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Streptozotocin-induced diabetes modulates presynaptic and postsynaptic function in the rat ileum

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Abstract

Altered gastrointestinal motility frequently occurs in diabetic patients and also in animal models of diabetes but the underlying causes are not clear. In the present study, contractile responses to agonists and electrical field stimulation (EFS) and the inhibitory actions of an adenosine A_1 receptor agonist were investigated on ilea from 8-week streptozotocin (STZ)-induced diabetic rats. Contractile responses to carbachol, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), the calcium ionophore A23187 and to EFS were increased in diabetic tissues compared to controls. In contrast, the inhibitory effects of a potent and selective adenosine A_1 receptor agonist N^6-cyclopentyladenosine (CPA) on electrical field stimulation-evoked contractions were decreased in diabetic tissues compared to controls but its ability to relax carbachol-contracted tissues was unaltered. These results suggest that diabetes may cause alterations at both pre- and postsynaptic sites and this may lead in turn to the gastrointestinal complications seen in diabetic patients.

Keywords: Streptozotocin-induced diabetes; Carbachol; Prostaglandin; Ca^{2+} ionophore A23187; Adenosine A_1 receptor

1. Introduction

Gastrointestinal motility disorders are common in patients with diabetes mellitus with up to 76% of patients suffering some problems (Mathison and Davison, 1988). Conflicting reports of alterations in gastrointestinal function have been published both in patients and in animal models of diabetes. Rapid gastrointestinal transit through the ileum and reduced intestinal tone were found in patients suffering from diabetic diarrhoea (Malins and French, 1957; McNally et al., 1969), while delayed transit through the ileum (Scarpello et al., 1976) or no change in the intestinal tone (Keshvazian and Iber, 1986) has been reported in patients with diabetic constipation. A number of studies have been performed on tissues from diabetic animals to investigate the mechanisms underlying these changes but again conflicting results have been obtained. At the level of the smooth muscle, increased responsiveness to the muscarinic agonist carbachol has been reported in ilea from streptozotocin-treated rats (Carrier and Aronstam, 1990) with similar results in the fundus of the stomach (Aihara and Sakai, 1989; Pinna et al., 1995) and antral smooth muscle (Maruyama et al., 1999). In contrast, decreased contractile responses to acetylcholine have been demonstrated in tissues from STZ-treated rats such as ileal longitudinal muscles (Lucas and Sardar, 1991), gastric fundal strips (Korolkiewicz et al., 1998) and gastric antral smooth muscle cells (Soulie et al., 1992). Decreased contractile responses to carbachol have also been found on the gastric circular smooth muscle taken from spontaneously diabetic BB/W rats (Takahashi et al., 1996).

At the neuronal level, Nowak et al. (1986) found defective cholinergic neurotransmission in the myenteric plexus of the distal small intestine of streptozotocin-induced diabetic rats. Decreased responses of terminal ileum longitudinal muscle taken from streptozotocin-treated rats to transmural nerve stimulation have also been reported (Lucas and Sardar, 1991). Very recently, Coulson et al. (2002) reported that presynaptic inhibitory muscarinic receptors on parasympathetic nerves were hyperfunctional in diabetic rats.

The majority of studies examining the responsiveness of intestinal tissues from diabetic animals have focussed on the

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cholinergic system, although Altan et al. (1987) found changes in β-adrenoceptor and 5-HT receptor activity in diabetic rat duodenum and ileum.

The aims of the present study were to extend the observations on altered responsiveness of diabetic rat ileal tissues using a range of contractile agonists and electrical field stimulation (EFS). It was hoped that this would help to identify the level at which any alterations occurred, i.e. neuronal, smooth muscle or pre- and post-synaptic receptors. To address this last level of control, studies were carried using an adenosine A_1 receptor agonist. Adenosine has been reported to have a number of effects on gut motility. Activation of presynaptic adenosine A_1 receptors has been demonstrated to inhibit electrically evoked acetylcholine release in the guinea-pig ileum (Lee et al., 2001) and in enteric neurons of the guinea-pig distal colon (Nitahara et al., 1995). Adenosine A_1 receptors located on post-synaptic sites have also been reported, activation of which causes relaxation of the rat ileum (Nicholls and Hourani, 1997).

Thus, the overall aim was to study the effect of diabetes on pre- and post-synaptic function in the rat ileum.

