

IMPAIRMENT OF BOTH ADENOSINE 5'-TRIPHOSPHATE AND ADENOSINE MEDIATED  
RELAXATIONS OF RAT ISOLATED DUODENUM BY EXPERIMENTAL DIABETES

DIABETİK SİÇANLARDAN İZOLE EDİLEN DUODENUMLARDA TRİFOSFAT VE  
ADENOZİNİN GEVŞETİCİ YANITLARININ BOZULMASI

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*Diabetes mellitus is associated with changes in gastrointestinal motility. The effects of experimental diabetes, induced by streptozotocin administration to rats 8 weeks previously, on the adenosine and adenosine 5'-triphosphate-mediated relaxation of the duodenum have now been investigated. Relaxant responses to adenosine and ATP were also reduced by diabetes. A defect in the intestinal purinergic innervation could thus contribute to the motility dysfunction observed in diabetes.*

*Diabet gastrointestinal motilitede değişiklikler oluşturmaktadır. 8 haftalık diabetik sıçanlarda adenozin ve ATP'yle gevşemeler de deneysel diabetik etkisini bu çalışmada araştırıldı. Adenozin ve ATP'yle oluşturulan gevşetici yanıtlar diabetle azaldı. Böylece, intestinal purinerjik innervasyondaki bir defekt diabette gözlenen motilite disfonksiyonuna yardım edebilir.*

**Keywords:** Adenosine; Duodenum; ATP; Diabet

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## Introduction

Physiological and pharmacological effects of purines have been investigated in a number of smooth muscles (1-3) and have become a matter of importance. These effects are mediated via two main classes of receptors, designated as P<sub>1</sub> and P<sub>2</sub>, which respond primarily to adenosine and Adenosine 5' triphosphate (ATP) respectively. P<sub>1</sub> purinoceptors in smooth muscles usually mediate relaxation (1), although in the number of smooth muscle preparations adenosine causes contraction via these receptors (4-5). Abnormalities of gastrointestinal motility are common in diabetes mellitus (6-8) and have been associated with degenerative changes in peripheral system.

It is also possible that the gut motility disorders of diabetics are associated with impaired relaxation induced by adenosine and ATP in the gastrointestinal tract.

## Materials and Methods

### *Animals induction of diabetes mellitus*

Male Albino rats (220-250) were obtained from Animal Laboratories (Ankara University) and housed under standart conditions from 1 week after delivery. Rats were fasted for 18 to 24 hr and then made diabetic under ether anaesthesia by intravenous injection of streptozotocin (45 mg.kg<sup>-1</sup>) into the tail vein. Streptozotocin was dissolved (60 mg/mL) in 0.1 M citrate buffer (pH 4.5) and injected within 5 min of preparation. Seventy-two hours after administration of streptozotocin, induction

of diabetes was confirmed by glucose testing of urine samples from each animal, using Keto-Diastix (Ames Co., Elkhart, Ind.). The diabetic state was also assessed at the time of death on the basis of analysis of indice of glycemc control, i.e., plasma glucose concentrations. The experimental animals were used after 8 weeks induction of diabetes.

Diabetes was considered successfully induced only if urine glucose concentrations were greater than 2 g/dl. Urine glucose concentrations in control animals were less than 100 mg/dl. Blood was collected in ice-chilled tubes and serum separated and analyzed for serum glucose concentration using an Ames Glucometer 3 (Bayer Diagnostics France).

### *Maintenance of animals*

Each group of animals was caged separately and kept under precisely the same conditions as other groups. All groups were kept in the controlled room (22 °C), artificially lit from 06 h 00 min- 18 h 00 each day. The animals were fed with standart laboratory diet (Purina rat chow) and provided with water ad libitum. After the initial weights had been noted, each rats routinely weighted once a week and weight recorded up to the end of the study.

### *Preparation of duodenum*

Rats were sacrificed by decapitation. Blood was collected in ice-chilled tubes and serum separated and analyzed for serum glucose concentration. The duodenum were dissected free and placed in Krebs solution of following composition (milimolar): NaCl, 113; KCl, 4.7; NaH<sub>2</sub>PO<sub>4</sub> 1.4, NaHCO<sub>3</sub> 16.3; MgSO<sub>4</sub> 0.6; CaCl<sub>2</sub> 2.5 and glucose 7.7. The organ-baths were constantly gassed with 95% O<sub>2</sub>/5%CO<sub>2</sub> and the organ baths and reservoirs of Krebs solution were maintained at 37°C.

