Electrical Properties of Colonic Smooth Muscle in Spontaneously Non-Insulin-Dependent Diabetic Rats

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Abstract

Electrical properties of colonic smooth muscle were investigated in the Otsuka Long-Evans Tokushima Fatty (OLETF) rat, a model animal for spontaneous non-insulin-dependent diabetes mellitus (NIDDM), and the results were compared with those obtained from the Long-Evans Tokushima Otsuka (LETO) rat, a control of OLETF rat. At experiments (aged 60-80 weeks), blood glucose level was about 171 mg/dl in LETO rats and 370 mg/dl in OLETF rats. Feces in the colon were restricted to the proximal region in LETO rats and distributed widely in the whole colon in OLETF rats. In both LETO and OLETF rats, the circular smooth muscle strips of the isolated distal colon revealed two types of spontaneous electrical response, slow wave and transient hyperpolarization. The resting membrane potential was smaller in OLETF rats than in LETO rats by about 3 mV, but it was not positively related with the blood glucose level. The amplitude of hyperpolarization produced by noradrenaline (NA) was smaller in OLETF rats than in LETO rats. Transmural nerve stimulation evoked a non-adrenergic, non-cholinergic (NANC) inhibitory junction potential (i.j.p.) in both LETO and OLETF rats; the amplitude of the i.j.p. was smaller in OLETF rats than in LETO rats, while the latency of the i.j.p. was longer in OLETF rats than in LETO rats. Thus, in the distal colon, NIDDM may cause a depolarization of the membrane, an attenuation of NANC inhibitory transmission and a reduction in reactivity of adrenoceptors to NA. These results suggest that the constipation appearing with diabetes mellitus involves dysfunction of both the enteric autonomic nerves and the smooth muscles in the colon.

Key Words: Colonic smooth muscle, NIDDM, OLETF, Inhibitory junction potential, Neuromuscular transmission

Introduction

Constipation is one of the most common gastrointestinal problem in diabetes mellitus (Feldman et al., 1983) and it is suggested that abnormalities in colonic motility relates with

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autonomic neuropathy of the gastrointestinal tract (Battle et al., 1980). Alteration of electrical properties of colonic smooth muscle in insulin-dependent diabetes mellitus (IDDM) has been reported in experimental model animals prepared by injection of streptozotocin (STZ), and the results indicate that IDDM causes enhanced sensitivity to noradrenaline (NA) with reduced chemical transmission from the enteric nerves to the smooth muscle (Hoyle et al., 1988). Although a large population of patients is non-insulin-dependent diabetes mellitus (NIDDM), the alteration of the properties of smooth muscle and neuromuscular transmission remains unclear.

Otsuka Long-Evans Tokushima Fatty (OLETF) rats are a genetically established hyperglycemic animal model and their properties are similar to NIDDM in human (Kawano et al., 1992; Kawano et al., 1994; Shima et al., 1994). We investigate the properties of colonic smooth muscle in OLETF rats, the objective being to find out possible alterations of smooth muscle during the development of NIDDM. Experiments were carried out by recording the membrane potential of isolated colonic smooth muscle intracellularly using microelectrode, and the electrical responses of the membrane were compared with those recorded from Long Evans Tokushima Otsuka (LETO) rats, control of OLETF rats. Junction potentials were evoked by transmural nerve stimulation (TNS) to assess functional alteration of chemical transmission from the enteric nerves to the colonic smooth muscles. A part of the results was reported briefly to the American Gastroenterological Association (Imaeda et al., 1998).

