıçan Aortundaki Hızlı, Yavaş ve Total Kontraktil Yanıtlarına Etkileri

Özet *: Periferal vasküler bozukluk, diabetin önemli komplikasyonlarındandır. Hipotirioidizm ise diabetle birlikte gelişmektedir ve diabetle ilişkili komplikasyonlarda rol oynadığı düşünülmektedir. Artan vazokonstriksiyon değişik vasküler diabetik preparatlarda gösterilmiştir. Biz de bu çalışmada, 10 haftalık kontrol, insulin bağımlı olan, insüline bağımlı olmayan diabetik ve hipotiroid sıçanların izole aortik striplerinde, 1 μM fenilefrine mekanik yanıtın, hızlı ve yavaş komponentlerinin değişip değişmediğini araştırmayı planladık. Ayrıca 1, 2, 4, ve 8 haftalık diabetik sıçanlarda da süreye bağlı olası bir değişiklik olup olmadığını da araştırdık. Bu bulgular bize, yalnızca, 10 hafta da Tip 1 ve Tip 2 diabetin, fenilefrinin hızlı ve yavaş komponentlerini ve aynı zamanda total yanıtını arttırdığını, ve bunun diabette gelişen hipotiroidizmle büyük olasılıkla ilişkili olmadığını göstermektedir.*

Anahtar kelimeler: *Diabet, hipotiroidizm, fenilefrin, aorta*

INTRODUCTION

It is generally known that vascular disease is associated with diabetes mellitus in man. Many studies concerning vascular dysfunction in the diabetic state have been performed using chemically induced diabetic rats. In previous studies on the response to norepineprine (NE) Jackson and Carrier[1] and MacLeod[2] reported an increase in the maximal response.

Various studies have demonstrated the presence of hypothyroidism in diabetic patients and rats[3,4]. A depression of myosin ATPase or a decreased number of β-adrenoceptors in the myocardium in diabetes mellitus are considered to result from deficiency of thyroid hormones[4,5]. However, the role of thyroid hormones in the changes of vascular reactivity has been examined in only a few studies[6].

Vascular response to catecholamines can be divided into fast and slow components from the mechanical point of view[7]. These components are caused by mobilization of Ca^{2+} from different sources (intracellular Ca^{2+} for the fast, extracellular for the slow), and both

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processes are mediated by well-identified second mesengers[8]. Ca^{2+} entry blockers affect only the slow component because it needs extracellular Ca^{2+}, whereas, the fast component is not altered due to its intracellular origin.

Although the enhanced vascular response to catecholamines in diabetes has been described, reports about the influence of the disease on the different components of contraction are scarce[9]. This could have important pathological implications, however, because the selective modification of any of the phases could point to a particular site and/ or mechanism of action of the disease.

This study investigated the effects of diabetes STZ-induced Type 1 diabetes, STZ-induced Type 2- (n2 neonatal) diabetes and hypothyroidism induced by propylthiouracil on the fast and slow components of the response to phenylephrine (PE) in the rat aorta.

MATERIALS AND METHODS

Animals

Male albino rats (diabetic: 10 weeks of age) were used. Type 1 insulin dependent diabetes, (IDD), was induced with a single injection of streptozotocin (STZ; 45 mg/kg, i.v.) in citrate buffer (pH 4.5).Animals were studied after 10 weeks injection of STZ.

For non-insulin dependent diabetes (NIDD), rats were injected intraperitonally (i.p.) at 2 days of age with 90 mg/kg streptozotocin (STZ) in 0.1 ml citrate buffer. Although animals exhibited little nonfasting (<350 mg/dl) or fasting (<140 mg/dl) hyperglycemia by 6 weeks of age, all STZ-injected rats were markedly intolerant when challenged with an i.p. injection of glucose (2 mg/ kg) and became more intolerant with age[9].

Animals (hypothyroid: 6 weeks of age) were made hypothyroid by being fed a diet containing 0.15 % 6-propyl-2-thiouracil for 10 weeks.

Age matched control rats were used for each group in our experiments.

