Diabetes Mellitus-Induced Hypersensitivity of Mouse Skeletal Muscles to Acetylcholine and Succinylcholine

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Abstract—The myopathy in skeletal muscles of genetically diabetic male KK-CA\(^{+}\) mice or alloxan-induced diabetic mice was investigated. In these diabetic mice, nerve-stimulated twitch tensions of in situ sciatic nerve-gastrocnemius muscle preparations were inhibited by intraarterially administered succinylcholine (SuCh) to a greater extent than in normal (non-diabetic) ones. Despite the high blood glucose level, at one week after alloxan administration, no hypersensitivity to SuCh was induced in mice, but mice at 2 weeks and 4 weeks after alloxan showed greater sensitivities. In isolated diaphragm muscles of diabetic KK-CA\(^{+}\) mice, the acetylcholine (ACh, iontophoretically applied) potential amplitude was greater than in KK-CA\(^{+}\) prediabetic muscles. SuCh in diabetic KK-CA\(^{+}\) muscles inhibited ACh potentials to a greater extent than in normal ddY muscles. Hill coefficients obtained from the inhibition curve by SuCh of the nerve-stimulation response were decreased by the diabetic state. The sensitivities to d-tubocurarine and \(\alpha\)-bungarotoxin were the same in both kinds of muscles. Both extrajunctional ACh receptors in denervated muscles of normal ddY and diabetic KK-CA\(^{+}\) mice revealed the lower sensitivity to SuCh than junctional receptors in non-denervated normal muscles. In conclusion, diabetic muscles showed the hypersensitivity restricted to SuCh. These phenomena are neither due to glycosylation nor to denervation supersensitivity.

Neuropathy in diabetes has attracted much attention recently, but few studies have been made on the functioning of terminal neuromuscular junctions (or regions) in diabetes mellitus, although failure of neuromuscular transmission occurs in many such patients. In animals with diabetes caused by alloxan, Minker and Koltai (1) reported that succinylcholine (SuCh) was more effective than in the controls. Schofield and others (2) reported no significant difference in the sensitivity to decamethonium of animals with and without experimental diabetes.

The genetically diabetic KK-CA\(^{+}\) mouse (NIDDM type) is an inbred diabetic strain established by Nishimura (3) by transferring the yellow obese gene (\(A^{Y}\)) into KK mice, a strain established by Kondo et al. (4) from native Japanese mice. Kimura et al. (5) reported in detail on the diabetic pattern in blood glucose and immunoreactive insulin levels of the KK-CA\(^{+}\) strain, and they showed that the Langerhans` islets could be seen to be disordered morphologically. The neuromyopathy of the skeletal muscles of these mice has not been investigated in terms of drug sensitivity. In this paper, we wish to examine the properties of the muscle membrane impaired by alloxan-induced or KK-CA\(^{+}\) diabetes. This study is restricted to the muscle sensitivity to electrical nerve stimulation, acetylcholine (ACh), SuCh, and d-tubocurarine (d-TC).

Materials and Methods

We used male ddY mice (7–8 weeks old, 20–36 g), diabetic male KK-CA\(^{+}\) mice (4–6 months old, 35–65 g; blood glucose (BG) level: 200–400 mg/dl), prediabetic male KK-CA\(^{+}\) mice (24–60 g, BG: 104–156 mg/dl),
female KK-CAY mice (45–65 g, BG: 120–148 mg/dl), male KK-C mice (29–39 g, BG: 118–182 mg/dl), and male C57BL/6 mice (21–26 g). We also used male mice with diabetes induced by alloxan on the 7th day after the administration of alloxan (21–29 g, BG: 400–664 mg/dl), the 14th day (24–33 g, BG: 332–570 mg/dl), and the 28th day (22–32 g, BG: 300–510 mg/dl). For this purpose, alloxan monohydrate (85 mg/kg) was dissolved in 0.9% NaCl solution and injected into the tail vein of the mice. Glucose levels were measured by the glucose oxidase method on a glucose analyzer (Beckman, Type II). Denervation procedures were as follows: A left unilateral phrenicotomy was done by removing 1–1.5 cm of the phrenic nerve at the plexus cervicalis of mice under urethane anesthesia. Then, 12–18 days later, the denervated diaphragm muscles were isolated.