Materials and methods

OLETF and LETO rats (males for both) were supplied at the age of 5 weeks and were bred in separate cages at constant humidity (60%) and temperature (25°C), with free access to water and food for 60 to 80 weeks in the Experimental Animal Sciences Center of Nagoya City University Medical School. Then, at 9:00-9:30 in the morning, the animals were weighed, anesthetized with carbon dioxide (CO₂) and then exsanguinated by bleeding from the femoral arteries. The blood glucose level was measured three times using a glucose test kit (Glutest E II, Kyoto Daichi Kagaku, Kyoto, Japan), and the mean value of the measurements was used. The location of feces was observed before dissection of the colon. The narrow part formed by the sphincter in the ascending colon was defined as the boundary between the proximal and middle regions. The portion attached by mesentery was defined as the distal region (Suthamnatpong et al., 1993). The distal colon was excised and placed in oxygenated Krebs solution. The muscle was cut in the longitudinal direction along the mesenteric border and pinned out in a dissecting chamber with the mucosal side up. The mucosa was removed, using microscissors, and transverse strips (1 mm wide and 10-15 mm long) were prepared in Krebs solution at room temperature.

Preparations were mounted in a recording chamber and superfused with warmed (35°C) Krebs solution at a constant flow rate of 2-3 ml/min. Muscle strips were immobilized on a rubber plate in the chamber with small pins. Conventional microelectrode techniques were used to record the membrane potential of the smooth muscle cells of the distal colon. Briefly, glass capillary microelectrodes made from borosilicate glass tube (out diameter 1.2 mm with a
Colonic smooth muscle in OLETF rat

filament inside, Hilgenberg, Malsfeld, Germany) were filled with 3 M KCl. The tip resistance of these electrodes ranged between 50-80 M Ohm. The intracellular potential recorded was displayed on a cathode-ray oscilloscope (SS-7602, Iwatsu, Tokyo, Japan) and also on a chart (Recticorder, RJG-4204, Nihon Kohden, Tokyo, Japan). Transmural nerve stimulation (TNS) was achieved using a silver wire (0.5 mm diameter) coated with enamel, except at the tip, which was located so as to just touch the tissue, and the second electrode (silver plate) was placed in the bath. Electrical pulses, 0.05-0.1 ms duration and 10-80 V intensity, were obtained from a stimulator (MES-8101, Nihon Kohden, Tokyo, Japan).

The ionic composition of the Krebs solution was (in mM); Na⁺ 137.4, K⁺ 5.9, Mg²⁺ 1.2, Ca²⁺ 2.5, H₂PO₄⁻ 1.2, HCO₃⁻ 15.5, Cl⁻ 134, glucose 11.5. The solution was aerated with 95% O₂ containing 5% CO₂, and the pH of the solution was maintained in the range 7.3-7.4.

Drugs used were apamin, atropine sulfate, noradrenaline hydrochloride (NA), propranolol hydrochloride, tetrodotoxin (TTX), (all from Sigma, St. Louis, MO), guanethidine sulfate (Tokyo Kasei, Tokyo, Japan), phenolamine mesylate (Ciba-Geigy, Basel, Switzerland), charybdoxin (CTX) and N⁶-nitro-L-arginine (L-NNA), (Peptide institute, Osaka, Japan). The drugs were dissolved in distilled water at concentrations at least 1,000 times higher than used in the experiments and diluted in Krebs solution to obtain the desired concentration. These procedures did not alter the pH of the Krebs solutions.

Measured values were expressed as means ± standard deviation (S.D.) unless otherwise indicated. Statistical significance of the values was determined using paired and unpaired Student's t test. Probabilities of less than 5% (P<0.05) were considered significant.

Results

Physical condition of experimental animals

Table 1 summarizes the body weight, blood glucose level and distribution of feces in colon observed in nine LETO and twenty OLETF rats. Body weight of LETO rats was significantly heavier than that of OLETF rats. Blood glucose levels of OLETF rats were about twice of LETO rats. Distribution of feces observed at laparotomy was restricted within the proximal colon in LETO rats, and it was expanded widely from the cecum to the distal colon in OLETF rats.

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<th>Table 1 Physical parameters of the experimental animals.</th>
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<td>Body weight (g)</td>
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Mean±S.D. (n=9 for LETO and 20 for OLETF).

