Vol. 184, No. 1, 1992 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 15, 1992 Pages 353-358

Ca^{2+}-CHANNELS AND ADRENOCEPTORS IN DIABETIC SKELETAL MUSCLE

Sheu L. Lee and Naranjan S. Dhalla

Division of Cardiovascular Sciences
St. Boniface General Hospital Research Centre
and Department of Physiology, University of Manitoba
Winnipeg, Canada R2H 2A6

Received February 27, 1992

SUMMARY: The status of Ca^{2+}-channels and adrenoceptors in the hind leg skeletal muscle was examined in rats 8 weeks after inducing diabetes by an intravenous injection of streptozotocin (65 mg/kg). Scatchard plot analysis of the data on specific binding of ^{3}H-nitrendipine with crude membranes from diabetic muscle revealed an increase in the density of Ca^{2+}-channels without any significant change in their affinity for the ligand. An increase in the density of beta-adrenoceptors without any alteration in their affinity, as measured by ^{3}H-dihydroalprenolol binding, was also evident in the diabetic muscle. The observed increase in the number of Ca^{2+} channels or beta-adrenoceptors seems specific since no change in the alpha-adrenoceptor density or affinity, as measured by ^{3}H-prazosin binding, was seen in the diabetic membranes. These results support the view that higher activities of Ca^{2+} transport systems or regulatory mechanisms may be associated with hyperfunction of the diabetic skeletal muscle.

A wide variety of functional, metabolic and biochemical changes have been reported to occur in the skeletal muscle in chronic diabetes (1-6). Earlier studies from our laboratory have revealed hyperfunction of the hind leg skeletal muscle in rats with chronic diabetes induced by streptozotocin (7). Likewise, both sarcoplasmic reticular Ca^{2+}-pump and myofibrillar Ca^{2+}-stimulated ATPase activities were higher in the diabetic skeletal muscle (7,8). While the sarcolemmal Ca^{2+}-pump and Na^+-Ca^{2+} exchange activities were increased, no changes in the sarcolemmal Na^+-K^+ ATPase and Mg^{2+}-ATPase activities were observed in the diabetic skeletal muscle (9). Thus it appears that Ca^{2+}-related mechanisms involved in muscle function are augmented in chronic diabetes. Although Ca^{2+}-channels in sarcolemma are intimately involved in Ca^{2+}-movements across the cell membrane and beta-adrenergic receptors are known to regulate Ca^{2+}-channels (10,11), no information concerning the characteristics of these structures in diabetic

0006-291X/92 $1.50

skeletal muscle is available in the literature. This study was therefore undertaken to examine whether or not these Ca^{2+}-transport and regulatory mechanisms in the skeletal muscle are altered in streptozotocin-induced diabetes in rats.

MATERIALS AND METHODS

Diabetes in Sprague-Dawley male rats weighing about 200 g each was induced by injecting streptozotocin (65 mg/kg; i.v.) in 0.1 M citrate buffer. Control animals received a similar injection of the vehicle alone. All animals were killed at 8 weeks. One group of the 4 weeks diabetic rats was given subcutaneous injections of 3 U protamine zinc insulin daily for 4 weeks before sacrifice. The plasma samples were analyzed for insulin and glucose by the radioimmunoassay technique (Amersham) and glucose reagent kit (Sigma), respectively. The hind leg skeletal muscle (hamstring muscle) was dissected out and crude membranes were isolated by methods described elsewhere (10). For the purpose of measuring Ca^{2+}-channels, total and nonspecific ^3H-nitrendipine binding with control and experimental preparations were measured in the absence or presence of 2 uM unlabelled nifendipine, respectively (10). The beta-adrenoceptor assay involved measurement of total and nonspecific ^3H-dihydroalprenolol binding in the absence and presence of 10 uM unlabelled l-propranolol, respectively (11). In experiments where alpha-adrenoceptors were determined, total and nonspecific ^3H-prazosin binding were measured in the absence or presence of 5 uM unlabelled phentolamine, respectively (11). Specific binding for each ligand was calculated by subtracting nonspecific binding from the respective total binding value. Results were evaluated according to the Scatchard plot analysis in terms of dissociation constant (K_d) and maximal density (B_{max}). The statistical difference between mean values for two groups was obtained by Student's t-test.

