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The effect of bupivacaine on gastrocnemius muscle contractility in rats with diabetes

A. Pelit*[1], B. Taştekin[1], A. Demirkazık[2] and K. Dağlıoğlu[3]

Çukurova University,
Medical Faculty Biophysics Department, Adana, Turkey.

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ABSTRACT

This study examined the changes in skeletal muscle contraction parameters after the injection of bupivacaine into the gastrocnemius muscle in diabetic rats. Forty male Wistar albino rats (230–270 g) were divided into four groups: Group I: Untreated healthy control; Group II: Healthy injected with Bupivacaine; Group III: Diabetic control; Group IV: Diabetic injected with bupivacaine. The diabetes was induced with streptozotocin in 0.1 M citrate buffer (pH 4.5) injection at 45 mg/kg in tail vein. On 7th day of streptozotocin injection, the rats in groups II and IV were injected with 0.25% bupivacaine at 8 mg/kg body weight into the gastrocnemius muscle. Three weeks post bupivacaine application, the animals were sacrificed and right leg gastrocnemius muscle was isolated for studying various parameters such as muscle twitch, tetanic force, contraction and relaxation time and maximum contraction and relaxation rates. The blood glucose estimation showed that the rats in group III and IV developed diabetes at two days after administration of streptozotocin. The administration of bupivacaine resulted in a significant ($p<0.05$) increase in the muscle twitch parameters both in Group II and Group IV. Thus, bupivacaine can increase muscle contractility in diabetic muscles also.

Key words: Bupivacaine, Diabetes, Muscle contractility, Rats.

INTRODUCTION

Bupivacaine is considered to be the first local anesthetic having a long duration of action. The drug had been used as a local anesthetic and to treat muscle pain for many years (Graboski *I*., 2005; Kamanli *et al*., 2005), despite its toxic effect on muscles (Hall-Craggs, 1980; Rosenblatt and Woods, 1992). Bupivacaine injections into the muscles of laboratory animals showed that the subsequent repair of satellite cell proliferation and eventual muscle hypertrophy occurred with some variability depending on the type of muscle fiber, fast or slow (Plant *et al*., 2003; Park *et al*., 2004).

Diabetes mellitus is a syndrome characterized by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism associated with absolute or relative deficiency in insulin secretion and action (Kannan *et al*., 2016). This is a world wide health problem affecting millions in both developed and developing countries and today it is recognized as an epidemic disease in most countries (Budhwar *et al*., 2010).

The chronic high glucose levels in diabetes, if not controlled over time, can cause irreversible organ damage, and results in pathologic changes in vascular and muscular structure (Johnson *et al*., 1986). The muscles lose their ability to contract but regain some degree of function during the subsequent period of regeneration. There have been studies on the contractile properties of regenerating rat skeletal muscles following massive damage in minced (Carlson and Gutmann, 1972) free and nerve-intact grafts (Carlson *et al*., 1981). It has been observed that the recovery of the contractile properties of the various graft preparations maintains fractions but never returns to normal values (Heavner, 2008).

In recent years, pediatric ophthalmologists have been treated strabismus by injecting bupivacaine into eye muscles. Bupivacaine injection improves eye alignment in strabismus by inducing changes in extraocular muscle structure generally with increased size (Miller *et al*., 2013).

The ultimate goal of this study is to investigate the effects of local administration of bupivacaine in the treatment of atrophy caused by diabetes. The present study evaluates the effect of bupivacaine on the muscle force in the diabetic rats.

MATERIALS AND METHODS

The 40 male Wistar albino rats (230–270 g) were divided into four groups; Group I: Untreated healthy control; Group II: Healthy injected with Bupivacaine; Group III: Diabetic control; Group IV: Diabetic injected with bupivacaine. The rats in group III and IV were injected into

*Corresponding author's e-mail:apelit@gmail.com; and address
[1]Çukurova University Medical Faculty Biophysics Department, Adana, Turkey.
[2]Cumhuriyet University Medical Faculty Biophysics Department, Sivas, Turkey.
[3]Çukurova University Research and Practice Center of Experimental Medicine, Adana, Turkey.