*P<0.05 vs LETO rats.

Location of feces was observed at laparotomy and the most distal position of feces was selected.
Fig. 1. Spontaneous activity of smooth muscle isolated from the distal colon.
Transient irregular hyperpolarization (A, B) and slow waves (C, D) were recorded from colonic smooth muscle of LETO and OLETF. The numbers shown in parentheses indicate positive rats versus total rats observed. Membrane potential: A, -43 mV; B, -39 mV; C, -42 mV; D, -41 mV.

Fig. 2. Relationship between membrane potential and blood glucose level.
The resting membrane potential was measured in the isolated smooth muscle of the distal colon, and the value was plotted as a function of blood glucose level (mg/dl). ○, LETO; ×, OLETF. The mean values of the resting membrane potential and the blood glucose level (with S.D.) were also shown in the figure (170.6 ± 38.5 mg/dl, -44.9 ± 1.6 mV, ○, LETO; 380.3 ± 87.5 mg/dl, -41.8 ± 2.5 mV, ■, OLETF). *P<0.05 vs LETO rats.

Spontaneous membrane activities
The resting membrane potential of the smooth muscles of the distal colon was -45±1.6 mV (n=8) in LETO rats and the value was significantly small in OLETF rats (-42±2.5 mV, n=18, P<0.05). The smooth muscle membranes were spontaneously active with generation of slow waves with occasional generation of spike potentials on top of the potential or transient
hyperpolarizations with irregular amplitude (up to 12 mV) and interval (mean interval, 1.5±1.5 s, n=50) (Fig. 1). In 3 out of 8 LETO rats, the amplitude and interval of slow waves were 17.4±17.0 mV (n=3) and 5.0±0.9 s, respectively, while in 5 out of 17 OLETF rats, similar amplitude and interval of slow waves were also observed (amplitude, 15.4±11.0 mV, n=6; interval, 5.9±0.7 s, n=6; P>0.1 for both). The membrane responses were the transient hyperpolarization in 5 out of 8 LETO rats and 12 out of 17 OLETF rats. The amplitude and interval of the transient hyperpolarization were irregular with up to 12 mV and 1-5 s respectively, and therefore the potential was not quantified. No significant difference was detected in the transient hyperpolarization between LETO and OLETF rats (Fig. 1).

Attempts were made to plot the membrane potential as a function of the blood glucose level, the objective being to determine any causal relationship between these two parameters. As shown in Fig. 2, although the mean value of the blood glucose level of OLETF rats (380±87 mg/dl, n=18) was significantly higher than that of LETO rats (171±39 mg/dl, n=8, P<0.05), the relationship of these two parameters estimated by the least square method was not positive (r=0.29).

Responses to noradrenaline

In colonic smooth muscle of the LETO and OLETF rats, noradrenaline (NA) hyperpolarized the membrane (Fig. 3, A). The threshold concentration of NA to hyperpolarize the membrane was 10⁻⁷ M for both LETO and OLETF rats, and the amplitude increased in a

![Figure 3](image_url)

Fig. 3. Noradrenaline(NA)-induced hyperpolarization in colonic smooth muscle
A: hyperpolarization produced by 10⁻⁷ M NA in LETO (a) and OLETF rats (b). NA was applied for 2 min (horizontal bar). Resting Membrane potential was -43 mV in (a) and -40 mV in (b). B: relationship between concentration of NA and amplitude of hyperpolarizations in colonic smooth muscle from LETO (○) and OLETF rats (■). Peak amplitude of hyperpolarization was measured. Mean±S.E.M. (n=6-12). *P<0.05 vs LETO rats.
concentration-dependent manner between $10^{-7}$ M and $10^{-5}$ M NA (Fig. 3, B). The net amplitudes of hyperpolarization produced by $10^{-6}$ M and $10^{-5}$ M NA were significantly smaller in OLETF rats than in LETO rats ($P<0.05$). The NA-induced hyperpolarization was inhibited by the $10^{-6}$ M phentolamine by $60.2 \pm 5.0\%$ ($n=5$) in LETO rats and $63.8 \pm 9.4\%$ ($n=3$) in OLETF rats; these values were not significantly different ($P>0.1$). In the presence of both $10^{-6}$ M phentolamine and $10^{-6}$ M propranolol, NA did not produce any detectable hyperpolarization ($n=3$). These results indicate that both phentolamine-sensitive $\alpha$-adrenoceptors and propranolol-sensitive $\beta$-adrenoceptors are involved in the NA-induced hyperpolarization. As the ratio of the inhibition of NA-induced hyperpolarization by phentolamine does not differ between LETO and OLETF rats, $\alpha$- and $\beta$-adrenoceptors may not be impaired in hyperglycemic OLETF rats.

