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EFFECT OF HYPOTHERMIA ON THE TWITCH AND TETANIC CONTRACTIONS OF DENERVATED RAT DIAPHRAGM MUSCLE UNDER ISOTONIC CONDITIONS

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ABSTRACT

Effect of hypothermia was studied on the twitch and tetanic contractions of the chronically denervated rat diaphragm muscles under isotonic conditions. The muscles were denervated for 15-20 days and the temperature was lowered from 35°C to 15°C. The results showed that hypothermia and chronic denervation had very little effect on the isotonic contraction parameters since these were found to be unaltered at all temperatures except at 15°C. It is suggested that denervation has no significant effect on the contractile component of our experimental muscles.

Introduction

It has been known for a long time that the twitch and satanic contractions of the skeletal muscles are altered when the experimental temperature is changed (Hill, 1951; Maclagan and Zaimis, 1957; Cullingham et al., 1960; Truong et al., 1964; Padsha, 1965). It has further been demonstrated that the normal and denervated mammalian skeletal muscles react differently in their twitch and tetanic contractions under hypothermic conditions (Padsha, 1968; Shaikh et al., 1974; Shaikh et al., 1979a). Other studies have further confirmed that the denervated rat diaphragm develops less tension (Miledi and Slater, 1969). Similar temperature dependence of contractile behaviour of fast and slow mammalian muscles has also been reported by others (Buller et al., 1968a; Close and Hoh, 1968; Ranatunga, 1977). In addition, chronic denervation alone has also been shown to be responsible for producing changes in the contractile characteristics of mammalian skeletal muscles (Lewis, 1972) which were found to be related to changes in the Ca^{++} activated myosin ATPase activity (Gutmann and Hanzlkova, 1972).

Most of the studies made so far on the effect of temperature on the mechanical contraction behaviour of skeletal muscles were carried out on muscles under isometric conditions. The isometric connection is characterized by the fact that it does not deform when the attached muscle develops tension as the muscle is fixed on both the sides and is not allowed to shorten. The model of skeletal muscle suggested by Hill (1949) assumes a two component system, i.e. contractile elements present in series with passive elastic part of the muscle. The contractile components can be completely described by the force velocity parameters while the elastic components arc given by that series elasticity of the muscle which, when coupled to the contractile component, results in the form of isometric tetanus generally observed in the skeletal muscles. Thus, under non-isotonic conditions, the contractile components stretch the elastic components. To study the contractile system alone, isotonic conditions are required so that the elastic effects are eliminated. The present work was therefore, carried out to study the effect of hypothermia on the contractile system alone of the denervated muscles using isotonic conditions to eliminate effects of the elastic component of the muscle.

Material and Methods

Adult white albino rats of both sexes of wister strain, weighing from 160-240g were used and the left diaphragm muscles were chronically denervated for 15-20 days. The procedure used for denervation and dissection of the muscle, temperature regulation and the general experimental setup was the same as has been described earlier (Shaikh et al., 1979a). A description of the method used for isotonic recording has also been given elsewhere (Shaikh & Shaikh, 1985). The solution used for bathing the diaphragm strips was a slightly modified Krebs Henseliet solution (Winograd and Shanes, 1962) and had the following composition: NaCl, 118mM; KCl, 4.1 mM; $CaCl_2$, 1mM; $MgSO_4$, 1.2mM; KH_2PO_4, 0.8mM; $NaHCO_3$, 25mM; glucose, 5.55mM with pH 7.4. In addition, l0mg/litre tubocurarine chloride was also added to avoid spontaneous twitching of the muscle fibres. A mixture of 95% O_2, and 5% CO_2, was also bubbled through the solution. The isotonic recordings were carried out at a constant load of 2 gins at temperatures ranging from 35°C to 15°C.