Tissues were allowed to equilibrate for 60 minutes. Isometric tension was recorded on Grass model 79D polygraph and a Grass FT10 force displacement transducers. The segments of duodenum were loaded with a tension of 1 g and were then allowed to equilibrate for approximately 60 min. with the bathing solution being changed every 15 min. Relaxations to the agonists were recorded in duodenum obtained from control and diabetic animals.

#### Drug used

Adenosine hemisulfate, adenosine 5'-triphosphate (ATP) (sodium salt) (ATP), streptozotocin were purchased from Sigma Chem. Co. (St. Louis MO. USA). All drugs were prepared freshly before each experiment by dissolving them in control Krebs solution.

#### Statistical analysis

All values are expressed as mean  $\pm$  s.e. Statistical evaluation of the data was done using Student's *t* test for unpaired observations. When  $P < 0.05$  and  $P < 0.01$ , the values were considered to be significantly different.

## Results and Discussion

### Changes in serum glucose, body and duodenum weight

Characteristics of animals and tissues used in these experiments are shown in Table 1. The classical symptoms of polyuria, polydipsia and polyphagia were also evident in the diabetic animals. The presence of an autonomic neuropathy was suggested by intestinal distention and diarrhea, poor peripheral blood supply (bleeding after cutting the tail vein was difficult) and high incidence of abnormal penile erection. The body weights of all rats were similar before induction of diabetes (Table 1). In the present study, diabetic animals in 8 weeks groups did not gain weight during the investigation period.

Table 1. Effect of streptozotocin-induced diabetes on rat body and duodenum weights and serum glucose concentrations

Data are expressed as mean  $\pm$  S.E.M; n=10 for all values.

	Control	Diabetic
Body weight	238 21	211 12
Serum glucose (mg/100 ml)	118 19	483 19*
Duodenum weight (mg)	132 14.2	185 31.1

\* $p < 0.05$  (vs controls).

By contrast, non-diabetic controls gained significant weight during this period (Table 1). At killing time, there was a significant difference in body weights and in serum glucose of 8 week diabetic animals (Table 1). There was no significant difference between control and diabetic groups for duodenum weights (Table 1). After 8 wk some mortality was encountered. Specifically, 3 of 12 rats injected with streptozotocin died between 40 and 50 days. 6 of the nine diabetic animals were used in the present study.

### Rat duodenum

#### Relaxant responses to ATP

ATP ( $10^{-8}$ - $10^{-3}$  M) produced concentration dependent relaxations of the acetylcholine-contracted duodenum from control and diabetic animals (Figure 4). Duodenum from diabetic animals exhibited decreased maximum response to ATP induced relaxation (Table 2).

Table 2. Acetylcholine-contracted duodenum from control and diabetic rats at 8 wk:  $PD_{20}$  values and maximum responses of the inhibitory responses of ATP and adenosine

	$PD_{20}$	Maximum response (% inhibition)
ATP		
Control	5.63 0.17	94.2 2.0
Diabetic	6.13 0.34	66.1 4.2*
Adenosine		
Control	5.68 0.17	95.7 3.6
Diabetic	5.65 0.21	55.2 2.1*

\*\* $P < 0.01$ , paired test against control

#### Relaxant responses to adenosine

Adenosine ( $10^{-8}$ - $10^{-3}$  M) produced concentration dependent relaxations of the acetylcholine-contracted duodenum from control and diabetic animals. Furthermore, there was a significant decrease in maximal relaxant effect in response to adenosine in duodenum from 8 week old diabetic rats. This was not accompanied by alteration in the sensitivity.

In this study, *in vitro* duodenum preparations have been used to study changes to ATP and

adenosine eight week streptozotocin-induced diabetes. Previous studies suggested a failure in the nitregeric modulation of intestinal tone, as demonstrated in the rat isolated duodenum (9). The present resut obtained may have clinical relevance, since they demonstrate the striking changes in duodenum function that occur after the onset of experimental diabetes.

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