In colonic smooth muscle of both the LETO and OLETF rats, acetylcholine (ACh), up to $10^{-5}$ M, did not alter the membrane potential ($n=3$, date not shown).

**Inhibitory junction potential**

In circular muscles from the distal colon, application of a brief current pulse transmurally evoked a transient hyperpolarizing response with 1.5-2.0 s duration. This response was considered an inhibitory junction potential (i.j.p.), since it was abolished reversibly by $10^{-7}$ M tetrodotoxin (TTX) (Fig. 5, A). The amplitude of the i.j.p. was a function of the intensity of stimuli (Fig. 4), and 20 V intensity stimuli were required to elicit the maximum i.j.p. amplitude of $21.0 \pm 3.3$ mV ($n=6$) in LETO rats and $15.8 \pm 4.4$ mV ($n=8$) in OLETF rats. The i.j.p. amplitudes were smaller in OLETF rats compared to LETO rats at 5, 10 and 20 V intensity stimuli ($P<0.05$).

![Fig. 4. Relationship between the amplitude of i.j.p. and intensity of stimuli in smooth muscle of the colon. Peak amplitudes of the i.j.p. evoked by single stimuli were recorded from the distal colon of both LETO (○) and OLETF (■) rats, and were expressed by the mean ± S.E.M. ($n=6-8$). *$P<0.05$ vs LETO rats.](image-url)
Fig. 5. Effects of inhibitors on the i.j.p. in colonic smooth muscle from LETO rats
A. The i.j.p. was evoked by transmural nerve stimulation (single pulse, 0.1 msec duration, 5-20 V intensity) in muscles from the distal colon of LETO rats, in the absence (Aa) and presence of atropine (10^-6 M) plus guanethidine (5x10^-6 M) for 20 min (Ab) and with the addition of TTX (10^-6 M) for 10 min (Ac). B. The i.j.p. evoked in the presence of atropine (10^-6 M) plus guanethidine (5x10^-6 M) (Ba), additional application of L-NNA (10^-4 M) for 20 min (Bb) and apamin (10^-7 M) for 10 min (Bc). C. The i.j.p. evoked in the presence of atropine (10^-6 M) plus guanethidine (5x10^-6 M) (Ca), additional application of charybdotoxin (10^-7 M) for 10 min (Cb) and apamin (10^-7 M) for 10 min (Cc). The membrane potential: A, -45 mV; B, -48 mV; C, -43 mV.

The effects of some drugs were observed on the i.j.p. evoked by supramaximal intensity of stimuli (equal to 20 V). The i.j.p. was not altered by the application of 10^-6 M atropine and 5x10^-6 M guanethidine, indicating that the potential was non-adrenergic, non-cholinergic (NANC) in nature (Fig. 5, A). The NANC i.j.p. (24.0±2.4 mV, n=14) was not altered by inhibiting nitric oxide synthase with 10^-4 M L-NNA (22.6±1.8 mV, n=14), but additional application of apamin (10^-7 M) reduced the amplitude of i.j.p. to 12.9±0.8 mV, (n=3, P<0.05) (Fig. 5, B). Charybdotoxin (CTX, 10^-7 M) did not significantly alter the i.j.p. (control, 23.4±0.8 mV, n=3; in CTX, 23.4±1.3 mV, n=3; P>0.1), but combined application of apamin and CTX significantly reduced the amplitude of i.j.p. (7.1±0.8 mV, n=3; P<0.05) (Fig. 5, C).