On the day of the experiments, the animals were anesthetized with 80 mg/kg body weight i.p. pentobarbital sodium, and the thoracic aorta was quickly excised and placed in a petri dish filled with ISS (Isotonic salt solution) with the following composition: 118 mM NaCl, 5.32 mM KCl, 1.54 mM NaH_2PO_4, 119 mM $MgSO_4$, 24.9 mM $NaHCO_3$, 1.35 mM $CaCl_2$, 0.01 mM EDTA, and 5.6 mM glucose. Rings 2 mm in length were cut and mounted isometrically in thermostattic (37°C) organ baths filled with ISS and continuously bubbled with mixture of 5% CO_2 / 95 % O_2. Force was recorded with force transducers (Ugo Basile) coupled to pen recorder (Ugo Basile 7006).

At the end of the experiments, rings were contracted with 1 μM PE in ISS. After the contraction stabilized, the presence of functional endothelium was confirmed by the addition of 1 μM acetylcholine, which produced a relaxation of 30-50 %.

After stabilization under a passive force of 2 g, the rings were exposed to PE until the contraction a reached a plateau (usually 15-20 min). The fast component of the response was measured from the baseline to the point at which the rate of force development decreased abruptly. The slow component was measured from that point to the top of the contraction. The total response was the sum of both components.

The results were expressed as means ± SE. Contractile response to PE were calculated as g/mm^2. Groups were compared by Student's t test for unpaired samples or analysis of variance (> 2 groups compared at the same time). $p< 0.05$ was significant.

RESULTS

In STZ diabetic animals at 1, 2, 4, 8, and 10 weeks, but not hypothyroid animals, plasma glucose levels were found to be elevated. The fast and slow components of these total responses were measured graphically (Figure 1). The fast, slow and total response to PE in aortic preparations from Type 1 diabetic rats did not seem to be changed at 1, 2, 4, and 8 weeks (Figure 2 a,b,c).

The total contractile response to PE at 10 weeks was significantly different in IDDM and NIDDM groups of rats, but not in hypothyroid rats (Fig 3a). The fast components of these total contractile responses were

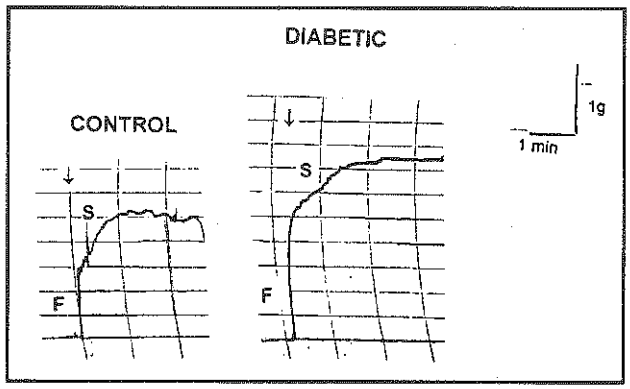

Figure 1. Actual records to phenylephrine (1 μM) in aortic rings of Type 1 diabetic and control rats at 10 weeks, showing that, in the diabetic preparation, F (fast) predominated over S (slow). Arrows, addition of 1 μM PE to bath.