Results and Discussion

The isotonic twitch and teanic tensions *were* recorded from both the normal and 15-20 days denervated hemidiaphragms under a constant load of 2 gms. The results showed that both the normal and denervated muscles developed maximum isotonic twitch tensions at 35°C (Table I). A decrease in temperature to 25°C and 15°C reduced the isotonic twitch tensions in both the normal and denervated muscles, with the minimum values being obtained at 15°C. It was further noted that the denervated muscles developed significantly less twitch tensions than the normal controls at all the three temperatures studied (Fig. 1), the decrease always being significant statistically ($P < 0.05$ & $P < 0.025$).

The isotonic tetanic tensions were also found to be generated maximally at 35°C in both the normal and denervated muscles which then decreased with a decrease in temperature, and the lowest values were obtained at 15°C in both the muscles (Table 2). However, in all the experiments done, the isotonic tetanic tensions generated by the denervated muscles were always significantly smaller than their normal controls (Fig, 2). To further clarify the differences in the isotonic tetanic tensions obtained from the normal

Table 1: Effect of temperature on isotonic twitch tension recorded from the right normal and left 15-20 days denervated diaphragm strips. For twitch recordings, the muscles were stimulated by 12V pulses and of 5mS duration.

S. No.	Temperatures	Isotonic Twitch Tensions (Kg/cm2) Mean + S.E.		P
		Normal	Denervated	
1.	35°C	0.28±0.03 (13)	0.19±0.03 (13)	(P< 0.025)
2.	25°C	0.24±0.03(13)	0.10±0.03(13)	(P<0.05)
3.	15°C	0.19±0.05(13)	0.0530.04(13)	(P<0.025)

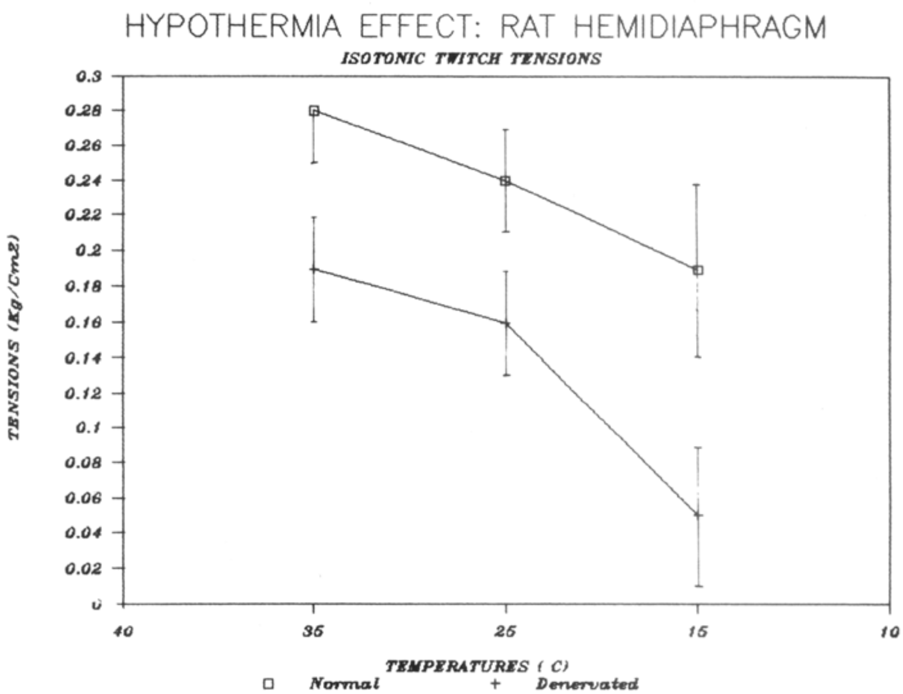

Fig. 1

Table 2: Effect of hypothermia on the isotonic tetanic tension recorded from the right normal and left 15-20 days denervated rat diaphragm strips. For tetanus recordings, the muscles were stimulated by 12V pulses and of 5mS duration at a frequency of 40/sec.