2. Materials and methods

Male Wistar rats weighing 200–350 g were used in the present study. Animals were assigned into one of two groups; one was the diabetic group and the other was age-matched controls. Housing conditions and all experimental work were conducted in accordance with the Animals (Scientific Procedures) Act 1986 under Project Licence Number PPL 70 4649 with a Project Title of Gastrointestinal Research.

2.1. Induction of diabetes and control animals

To induce diabetes, rats received a single intra-peritoneal injection of streptozotocin (65 mg kg^{-1}; 1 ml 100 g^{-1} body weight), freshly dissolved in 20 mM citrate buffer solution at pH 4.5. Age-matched control rats were injected with equal volume of citrate buffer vehicle alone. Diabetes was verified by an increase in the non-fasting blood glucose levels of at, or above, 200 mg/dl, which were measured by puncturing the vein at the tip of the tail with a needle. The blood glucose levels were determined 8 weeks after STZ administration using BM-test 1–44 blood glucose test strips with Reflolux Blood Glucose Meter (Boehringer Mannheim, UK or Roche Diagnostics). The rats with blood glucose levels of less than 200 mg/dl were rejected. To prevent the initial hypoglycaemia phase, 2% sucrose was added to the drinking water for the first 48 h after the injection of streptozotocin.

2.2. Tissue preparation

Eight-week STZ-treated rats and age-matched controls were killed by carbon dioxide (CO_2) asphyxiation after measuring the blood glucose levels. The abdominal cavity was opened via a midline incision. A 20-cm section of the distal ileum just proximal to the ileocaecal valve was immediately removed and kept in Krebs solution gassed with 95% O_2–5% CO_2.

Segments of distal ileum, approximately 2 cm in length, were isolated free from the surrounding mesentery. After washing out the gut contents with Krebs solution, the ileum was mounted vertically between paralleled platinum electrodes, and suspended with one end attached to the base of a stimulating electrode and the other end attached by a silk thread to a force displacement transducer. The transducer (Dynamometer UF1) was connected to a recorder via a preamplifier (Lectromed 3552) to record changes in isometric tension and recorded on a Lectromed 5041 recorder. The ileum preparations were mounted in 30 ml organ baths containing a Krebs solution of the following composition (in mM): NaCl 118.3, KCl 4.7, $MgSO_4$ 1.2, K_2HPO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, and glucose 11.1. The solution was maintained at 37 °C and gassed with 95% O_2–5% CO_2. The preparations were allowed to equilibrate under the resting tension of 1 g for at least 1 h before the experiments were commenced.

2.3. Experimental design

2.3.1. Contractile responses to electrical field stimulation

To study the contractile response of the ileum to electrical field stimulation, a train of pulse (0.25–5.00 ms, 30 V, 10 Hz for 10-s and 5-min interval) was applied via parallel platinum electrodes using a Harvard Grass S11 stimulator.

2.3.2. Contractile responses to carbachol, prostaglandin $F_{2\alpha}$ and calcium ionophore A23187

The contractile responses of the ileum to carbachol, prostaglandin $F_{2\alpha}$ and calcium ionophore A23187 (10^{-9} to 10^{-5} M) were studied by cumulatively adding the individual contractile agents to the organ baths.

2.3.3. Inhibitory effect of N^6-cyclopentyladenosine

To study the inhibitory effect of the selective adenosine A_1 receptor agonist, N^6-cyclopentyladenosine on electrically evoked contractions of rat ileum, stimuli of (30 V, 10 Hz, 1 ms for 10-s and 5-min interval) were used. Once a stable contractile response to electrical field stimulation was established, increasing concentrations of N^6-cyclopentyladenosine (10^{-9} to 5×10^{-5} M) were added to produce a cumulative dose–response curve. Each addition of N^6-cyclopentyladenosine was performed after a plateau of inhibition to the previous concentration was established.