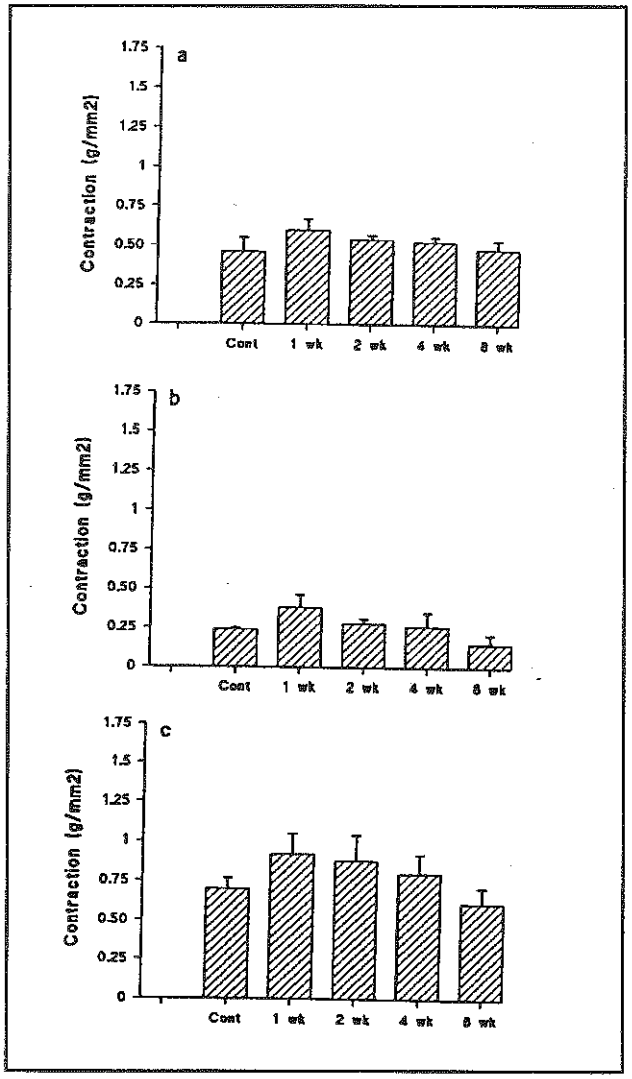

Figure 2. Fast (a), slow (b) and total (c) contractile response to 1 μM phenylephrine in aortic preparations obtained from STZ-induced Type 1 diabetes at 1, 2, 4 and 8 weeks.

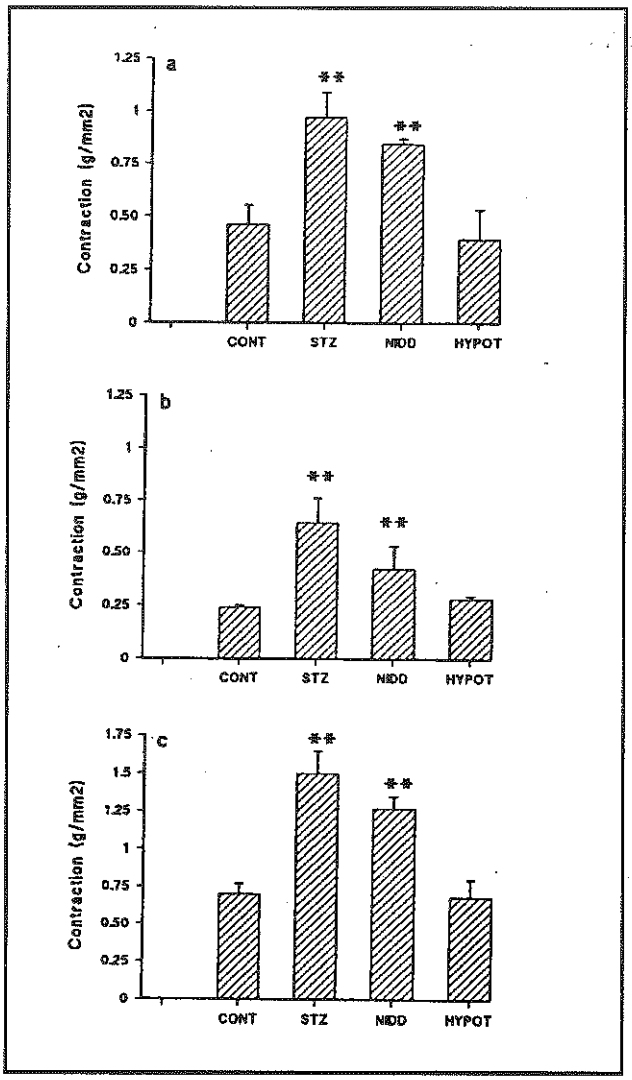

Figure 3. Fast (a), slow (b) and total (c) contractile response to 1 μM phenylephrine in aortic preparations obtained from STZ-induced Type 1 diabetes, neonatal Type 2 diabetes and hypothyroid rats at 10 weeks.(** $p < 0.01$ significantly different from control, n=6).

presented in Figure 2b. Although the fast component did not altered in hypothyroid animals, it was significantly greater in STZ-diabetic (Type 1) and neonatal diabetic rats than in their age matched controls. Slow responses were also significantly greater in STZ - diabetic and neonatal diabetic , but not in hypothyroid rats (Figure 3c).

DISCUSSION

It is well known that diabetes is frequently associated with peripheral vascular disease[1,2]. The objectives of this study were to determine whether chemically in-

duced adult and neonatal diabetes are associated with changes in the fast, slow and total responses of phenylephrine induced contraction in vascular smooth muscle preparations and whether there was a possible relationship between diabetes and hypothyroidism.