Measurement of twitch tension: In in situ experiments, all mice were anesthetized by an intraperitoneal injection of urethane (1.7 g/kg). Each mouse was transferred to a warm experimental table (36±1°C). A tracheal cannula was inserted and was connected to a Harvard rodent respirator (Type 680) operating at 1.3 ml and 170 strokes/min. The drug at the dose of 0.1 ml/10 g body weight was given through the left femoral artery, which was cannulated by a glass microtubule (with a diameter of less than 0.1 mm) connected via a polyethylene catheter (ATOM, diameter 1 mm) to a glass syringe. The left gastrocnemius muscle was freed from the adjacent muscles, leaving the vascular vessels intact. The sciatic nerve was separated and was cut off as proximally as possible, except the branch that innervates the gastrocnemius muscle. We always verified that there was no thrombi in the femoral artery after the experiment. The sciatic nerve was stimulated at 0.2 Hz by square wave pulses (supramaximally, 0.5–1.0 V) of 1.0 msec duration. The twitch responses of the muscles were recorded with an isometric transducer (Nihon Kohden, SB-1T-H) on a Biophysiograph 110 system (San-ei). A resting force of 1 g was applied to each tissue. Inhibitory responses by drugs were presented as percentage of the control value for 1 min, and averaged. Hill coefficient values (6) were calculated on the basis of the log dose-Logit (response) curve of averaged points.

Intracellular recording: Standard glass capillary electrodes filled with 3 M KCl and with the resistance of 20 to 40 MΩ were used for intracellular recording of membrane potentials using a microelectrode amplifier (Nihon Kohden, MEZ 8101). Only those cells having miniature end-plate potentials (mepp) were considered acceptable for study. ACh chloride was applied iontophoretically through an electrode filled with 1 M ACh using a micro-iontophoresis unit (Diamedical, DPI-25) and a V-I conversion unit (DPI-25 T).

Drugs: ACh chloride (Dai-ichi), SuCh chloride 2 H2O, d-TC chloride (Nakarai), α-bungarotoxin (α-BuTX, Biotoxin, Inc.), and alloxan monohydrate (Nakarai) were used.

Results

Hypersensitivity in nerve-stimulated twitchings of diabetic muscles in situ to SuCh: The effects of SuCh on indirectly stimulated twitching in in situ motor nerve-skeletal muscle preparations were compared in normal (non-diabetic) ddY and diabetic KK-CAY mice or alloxan-induced diabetic mice. As hypersensitivity to SuCh in the inhibition of nerve-stimulated twitchings was difficult to see in vitro (data not shown), we looked for it using preparations of sciatic nerve and gastrocnemius muscle in situ (the drug seems to be effectively transferred to end-plate regions). The log dose of SuCh against the percentage of inhibition of nerve-stimulated twitch tension was plotted (Fig. 1a and b). The sensitivity to SuCh of neuromuscular junction in diabetic KK-CAY muscles was approximately 2 times that in prediabetic KK-CAY muscles or normal ddY muscles. The ID50 for SuCh in male KK-C, in female KK-CAY, and in male C57BL/6 mice were not significantly different from the ID50 value in prediabetic KK-CAY mice (Table 1). Prediabetic KK-CAY muscles and normal ddY muscles had the same sensitivity to SuCh. This, therefore, justified the appropriateness of ddY (normal) as a control throughout this study. On the other hand, no
A difference was observed in the sensitivity to d-TC at the neuromuscular junction (data not shown). The hypersensitivity, therefore, seems to be restricted to SuCh.

Whether the hypersensitivity to SuCh is dependent on the blood glucose level or not was studied using muscles of ddY mice with alloxan-induced diabetes. On the 7th day after alloxan was given, the inhibition curve still overlapped that of control muscles despite the high blood glucose levels in the mice. The hypersensitivity to SuCh was observed on the 14th day after the alloxan administration, and it increased by the 28th day. These results showed that SuCh hypersensitivity is not due to the high level of blood glucose, but rather may be caused by the secondary effect due to the deficiency of insulin-like growth factor.