S. No.	Temperatures	Isotonic Tetanic Tensions (Kg/cm2) Mean + S.E		P
		Normal	Denervated	
1.	35°C	1.34 ± 0.03 (13)	0.91 ± 0.10 (13)	(P <0.01)
2.	25°C	1.20±0.12(13)	039 ± 0.09 (13)	(P< 0.01)
3.	15°C	0.8930.10(13)	0.50±0.07(13)	(P< 0.0025)

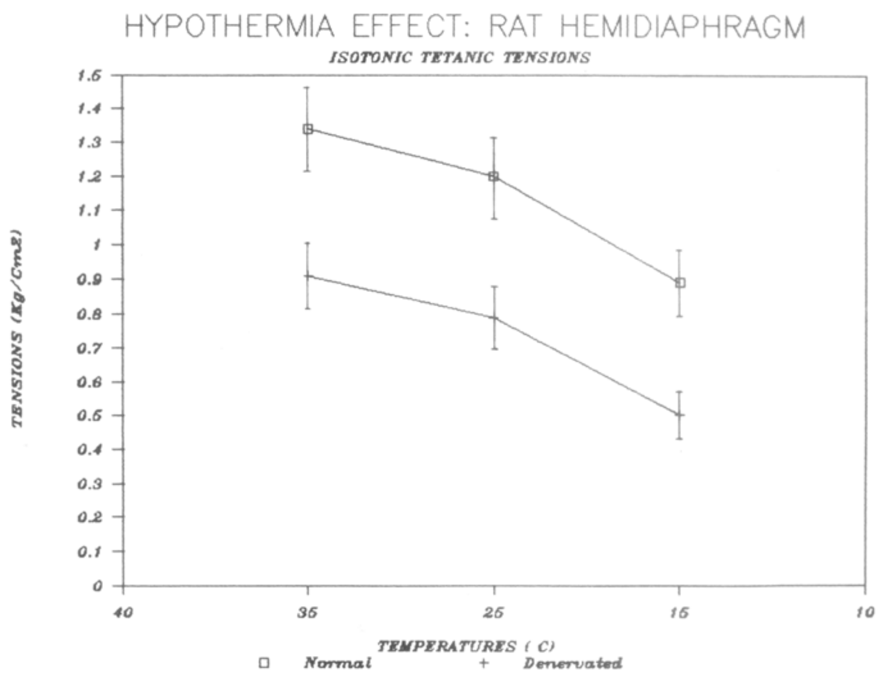

FIG. 2

and denervated muscles, the data shown in Table 2 was so treated that the values of tetanic tensions at 35°C were taken as unity, i.e. were considered to be equal to 1, and the tetanic tension values obtained at 25°C and 15°C were calculated as relative to 1 (Table 3). These results are shown in Fig. 3. It was noted that the differences between the isotonic tetanic tensions of the normal and denervated muscles became less marked as compared to those seen in Fig. 2. However, the denervated muscles still showed lesser tensions than their normal controls at lower temperatures. This again suggested that the ability of the denervated muscles to develop tension was reduced.

The isotonic twitch/tetanus tension ratios were also calculated and these results are shown in Table 4. It is to be noted that the ratios remained more or less constant in both the normal and denervated muscles at all the temperatures studied. Generally, the average of these ratios was of the order of 0.21 for all the experiments done. However, at 35°C, the isotonic twitch/tetanus ratio for the denervated muscles was very small, i.e. only 0.09. Further, there was no significant difference between these ratios calculated from the normal and denervated muscles ($P > 0.05$).

The application of an adequate electrical stimulus converts the resting state of the muscle into an active state which is characterized by an abrupt onset of the ability of the muscle to develop mechanical tension. If free to do so, the active muscle will begin to shorten and develop tension under a fixed load throughout its contraction and it is regarded as isotonic contraction. The active muscles behave in part as though they consist of a contractile component in series with an elastic component. Thus, under non-isotonic conditions, the contractile components stretch the elastic component. To study the contractile system alone, isotonic conditions are required so that the elastic effects are eliminated. Under isotonic conditions, the initial velocity of shortening, which is also the maximum velocity of shortening for any given load and the load applied, the muscles obey the following relationship:

$$(P+a)\ (V+b)=(P_o+a)b$$

where P is the load applied, V is the initial velocity of shortening, Po is the maximum tetanic tension the muscle can generate and 'a' and b' are two constants for the muscles: It is obvious from the above equation that with less load, the muscles shorten rapidly and with heavy loads, the muscles shorten slowly. In our experiments using isotonic conditions, an initial load of 2 gm was applied to the muscles.