RESULTS

Rats injected with streptozotocin for 8 weeks exhibited less body wt, high plasma glucose and decreased plasma insulin levels in comparison to the control animals (Table 1). These alterations are similar to those reported earlier by employing the streptozotocin-rat model of diabetes (12,13). Specific binding of ^3H-nitrendipine at different concentrations of the radioligand was reduced in diabetic skeletal muscle membranes (Fig. 1). Scatchard plot analysis of the data from diabetic animals revealed a significant increase in B_{max} value without any change in the K_d value (Fig. 1 and Table 1). All these changes were prevented upon treating the diabetic animals with insulin (data not shown).

In another set of experiments, specific binding of ^3H-dihydroalprenolol with control and diabetic preparations was monitored to assess the status of beta-adrenoceptors. Scatchard plot analysis of the data

Table 1. Characteristics of diabetic rats 8 weeks after injecting streptozotocin (65 mg/kg, i.v.) and specific binding of ^{3}H-nitrendipine with crude membranes from the hind leg skeletal muscle

	Control	Diabetic
Body weight (g)	420 ± 12	275 ± 8*
Plasma glucose (mg/dl)	145 ± 10	604 ± 25*
Plasma insulin (uU/ml)	28 ± 1.4	13 ± 1.3*
^{3}H-nitrendipine binding:		
K_d (nM)	6.78 ± 2.13	7.18 ± 1.84
B_{max} (pmol/mg)	4.84 ± 0.23	7.36 ± 0.82*

Each value is a mean ± SE of 5 to 9 experiments. Values for both K_d and B_{max} were obtained by the Scatchard plot analysis of the data for ^{3}H-nitrendipine binding with crude membranes. * - Significantly ($P < 0.05$) different from the control values.

on specific binding of ^{3}H-dihydroalprenolol at different concentrations of the radioligand revealed a marked increase in B_{max} value without any change in K_d value in diabetic membranes (Fig. 2 and Table 2). Treatment of diabetic animals was found to prevent the observed increase in the density of

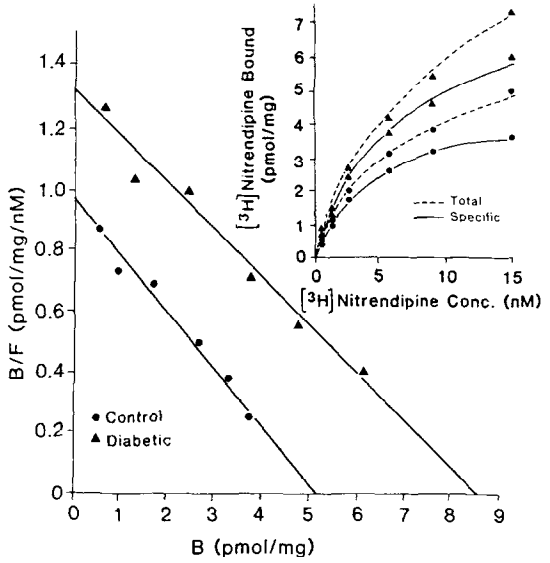

Figure 1. Scatchard plot analysis of the data for specific ^{3}H-nitrendipine binding with hind leg skeletal muscle crude membranes from control and streptozotocin-diabetic rats. Total and nonspecific ^{3}H-nitrendipine bindings were determined in the absence and presence of 2 μM unlabelled nifedipine, respectively. Specific ^{3}H-nitrendipine binding was calculated by subtracting nonspecific binding from total binding. The results are from a typical experiment with control and diabetic preparations.