On the other hand, whether the SuCh hypersensitivity is specific for genetically diabetic muscles or not was studied using...
muscles of male KK-C, female KK-CA\textsuperscript{y} and male C57BL/6 mice. The sensitivities (ID\textsubscript{50}) and the respective Hill coefficients are shown in Table 1. Hill coefficient values were calculated on the basis of the SuCh log dose-Logit response curves of averaged points (partly from Fig. 1a and b). These results showed that the neuromuscular junctions of both alloxan-induced diabetic mice and the genetically diabetic KK-CA\textsuperscript{y} mice were more sensitive to SuCh. So SuCh hypersensitivity was not a genetic state but rather a state of secondary modifications in the peripheral synapses caused by the diabetes. It is also supported by the gradual increase in the Hill coefficients due to diabetic states (Table 1).

Hypersensitivity in inhibition of ACh potential amplitudes in diabetic KK-CA\textsuperscript{y} muscles to SuCh: To exclude the influence of diabetic nerves, ACh was applied iontophotically at diaphragm muscles from normal ddY, KK-CA\textsuperscript{y} prediabetic and diabetic male mice by rectangular pulses (from 0.1 to 3.2 nano-Coulomb (nC) in an increasing order). The curves of ACh potential amplitude and ACh pulses in both kinds of normal ddY mice and prediabetic KK-CA\textsuperscript{y} mice were not significantly different from each other, but the curve for diabetic KK-CA\textsuperscript{y} diaphragm muscles was shifted to higher amplitudes of ACh potentials than that for prediabetic KK-CA\textsuperscript{y} muscles (Fig. 2). Thus, the sensitivity to ACh was actually higher in diabetic muscles.

We also investigated the sensitivity to the antagonists SuCh, d-TC and \(\alpha\)-BuTX against ACh potentials. SuCh was used at various concentrations (0.1, 0.3 or 1 \(\mu\)g/ml),

![Fig. 2. Acetylcholine (ACh) potential amplitude-log ACh pulse curves in end-plate regions of diaphragm muscles from male ddY mice (O, —) and diabetic (●, —) or prediabetic (○, ---) KK-CA\textsuperscript{y} mice (Means±S.E.M., n=4-6).](image)

![Fig. 3. Effects of neuromuscular blockers on ACh potential amplitude-log ACh pulse curves in end-plate regions of diaphragm muscles from male ddY mice (O, —) and diabetic KK-CA\textsuperscript{y} mice (○, —). a: SuCh (0.1, 0.3 and 1 \(\mu\)g/ml) effects. Values are means±S.E.M. (n=4). b: d-Tubocurarine (0.1, 0.3 and 1 \(\mu\)g/ml) effects. Values are means±S.E.M. (n=3-4).](image)
and ACH was applied after the resting membrane potential recovery from the depolarized state by SuCh. In Fig. 3, we plotted the percentage amplitude of ACH potentials against ACH pulses. In the absence of SuCh, the response to 0.1 nC of ACH pulse was regarded as 100% for both diabetic KK-CAY and normal ddY end-plates, and it corresponded to an amplitude of 5 mV. As 0.3 μg/ml SuCh in diabetic KK-CAY muscles showed the same potency as 1 μg/ml SuCh in normal ddY muscles, SuCh had approximately 3-fold potency of antagonistic action in diabetic KK-CAY muscles when compared with that in normal ddY muscles (Fig. 3a). On the other hand, the diabetic state did not alter the sensitivity to d-TC (used at 0.3, 1 and 3 μg/ml), as shown in Fig. 3b. The sensitivity to α-BuTX (30, 100 and 300 ng/ml) in diabetic KK-CAY muscles was the same as in ddY muscles (data not shown). These results indicated that the diabetic hypersensitivity occurred with an agonist or depolarizing neuromuscular blocking agents, but not with competitive blocking agents, suggesting that there is some damage caused by diabetes mellitus related to the depolarization mechanism in muscle membranes.