the tail vein with streptozotocin (STZ) at 45 mg/kg in 0.1 M citrate buffer (pH 4.5) for induction of diabetes. The rats in control groups (I and II) were administered with similar volume of cold 0.1 M citrate buffer. on the 7th day after streptozotocin application, the rats in Group II and IV were injected with 0.25% bupivacaine (0.5% bupivacaine diluted with % 0.9 NaCl physiologic saline, Astra Zeneca, UK) into the gastrocnemius muscle at 8 mg/kg body weight. The development of diabetes was confirmed on the basis of blood glucose level measured at 48 h of experiment; the levels of 250 mg/dL glucose or above were accepted as diabetic. The rats were housed for 30 days before they were sacrificed by decapitation. The right leg gastrocnemius muscle was isolated and evaluated for Isometric twitch parameters. The muscle length was determined after a 30-min period of resting and equilibration with Kreps solution. Square frequencies of 0.05 Hz (15–20 V) of 0.5 ms duration were applied for 20 min supramaximal to directly stimulate the muscles during the entire preparation period. A force-displacement transducer (FDT 10-A 500 g, Commat, Ankara, Turkey), stimulator (STPT02-A, Commat, Ankara, Turkey), research tissue organ bath and circulator (WBC 3044, Commat, Ankara, Turkey) and Biopac Systems MP30 (Goleta, CA, USA) were used to stimulate the muscle and record the response (Pelit *et al*., 2008; Pelit *et al*., 2013; Tastekin *et al*., 2018). Fig 1 displays the initial and final weights of the animals within each group. The muscle isometric twitch tension (Ps; g.F, Fig 2), contraction time (CT) and half-relaxation time (HRT) (ms) were determined (Fig 3). The muscle cross-sectional area was estimated using muscle weight and length. The length was measured before the distal tendons were cut. Additionally, the muscle dry weights were measured (Fig 4).

The Biopac MP30 system (Biopac Student Lab Pro ver. 3.7) was used to transfer and analyze the muscle isometric twitch tension curves. The isometric twitch parameters were expressed as averages ± SEM. and compared by one-way ANOVA subtest using the SPSS program (ver. 21.0). The differences were considered statistically significant at P<0.05 level.

RESULTS AND DISCUSSION

The captured data were analyzed, described, and interpreted in a systematic manner as the step of the research process. The documentation process in the figures aimed to present data in an intelligible and interpretable form in accordance with the research aims.

The noteworthy decrease observed in the weight of Group III displayed a statistically significant difference between Group I and Group III (p < 0.05, Fig 1). However, the weight improvement in the Group IV showed a meaningful difference between Group III and Group IV (P <0.05, Fig 1).

Twitch force comparison of Group I in Group II were observed to be statistically significant due to a 43% increase in Group II (p<0.05, Fig 2). We also observed that a 44% decrease twitch force in Group III as compared to that of Group I and 32% twitch force increase in Group IV according to that of Group III (P<0.05, Fig 2).

In addition, noteworthy extension of contraction time found in Group III resulted in a statistically significant difference between Group I and Group III (P< 0.05, Fig 3) and the half relaxation time (HRT) of Group IV as compared to Group I seemed to have been extended (P<0.05, Fig 3).

The muscles were left for 12 hours at room temperature after the evaluation of the contractility parameters and the dry weight of the muscles was measured. Cross comparison of the muscles revealed that Group II was expanded more than that of Group I (P<0.05, Fig 4) and the muscles in Group IV was expanded more than that of and Group III (p<0.05, Fig 4).

An association between skeletal muscle atrophy and Diabetes Mellitus (DM), especially in type 1 DM has been reported in different studies (Frier *et al*., 2008; Wang *et al*., 2006; Nair *et al*., 1995). It has also been reported that style 1 DM affect the growth and development of muscles and causes skeletal muscle atrophy by the disturbance of protein metabolism (Hasselgren *et al*., 2002; Lecker *et al*., 1999;

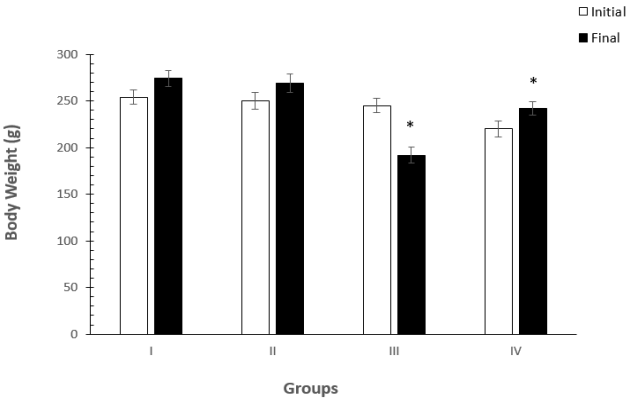


Fig 1: The weights given according to the mean ± standard error (*P<0.05).