The most important finding in the present results was that chronic denervation had very little effect on the isotonic contraction parameters of the muscles since isotonic twitch and tetanus were found to be unaltered by chronic denervation except as 15°C. It was therefore, concluded that denervation had no significant effect on the contractile components of our experimental muscles. However, as the isometric twitch and tetanus were found to be altered by denervation (Shaikh et al., 1979a, 1979b), it was assumed that these alterations must have been either due to changes in the series elasticity or due to changes in the duration of active state or both. Similarly, our earlier studies indicated that the force-velocity relationship of the muscles was not altered by denervation at 25°C to 35°C (Shaikh & Shaikh, 1985). Thus, a change in elasticity would have to be such that following denervation, the series elasticity was decreased.

Table 3: Effect of hypothermia on the isotonic tetanic tension recorded from the right normal and left 15-20 day denervated rat diaphragm strips calculated as unity from tetanic tensions at 35°C.

S. No.	Temperatures	Isotonic Tetanic Tensions RELATIVE TO UNITY	
		Normal	Denervated
1.	35°C	1.00	1.00
2.	25°C	0.896	0.868
3.	15°C	0.664	0.549

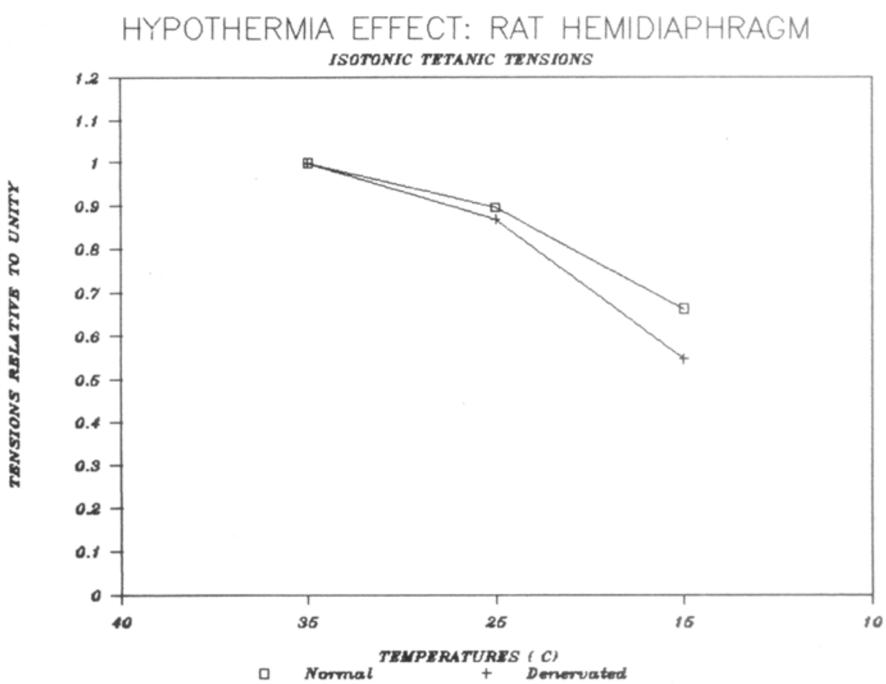

Fig. 3

Table 4: Effect of hypothermia on the twitch/tetanus ratios calculated from the isotonic twitch and tetanus records obtained from the normal and 15-20 days denervated rat diaphragm strips.

S. No.	Temperatures	Isotonic Twitch/Tetanus Tension Ratios Mean + S.E.		P
		Normal	Denervated	
1.	35°C	0.21±30.04(13)	0.21±30.06(13)	(P>0.05)
2.	25°C	0.20±0.04(13)	0.20±0.06(13)	(P>0.05)
3.	15°C	0.21±0.07(13)	0.09±30.01(13)	(P>0.05)

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