The latency of the NANC i.j.p. evoked by supramaximal intensity of stimuli was 133±9.3 ms (n=5) in LETO rats, and the value was significantly longer in OLETF rats (145±4.2 ms, n=5; P<0.05).

Discussion

The present studies were designed to evaluate the alteration of the electrical properties and autonomic neural transmission in smooth muscle of the distal colon during development of diabetes mellitus using OLETF rats, model animals for spontaneous NIDDM. Constipation
accompanying by the diabetes mellitus relates to the disorder of colonic motility, which appears in the dilated tracts and delayed movements of fecal contents in the middle and distal colon toward the anus, possibly because of reduced peristaltic movements (Christensen, 1989). The present experiments revealed that the distribution of feces is wide in OLETF rats, instead of restricted distribution in the proximal colon in LETO rats, the observations may reflect the disorder of peristaltic movements in OLETF rats. Thus, disorder of peristalsis in the colon seems to be induced both in IDDM and NIDDM model animals.

Gastrointestinal dysfunction appearing in diabetic patients is causally related to autonomic neuropathy (Battle et al., 1980; Schmidt et al., 1981; Lincoln et al., 1984; Belai et al., 1987; Belai et al., 1991). However, an involvement of any alteration of the properties in gastrointestinal smooth muscles remains unclear. Although distinctive morphologic abnormality in the intestinal smooth muscle of diabetic patients has been noted (Duchen et al., 1980), the absence of morphological alteration in the gastric smooth muscle of patients with diabetic gastroparesis is also reported (Yoshida et al., 1988). In our study, alterations of the membrane properties of colonic smooth muscle were observed: the depolarization of resting membrane potential and reduction of the sensitivity to NA. In the STZ-induced IDDM rats, the reduction of Na⁺-K⁺ ATPase activity is found in the small intestine (Fedorak et al., 1991), bladder (Hashitani et al., 1996) and stomach (Xue et al., 1997). Although we did not measure the activity of Na⁺-K⁺ ATPase in the distal colon of OLETF rats, it is assumed that the long term hyperglycemia would cause the difference in contribution of Na⁺-K⁺ ATPase activity to the resting membrane potential.

Alteration of the response to NA in intestinal smooth muscle during the development of diabetes mellitus is equivocal, and in STZ-induced IDDM rats the NA-induced hyperpolarization is increased in the cecum (Hoyle et al., 1987) or reduced in the stomach (Xue et al., 1997). In smooth muscle of the rat cecum, the NA-induced hyperpolarization is produced through an activation of α-adrenoceptor alone and the increased response to exogenously applied NA is considered due to an increase in both the sensitivity and efficiency of NA to α-adrenoceptors (Hoyle et al., 1987). The present study found that in smooth muscle of the distal colon of LETO and OLETF rats, the NA-induced hyperpolarization is produced by stimulation of both α- and β-adrenoceptors. Selective impairment of β-adrenoceptors is induced in the gastric smooth muscle of the STZ-induced IDDM rats (Sakai et al., 1991). However, the ratio of α- and β-adrenoceptors contributing to the generation of NA-induced hyperpolarization remained unaltered in OLETF rats, suggesting that both α- and β-adrenoceptors were equally impaired. It remains unclear whether these differences are causally related to the types of diabetes mellitus (experimental or spontaneous, or IDDM or NIDDM), the time course of developing diabetes mellitus (acute or chronic), or the region in the digestive tract (distal colon, ileum or stomach).