2.3.4. Relaxant responses to N^6-cyclopentyladenosine

To establish whether diabetes had any effect on the responsiveness of postsynaptic adenosine A_1 receptors, N^6-

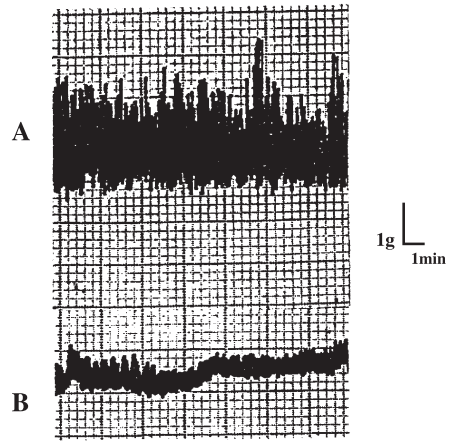

Fig. 1. Original trace illustrating the spontaneous activity of ileum from (A) streptozotocin-treated and (B) control rats.

cyclopentyladenosine at a concentration of 10^{-6} M was added to the organ bath to induce relaxant responses in ilea precontracted with carbachol (10^{-6} M).

2.4. Drugs

Atropine sulphate, carbamylcholine chloride (carbachol), N^6-cyclopentyladenosine (CPA), *N*-methylnitrocarbamyl-D-glucosamine (streptozotocin; STZ), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), and tetrodotoxin (TTX) were obtained from Sigma, UK. Calcium ionophore A23187 was purchased from Tocris, UK.

2.5. Analysis of results

The contractile responses of ileum to carbachol, prostaglandin $F_{2\alpha}$, calcium ionophore A23187 were expressed as contraction in g/g tissue wet weight using the plateau height of contraction at each concentration of the agonist. For electrical field stimulation, the plateau height of contraction at each pulse width was used. The development of tension was that measured above baseline. The inhibitory effects of N^6-cyclopentyladenosine was expressed as a percentage inhibition of the electrical field stimulation-evoked contraction, calculated by comparing the size of contraction immediately before adding N^6-cyclopentyladenosine with the size of contraction at plateau height of inhibition. Spontaneous activity of the tissues was expressed as the magnitude of contraction in grams during equilibration of the tissues. The tissues were weighed after each experiment was finished.

Results are shown as means ± S.E.M., where *n* indicates the number of animals used. Differences between means were determined using Student's *t*-test for unpaired data. Probability levels of less than 0.05 ($P < 0.05$) were taken to indicate statistical significance.

3. Results

3.1. Glucose levels

The mean blood glucose levels were significantly ($P < 0.05$) elevated in streptozotocin-induced diabetic rats