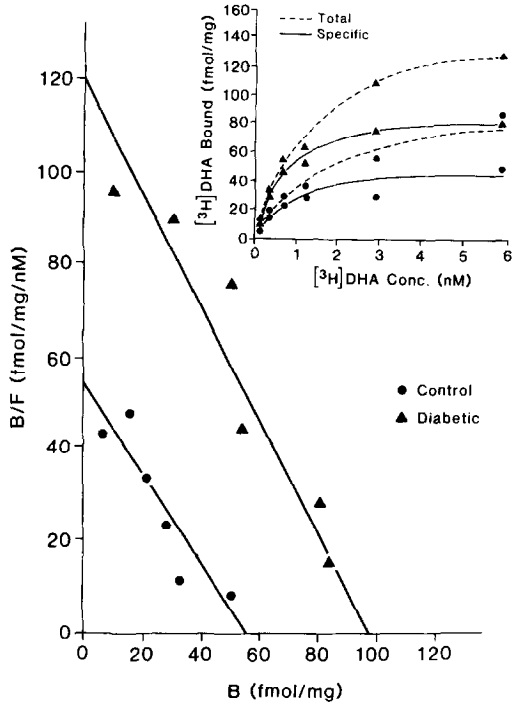

Figure 2. Scatchard plot analysis of the data for specific ^{3}H-dihydroalprenolol binding with hind leg skeletal muscle crude membranes from control and streptozotocin-diabetic rats. Total and nonspecific ^{3}H-dihydroalprenolol bindings were determined in the absence and presence of 10 μM unlabelled l-propranolol, respectively. Specific ^{3}H-dihydroalprenolol binding was calculated by subtracting nonspecific binding from total binding. The results are from a typical experiment with control and diabetic preparations.

Table 2. Characteristics of ^{3}H-dihydroalprenolol and ^{3}H-prazosin binding with the hind leg skeletal muscle membranes from control and diabetic rats

	Control	Diabetic
^{3}H-dihydroalprenolol binding:		
K_d (nM)	0.65 ± 0.73	0.73 ± 0.18
B_{max} (fmol/mg)	37.9 ± 7.1	66.3 ± 10.9*
^{3}H-prazosin binding:		
K_d (nM)	0.96 ± 0.12	0.97 ± 0.14
B_{max} (fmol/mg)	18.8 ± 0.56	21.2 ± 1.74

*Each value is a mean ± SE of 4 to 5 experiments. Skeletal muscle crude membranes were prepared from diabetic animals 8 weeks after injecting streptozotocin as well as from control animals. * - Significantly ($P < 0.05$) different from the control values.*

beta-adrenoceptors in diabetic membranes (data not shown). Both B_{max} and K_d values for alpha-adrenoceptors, as determined by ^{3}H-prazosin binding, were not altered in the diabetic skeletal muscle membranes (Table 2).

DISCUSSION

In this study we have shown a significant increase in the density of Ca^{2+} channels, as measured by the ^{3}H-nitrendipine binding assay, without any change in their affinity for the radioligand in the skeletal muscle membranes from the diabetic animals. Likewise, ^{3}H-dihydroalprenolol binding with diabetic preparations revealed a marked increase in the number of beta-adrenoceptors without any change in their affinity for the radioligand. These changes seem specific in nature since the characteristics of alpha-adrenoceptors, as determined by ^{3}H-prazosin binding, were not altered in diabetic membranes. Furthermore, the increased number of Ca^{2+}-channels and beta-receptors is likely to be a consequence of insulin deficiency in chronic diabetes since these changes were prevented upon treating the diabetic animals with insulin. Nonetheless, the observed alterations in Ca^{2+}-channels and beta-adrenoceptors in diabetic skeletal muscle are opposite to those reported in the diabetic heart under identical experimental conditions (14,15). Such results are consistent with our earlier observations regarding the opposite behaviour of the skeletal and cardiac muscles from the streptozotocin-induced diabetes in rats with respect to their contractile function, myofibrils, sarcoplasmic reticulum and sarcolemma (7-9,13,16,17). Such opposite changes in the responses of the skeletal and cardiac muscles to insulin deficiency in diabetic animals have been explained on the basis of differences in the innervation of these organs.