Smooth muscles of the colon were spontaneously active with slow waves or transient hyperpolarization, both in OLETF and LETO rats, indicating that NIDDM did not significantly impair excitability and pace making mechanisms. This is in good contrast with the impaired spontaneous activity in gastric smooth muscle of OLETF (Takano et al., 1998) and STZ-induced rats (Xue et al., 1997). Although the reason for these differences remains unclear, it
is speculated that the response of smooth muscle to hyperglycemia may differ between the stomach and the colon. However, the distribution of feces in the colon (Table 1) clearly indicates significant disorders in the peristaltic movement of the colon in OLETF rats. Possible contribution of middle or proximal colon in addition to distal colon has to be considered involved in the transport of feces in the colon.

Two types of spontaneous activity, slow wave and transient hyperpolarization, may be generated by different mechanisms; the slow wave may be produced by activation of cation channels as a result of electrical propagation of potentials generated in pacemaking cells such as interstitial cell of Cajal (Sanders, 1996) or activation of Cl⁻ channels activated by spontaneous release of Ca²⁺ in smooth muscle (Van Helden, 1993). The transient hyperpolarization may be generated by the activation of Ca²⁺-sensitive K⁺ channels as the result of intracellular calcium increase, since the transient hyperpolarization is attenuated by apamin, a small conductance Ca²⁺-activated K⁺ channel inhibitor (Romey et al., 1984) or CTX, a large conductance Ca²⁺-activated K⁺ channel inhibitor (Nelson et al., 1990).

Transmitter substances involved in the generation of the NANC i.j.p. remain unclear, and ATP (Crist et al., 1992; Zagorodnyuk et al., 1996; Ohno et al., 1993), vasoactive intestinal polypeptide (VIP) (Crist et al., 1992; He XD et al., 1993), pituitary adenylate cyclase activating peptide (PACAP) (McConalogue et al., 1995; Zagorodnyuk et al., 1996) or NO (Conklin et al., 1993; Thornbury et al., 1991) is considered involved. The NANC i.j.p. observed in the rat colon is not attenuated by L-NNa, an inhibitor of NO synthase, indicating this potential is not produced by NO, unlike the smooth muscle of small intestine of the dog (Sanders, 1996) or the rat stomach (Xue et al., 1996). The i.j.p. may be produced by the activation of Ca²⁺-sensitive K⁺ channels, due to partial inhibition by apamin or significant inhibition by combined application of apamin and CTX. Interestingly, CTX alone did not inhibit the NANC i.j.p. in the rat colon, suggesting that these two types of K⁺-channel are required to inhibit simultaneously to prevent the junction potential. The amplitude of the NANC i.j.p. was smaller in OLETF rats in comparison with that in LETO rats, suggesting that the neuromuscular transmission is impaired by NIDDM. In experimental IDDM rats prepared by injection of STZ, dysfunction of peristalsis in the colon is related to neuropathy (Schmidt et al., 1981). These results suggest a development of neuropathy in OLETF rats. The increase in the latency of the NANC i.j.p. also supports this concept. However, immunohistochemical study indicates that there is no evidence of degenerative change in adrenergic and peptidergic nerves in the myenteric plexus of the distal colon in STZ-induced IDDM rats (Belai et al., 1991). It is speculated that in diabetes mellitus, the functional alteration appears before detection of structural changes.

It is concluded that the depolarization of the membrane, the attenuation of NANC inhibitory neurotransmission and the reduced responses to adrenergic receptors stimulation with NA observed in smooth muscle of the distal colon of OLETF rats may be causally related to NIDDM. Constipation appearing with diabetes mellitus may involve a dysfunction of both smooth muscle and enteric autonomic nerves.
Acknowledgments

The OLETF and LETO rats were supplied by the Otsuka Pharmaceutical Co. The authors are grateful to Prof. K. Tanigawa and Dr. M. Shimizu for their helpful suggestion about the OLETF rats. Prof. M. Itoh encouraged these experiments throughout.

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(Received June 25, 1998; Accepted July 27, 1998)