Denervated muscles of diabetic KK-CAY mice were not significantly different from or rather apt to be less sensitive to SuCh only at 3 μg/ml than denervated muscles of normal ddY mice (Fig. 4). This result indicates that the extrajunctional ACH receptors developed by denervation were less sensitive to SuCh than non-denervated muscles, despite the high sensitivity to ACH. There were differences between the non-denervated muscle membrane in diabetic mice and the denervated one in non-diabetic mice in both their electrical properties such as ACH potentials or in their SuCh hypersensitivity.

Discussion

Diabetic polyneuropathy is probably caused secondarily by damage due to diabetic metabolic disorders. Observations that abnormal peripheral nerve function consistently follows the onset of hyperglycemia in non-insulin-dependent diabetes mellitus in both man and rodent strongly support this notion as reviewed by Clements (7). Based on in vitro studies of contractile and electrical properties of the extensor digitorum longus muscle from rats treated with alloxan, Grossie (8) reported that the specific twitch tension was unchanged in both direct (for muscles) and indirect (for nerves) contractions in response to stimuli, that resting membrane potentials were only slightly reduced, and that specific membrane resistance was significantly increased. There are three possible explanations for the muscle hypersensitivity: 1) an increase in ACH release from nerve terminals, 2) a decrease in the level or activity of cholinesterase, and 3) a hypersensitivity of the muscle cell itself. Concerning plasma pseudo-cholinesterase, the activity measured using butyrylthiocholine as a substrate was increased by diabetic states in KK-CAY mice (9). The change in the activity of muscle cholinesterase by diabetic states is still awaiting further studies. In our present study, in addition to diabetic damage to the nerves, the muscle sensitivity, in particular the ACH receptor sensitivity, is increased; the ACH pulse-ACH potential amplitude curve in diabetic mice was shifted above and to the left compared to the prediabetic control. The reactivity of these sensitized muscles to neuromuscular blocking
agents was necessarily altered. There have been two contradictory reports on the effect of depolarizing neuromuscular blocking agents. Schofield et al. (2) reported that neuromuscular preparations from rats had reduced sensitivity to d-TC, but there were no changes in sensitivity to decamethonium. Minker and Koltai (1) reported that in such rats, the susceptibility of the motor end-plate to d-TC decreased, whereas SuCh was more effective than in the control animals (a prolongation of the action of SuCh). The present results showed that sensitivity to d-TC and α-BuTX did not alter indirectly stimulated twitchings and also did not alter ACh potentials. On the other hand, the inhibitory sensitivity (against ACh potentials or twitchings) to SuCh in isolated or in situ preparations of diabetic mice was higher than in the non-diabetic controls. As increased sensitivity to SuCh was generated both in genetically diabetic KK-CA\textsuperscript{y} mice and also in mice with diabetes caused by alloxan, these properties do not seem to be inherited genetically, but seem to be secondary to the metabolic disorders. This is supported by the finding that the hypersensitivity was not necessarily correlated directly and immediately with increased blood glucose levels (glycosylation) since hypersensitivity had not yet occurred in mice with alloxan-induced diabetes of 1-week duration.

Denervation-induced hypersensitivity is known to correspond to the development of extrajunctional ACh receptor (10). Both kinds of denervated muscles in normal ddY mice and diabetic KK-CA\textsuperscript{y} mice were highly sensitive to ACh. In denervated muscles of diabetic mice, the sensitivity to SuCh was slightly greater than in muscles of non-diabetic controls at a dose level of 3 \(\mu g/ml\). These results suggest that diabetic states are quite different from denervated states in regard to SuCh sensitivity.

In conclusion, the hypersensitivity to a depolarizing agent (ACh or SuCh) in diabetic muscles may be partly caused by changes in the muscle membrane surrounding the ACh receptors. SuCh hypersensitivity is secondary to the diabetic metabolic disorders such as the blood glucose level and is accompanied with lessened Hill coefficients, suggesting the alteration of binding sites for SuCh.

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References