Although the exact role of Ca^{2+}-channels in the cell membrane of the skeletal muscle is not clear, these channels as measured by ^{3}H-nitrendipine binding are considered to participate in Ca^{2+}-influx (18). Likewise, beta-adrenoceptors through cyclic AMP-protein kinase dependent phosphorylation of membrane Ca^{2+}-channels are known to regulate Ca^{2+}-influx (18). Thus the observed increase in the number of both Ca^{2+}-channels and beta-adrenoceptors

in diabetic skeletal muscle will favour enhanced entry of Ca^{2+} into the myocytes. Such an increase in the availability of intracellular Ca^{2+} along with increased level of calmodulin (4) are consistent with the augmented contractile activity of the skeletal muscle in diabetic rats (7,9). However, it remains to be established whether the observed increase in the number of Ca^{2+}-channels and beta-adrenoceptors serves as an adaptation to hyperactivity of the hyperphagic animals in chronic diabetes or contributes in explaining the hyperfunction of diabetic skeletal muscle.

ACKNOWLEDGMENT

The research work reported in this paper was supported by a grant from the Juvenile Diabetes Foundation International.

REFERENCES

1. Randle, P.J., Hales, C.N., Garland, P.B. and Newsholme, E.A. (1963) Lancet 1, 785-789.
2. Pain, V.M. and Garlick, P.J. (1974) J. Biol. Chem. 249, 4510-4514.
3. Olson, E.N., Kelly, D.A. and Blaise-Smith, P. (1981) Exp. Neurol. 73, 154-172.
4. Morley, J.E., Levine, A.S., Brown, D.M. and Handwerger, B.S. (1982) Biochem. Biophys. Res. Commun. 108, 1418-1423.
5. Chen, V. and Ianuzzo, D. (1982) Arch. Biochem. Biophys. 217, 131-138.
6. Taira, Y., Ganguly, P.K., Panagia, V. and Dhalla, N.S. (1988) Am. J. Physiol. 255, E347-E352.
7. Ganguly, P.K., Mathur, S., Gupta, M.P., Beamish, R.E. and Dhalla, N.S. (1986) Am. J. Physiol. 251, E515-E523.
8. Ganguly, P.K., Taira, Y., Elimban, V., Roy, M. and Dhalla, N.S. (1987) Am. J. Physiol. 253, E395-E400.
9. Taira, Y., Hata, T., Ganguly, P.K., Elimban, V. and Dhalla, N.S. (1991) Am. J. Physiol. 260, E626-E632.
10. Dixon, I.M.C., Lee, S.L. and Dhalla, N.S. (1990) Circ. Res. 66, 782-788.
11. Dixon, I.M.C. and Dhalla, N.S. (1991) Coronary Artery Disease 2, 805-814.
12. Pierce, G.N., Kutryk, M.J.B. and Dhalla, N.S. (1983) Proc. Natl. Acad. Sci. USA 80, 5412-5416.
13. Makino, N., Dhalla, K.S., Elimban, V. and Dhalla, N.S. (1987) Am. J. Physiol. 253, E202-E207.
14. Lee, S.L., Ostadalova, I., Kolar, F. and Dhalla, N.S. (1992) Mol. Cell. Biochem. in press.
15. Heyliger, C.E., Pierce, G.N., Singal, P.K., Beamish, R.E. and Dhalla, N.S. (1982) Basic Res. Cardiol. 77, 610-618.
16. Pierce, G.N. and Dhalla, N.S. (1985) Am. J. Physiol. 248, E170-E175.
17. Ganguly, P.K., Pierce, G.N., Dhalla, K.S. and Dhalla, N.S. (1983) Am. J. Physiol. 244, E528-E535.
18. Sulakhe, P.V. and St. Louis, P.J. (1980) Prog. Biophys. Mol. Biol. 35, 135-195.