Contrary to the findings of several investigators [10,11,12] we did not find any significant alterations in the enhanced total response in STZ induced Type 1 diabetes at 1,2, 4 and 8 weeks. Most of these investigators have suggested an increased response to PE and other vasoconstrictors, and the reason for the discrepancy between our results and theirs is not evident. The difference could be partly due to the different species and/or etiology of diabetes. For example, the STZ dose was greater than our study (45 mg/kg, body wt.)[10,11]. The other studies used alloxan induced diabetes[11,12]. Furthermore we did not find any significantly altered responses when the response was separated into fast and slow components of response in previous studies. In addition, experiments carried out in vessels in which the fast component predominates could lead to overestimation of the response to PE. Similarly, if the fast component is measured too early during PE contraction, part of the slow component can be overlooked.

In this study we found that the fast, slow and total responses to PE contraction were enhanced in Type 1 and Type 2 diabetes at the chronic stage. Previous studies demonstrated that fast and slow phases of response to PE involve different types of Ca^{2+} [7]. Although the slow component reflects Ca^{2+} influx through receptor-operated channels, the fast component is caused by release of NE-sensitive intracellular Ca^{2+} stores[8]. Therefore, an increase of the fast component in diabetes could reflect an accumulation of intracellular Ca^{2+} release due to increased formation of inositol triphosphate and other intracellular mediators[13]. Our data suggest that diabetic state could enhance intracellular Ca^{2+} release from stores and Ca^{2+} influx through receptor operated channels.

In this study we examined whether hypothyroidism associated with diabetes could alter fast, slow and total responses to PE. A recent study in our laboratory indicated that diabetes and hypothyroidism each changed cardiac adenosine responses[14]. In contrast, in vascular preparations altered contractile response to PE were not related to hypothyroidism according to our finding. Future studies are required to investigate other Ca^{2+} - related mechanisms in diabetic vascular preparations.

REFERENCES

1) Jackson P, Carrier GO. Supersensitivity of isolated mesenteric arteries to noradrenaline in the long- term experimental diabetic rats. *J. Auton. Pharmac.*, 1, 399-405, 1981.
2) MacLeod KM. The effect of insulin treatment on changes in vascular reactivity in chronic, experimental diabetes. *Diabetes*, 34, 1160-1167, 1985.
3) Pittman CS, Suda AK, Chambers JB, Ray GY. Impaired 3,5,3'-triiodothyronine (T3) production in diabetic patients. *Metabolism*, 28, 333- 338, 1979.
4) Dillmann WH. Influence of thyroid hormone administration on myosin ATP ase activity and myosin isoenzyme distribution in the heart of diabetic rats. *Metabolism*, 31, 199-204, 1982.
5) Sundaresan PR, Sharma VK, Gingold SI, Banerjee S P. Decreased (-adrenergic receptors in rat heart in streptozotocin-induced diabetes: role of thyroid hormones. *Endocrinology*, 114, 1358-1363, 1984.
6) Takiguchi Y, Satoh N, Hashimoto H, Nakashima M. Changes in vascular reactivity in experimental diabetic rats: Comparison with hypothyroid rats. *Blood Vessels*, 25, 250-260, 1988.
7) Bohr DF. Vascular smooth muscle: Dual effect of calcium. *Science*, 139, 597-99, 1963.
8) Berridge MJ, Irvine RF. Inositol triphosphate, a novel second messenger in cellular signal transduction. *Nature*, (Lond), 312, 315-21, 1984.
9) Sakai Y, Aihara K, Honda H, Inazu M. Calcium mobilization and phosphatidylinositol turnover in vas deferens smooth muscle of diabetic rats. Eur. J. *Pharmacol.*, 162, 475-81, 1981.
10) Owen MP, Carrier GO. Calcium dependence of norepinephrine-induced vascular contraction in experimental diabetes. *J. Pharmacol. Exp. Ther.*, 212, 253-258, 1980.
11) Brody MJ, Dixon RL. Vascular reactivity in experimental diabetes mellitus. *Circ Res.*, 14, 494-501, 1964.
12) Fortes ZB, Scivoletto R, Garcia-Leme J. Functional changes in the microcirculation of alloxan-induced diabetic rats. *Gen. Pharmacol.*, 20, 615-620, 1989.
13) Sakai Y, Aihara K, Honda H, Inazu M. Calcium mobilization and phosphatidylinositol turnover in vas deferens smooth muscle of diabetic rats. *Eur J. Pharmacol.*, 162, 475-481, 1989.
14) Gür S, Arı N, Öztürk Y. Increased response to adenosine in isolated left atria from streptozotocin-diabetic rats: Evidence for the involvement of hypothyroidism. *J.Cardivasc. Pharmacol.*, 29, 174-179, 1997.