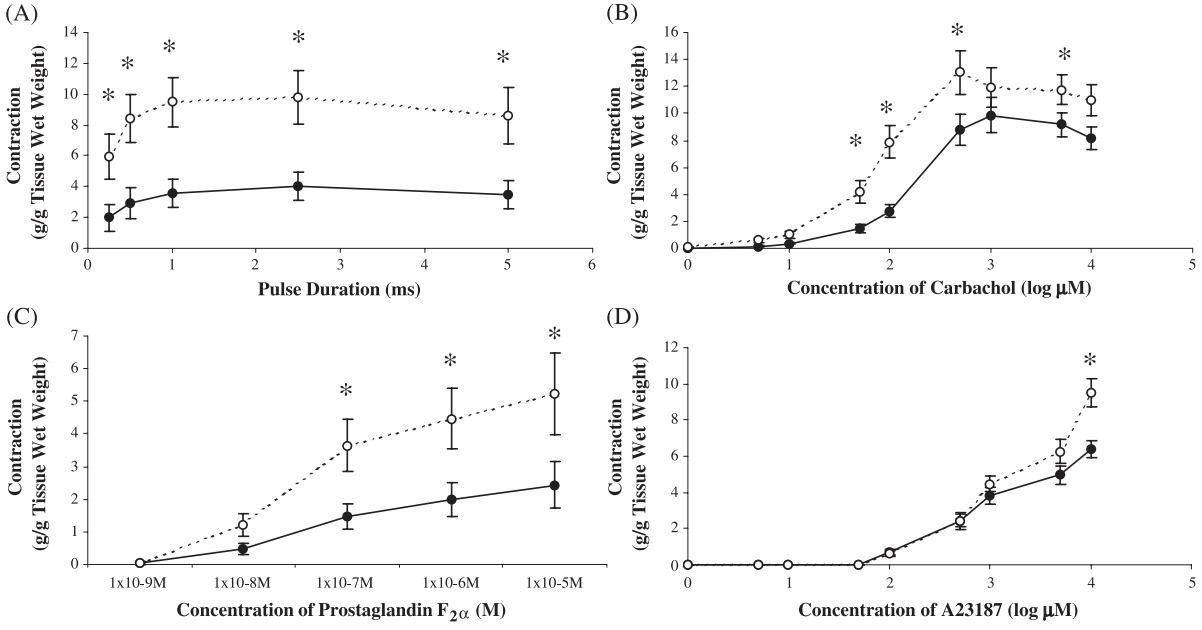

Fig. 2. Contractile responses of the ileum from streptozotocin-treated and control rats to (A) electrical field stimulation ($n = 7$), (B) carbachol ($n = 10$), (C) prostaglandin $F_{2\alpha}$ ($n = 6$), and (D) A23187 ($n = 7$). Values represent the mean ± S.E.M. *$P < 0.05$ is significantly different from control rats (Student's *t*-test for unpaired observations).

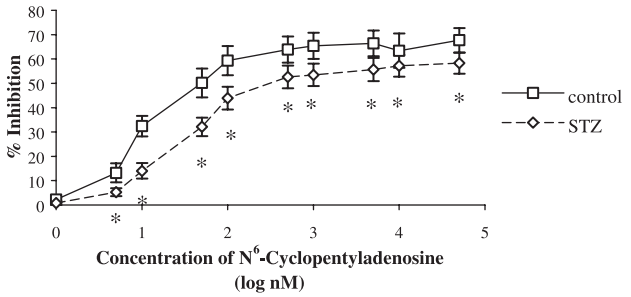

Fig. 3. The inhibitory effect of N^6-cyclopentyladenosine on electrical field stimulation evoked contraction of the ileum from streptozotocin-treated and control rats. Values represent the mean ± S.E.M. ($n=7$). *$P<0.05$ is significantly different from control rats (Student's *t*-test for unpaired observations).

(396 ± 16.17 mg/dl, $n=30$) compared to controls (92.57 ± 5.43 mg/dl, $n=30$). The streptozotocin-induced diabetic rats also showed other symptoms of diabetes such as polydipsia, polyuria and distension of the stomach and intestine.

3.2. Spontaneous activity

The ileum taken both from STZ-treated and control rats showed spontaneous activity, which was atropine (10^{-6} M) and tetrodotoxin (10^{-6} M) insensitive, but abolished by nifedipine (10^{-5} M). The spontaneous activity was significantly ($P<0.05$) greater in tissues from diabetic animals (1.53 ± 0.15 g/g, $n=30$) than controls (0.54 ± 0.05 g/g, $n=30$) (Fig. 1).

3.3. Contractile responses to electrical field stimulation

Electrical field stimulation over the pulse range 0.25–5.0 ms produced a shallow contractile response curve, which reached a maximum at 2.5 ms. At all pulse durations, the responses to electrical field stimulation were abolished by atropine (10^{-6} M) and were significantly ($P<0.05$, $n=7$) greater in the tissues taken from the streptozotocin-treated animals than those from controls.

3.4. Contractile responses to carbachol

Carbachol (10^{-9}–10^{-5} M) produced concentration-related contractile responses of the ileum. At concentrations of 5×10^{-8} to 5×10^{-7} M, the responses curve in tissues taken from streptozotocin-treated animals was significantly ($P<0.05$, $n=10$) greater than in control tissues (Fig. 2B).

3.5. Contractile responses to prostaglandin $F_{2\alpha}$

Prostaglandin $F_{2\alpha}$ (10^{-9}–10^{-5} M) produced concentration-related contractile responses of the ileum, which were greater in tissues from streptozotocin-treated animals than controls at concentrations of 10^{-7} M and above ($P<0.05$, $n=6$, Fig. 2C).

3.6. Contractile responses to Ca^{2+} ionophore A23187

The calcium ionophore A23187 (5×10^{-8}–10^{-5} M) produced concentration-related contractile responses of the ileum. In the tissues taken from streptozotocin-treated animals, the responses to concentrations of 10^{-4} and 10^{-5} M were greater than in control tissues, but this only reached statistical significance ($P<0.05$, $n=7$) at the highest concentration used (10^{-5} M, Fig. 2D). In neither set of tissues could the maximum response be established because of limited solubility of the ionophore.