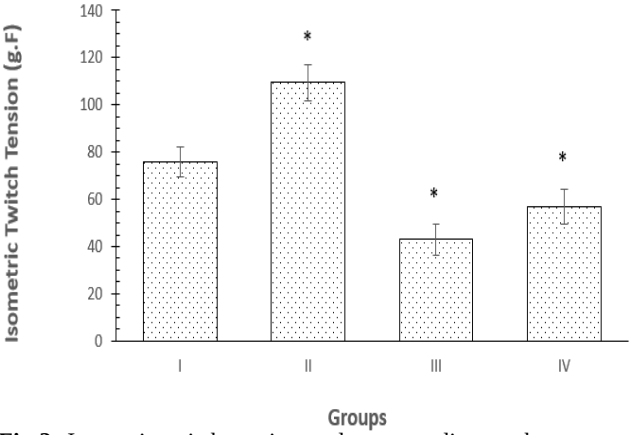


Fig 2: Isometric twitch tensions values according to the mean ± standard error (*P<0.05).

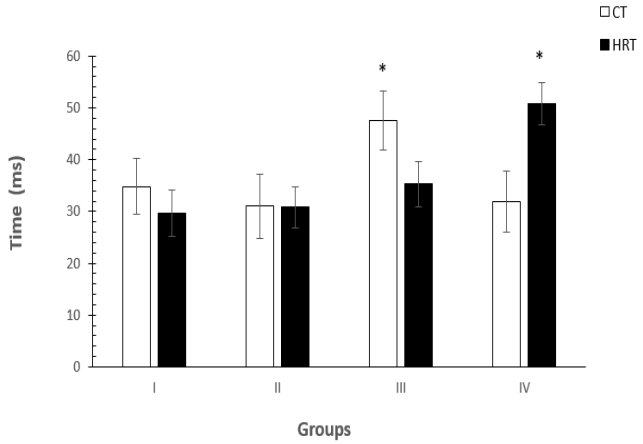


Fig 3: Contraction and Half Relaxation Times values according to the mean ± standard error (*P<0.05).

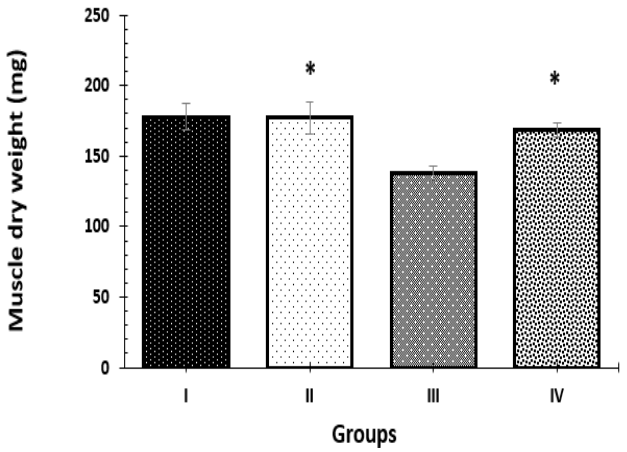


Fig 4: The changes of muscle dry weight according to groups. Values according to the mean ± standard error (*P<0.05).

Acharyya *et al*., 2007). The maintenance of skeletal muscle mass is dependent upon the balance between protein synthesis and protein degradation. Such stimulation of muscle hypertrophy leads to increases in muscle weight, increases muscle protein synthesis and induces muscle hypertrophy, whereas catabolic diseases increase protein degradation and cause muscle atrophy (Bodine *et al*., 2001).

Skeletal muscle hypertrophy has been reported as a side effect of bupivacaine, which has an impact on sarcoplasmic reticulum Ca-ATPase in the fast-twitch skeletal muscles. The common findings reported in the studies are degeneration and inflammation followed by a regeneration with an increased length and thickness of the muscle fibers after the bupivacaine injection (Park *et al*., 2004; Carlson *et al*., 1992).

Bupivacaine is especially lipophilic and strongly penetrates the sarcoplasmic reticulum of muscle fibers where it causes myotoxic damage. Within a few hours of exposure to BUP, autocrine growth factor molecules such as insulin growth factors IGF-I and IGF-IEc or mechano growth factor (MGF) are released from the damaged area. These molecules activate the satellite cells that are scattered around each muscle fiber. The satellite cells proliferate and coalesce to form new muscle fibers and myocytes to replace the damaged muscle, a process taking 3 weeks. Within a few weeks the muscle rebuilds, generally with increased size (Scott *et al*., 2013).

CONCLUSION

To our knowledge, this study was the first experimental trial of the contractile properties of bupivacaine-injected gastrocnemius muscles in diabetic rats since there do not exist any study the literature on streptozotocin-induced diabetes effects on the contractile properties of bupivacaine-injected muscles.

In conclusion, the results of the present suggest that intramuscular administration of bupivacaine into the diabetic rats can enhance muscle contractility. Further studies are needed to clarify whether there are long-term muscular effects after a single injection of bupivacaine.

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