3.7. Inhibitory effect of N^6-cyclopentyladenosine

N^6-cyclopentyladenosine in a concentration range of 10^{-9}–5×10^{-5} M inhibited the electrical field stimulation-evoked contractions of the ileum. The inhibition was significantly ($P<0.05$, $n=7$) less in tissues from streptozotocin-treated rats than that observed in controls over the whole concentration range (Fig. 3). The EC_{50} values were 24.0 ± 6.3 nM for control tissues and 58.5 ± 13.5 nM for the streptozotocin-treated tissues. Overall, the electrical field stimulation-evoked contraction of streptozotocin-treated ileum was greater than in controls and this may have influenced the inhibitory effect of N^6-cyclopentyladenosine. However, in a separate selected subgroup of experiments where the electrical field stimulation-evoked contractions from both control and diabetic tissues were not significantly different, the inhibitory effect of N^6-cyclopentyladenosine was still significantly less ($P<0.05$, $n=3$) in tissues taken from streptozotocin-treated rats than in controls.

3.8. Relaxant responses to N^6-cyclopentyladenosine

N^6-cyclopentyladenosine at a concentration of 10^{-6} M induced a relaxant response of the carbachol-contracted ileum, which was not significantly ($n=7$) different between tissues from streptozotocin-treated rats and controls (Fig. 4).

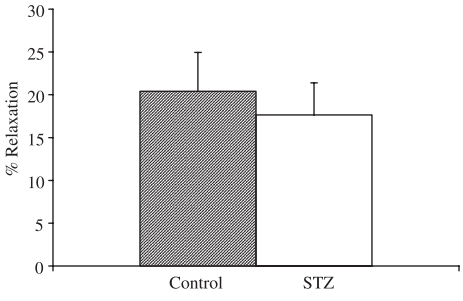

Fig. 4. Relaxant responses of carbachol-contracted ileum from streptozotocin-treated and control rats to N^6-cyclopentyladenosine (10^{-6} M). Values represent the mean ± S.E.M. ($n=7$).

4. Discussion

In the present study, rats were rendered diabetic by a single injection of streptozotocin. Eight weeks after injection, diabetes was confirmed in each rat by a significant elevation of glucose in whole blood.

Ileal tissues from both control and streptozotocin-treated rats showed spontaneous contractile activity, but the amplitude of this activity was significantly greater in tissues from the latter group. The spontaneous activity was totally refractory to inhibition by atropine and tetrodotoxin indicating that it was non-cholinergic and non-neuronal in origin, but it was abolished by the L-type Ca^{2+} channel blocker nifedipine. Spontaneous activity in the gut is controlled by a combination of intrinsic neurons, the interstitial cells of Cajal (ICCs) and the smooth muscle itself (Huizinga, 1999). Therefore, the increase in spontaneous activity found in the diabetic tissues is possibly due to an increased activity of the interstitial cells of Cajal or, more likely, increased myogenic activity.

There have been conflicting reports on the level of spontaneous gastrointestinal activity in diabetic tissues. Maruyama et al. (1999) found that the rhythmic spontaneous contraction of the gastric antral smooth muscle from streptozotocin-induced diabetic rats was increased compared to controls. In contrast, the spontaneous activity (expressed as resting tension) of streptozotocin-treated rat gastric fundal strips was less than in controls (Aihara and Sakai, 1989; Xue and Suzuki, 1997). Using a different model of diabetes, Takano et al. (1998) found that the basal activity of gastric antral smooth muscle from spontaneously diabetic Otsuka Long Evans Tokushima Fatty (OLETF) rats (diabetic Type II) was reduced and more irregular compared to Long Evans Tokushima Otsuka (LETO) control tissues. Finally, Imaeda et al. (1998) reported that there was no difference in the level of spontaneous activity in colonic tissues from Otsuka Long Evans Tokushima Fatty rats compared to controls. Clearly, the choice of model and the tissue studied influences the results obtained and the present study is the first to describe increased spontaneous activity in the ileum from diabetic animals.

In the present study, contractile responses to electrical field stimulation were larger in the ileum from streptozotocin-treated animals then controls. In contrast, Nowak et al. (1986), Lucas and Sardar (1991) and Coulson et al. (2002) all found impaired contractile responses to electrical field stimulation in the rat distal small intestine from streptozotocin-treated animals. The most likely explanation for these conflicting data is the duration of diabetes before the tissues were taken which was 7 days in the Coulson et al. study, 30 days in the Nowak et al. and Lucas and Sardar studies, whereas 8 weeks (56 days) were used in the present study. Another possible explanation is that different stimulus parameters were used in the individual studies. However, the fact that in all the studies, the contractile responses were virtually abolished by atropine, indicating a cholinergic origin, renders this unlikely. Since they are cholinergically mediated, the increased size of the electrical field stimulation-evoked contractions in diabetic tissues could be due to a number of reasons, i.e. increased acetylcholine availability (due to increased synthesis or reduced metabolism), increased acetylcholine release or changes at the postsynaptic level in term of tissue responsiveness. The last appears the most likely since the responses to exogenously applied carbachol were greater in streptozotocin tissues than controls.

Increased responsiveness and sensitivity of diabetic tissues to cholinergic stimulation have been reported previously. Carrier and Aronstam (1990) found an increased responsiveness of streptozotocin diabetic rat ileum longitudinal muscle to acetylcholine, carbachol and bethanechol. Similarly, increased sensitivity to cholinergic agonists have been reported in gastric tissues taken from streptozotocin-treated rats (Aihara and Sakai, 1989; Kamata et al., 1988; Maruyama et al., 1999). However, reduced sensitivity of diabetic gastrointestinal tissues to cholinergic stimulation has also been reported (Korolkiewicz et al., 1998; Lucas and Sardar, 1991; Soulie et al., 1992). The reasons for the differences between the various studies are not clear but may relate to the duration and severity of the diabetic state. The results of the present study suggest that the increased contractile responses of tissues taken from streptozotocin-treated rats to electrical field stimulation may be due to a combination of increased tissue sensitivity to acetylcholine and increased acetylcholine release since the differences between control and treated tissue responses are greater for electrical field stimulation than exogenously applied carbachol.

The increase in tissue sensitivity to cholinergic agonists could reside at the muscarinic receptor level but this seems unlikely, as there are also increased responses to prostaglandin $F_{2\alpha}$ and the Ca^{2+} ionophore A23187. The former result indicates that the increases are not restricted to the muscarinic receptor and the latter suggests that, in part at least, the alterations are occurring beyond the receptor level. Interestingly, Aihara and Sakai (1989) found increased responsiveness of gastric tissues from streptozotocin-treated rats to another non-receptor-mediated contractile agonist, KCl. From studies using radiolabelled Ca^{2+}, the authors concluded that the increased responses to acetylcholine and KCl were a consequence of increased Ca^{2+} influx and this may also underlie the increase in responsiveness to the Ca^{2+} ionophore, which causes transmembrane influx of Ca^{2+} reported here.

The inhibitory effects of the adenosine A_1 receptor agonist N^6-cyclopentyladenosine on electrically evoked contraction of the ileum were decreased in tissues from streptozotocin-treated rats compared to controls. This action of N^6-cyclopentyladenosine is known to be the result of activation of presynaptic adenosine A_1 receptors leading to a reduction of acetylcholine release and hence a reduction in the size of the contraction to electrical field stimulation (Lee et al.,

2001). Thus, it appears likely that the diabetic state is causing impairment at the level of the presynaptic adenosine A_1 receptor, which could involve a reduction in receptor number or alteration in agonist affinity or efficacy. Evidence for alteration in adenosine A_1 receptors in gastrointestinal tissues from diabetic animals has not been previously reported. However, a study on adipocyte cells from streptozotocin-treated rats found that the density of adenosine A_1 receptors was not altered (Green and Johnson, 1991). Alteration in several other receptor types has been reported in tissues from diabetic animals. For example, a decreased muscarinic receptor content of ileal smooth muscle from diabetic rats has been described (Carrier and Aronstam, 1990). Conversely, increased function of inhibitory presynaptic neuronal muscarinic M_2 receptors has recently been reported in the ileum of streptozotocin-treated rats (Coulson et al., 2002).

Postsynaptic adenosine A_1 receptors mediating relaxant responses of the rat ileum have been reported (Nicholls and Hourani, 1997) and N^6-cyclopentyladenosine caused relaxations of carbachol-contracted ileum in the present study. However, there was no difference between the size of relaxation induced by N^6-cyclopentyladenosine between the control and treated tissues making it likely that the effects on streptozotocin-evoked contractions are mediated via presynaptic receptors.

Clearly 8-week streptozotocin-induced diabetes leads to a number of significant alterations in the functional responses of the rat ileum. Further studies such as radioligand binding and transmitter release will be needed to establish the mechanisms underlying these changes, but they may be relevant to the gastrointestinal complications seen in diabetic patients.

Acknowledgements

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