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Inhibitory effect of streptozotocin-induced diabetes on non-cholinergic motor transmission in rat detrusor and its prevention by sorbinil

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1 Non-cholinergic motor transmission in the urinary bladder of streptozotocin (STZ)-diabetic rats was studied by recording contractile activity of strips of detrusor *in vitro*.

2 The neurogenic contractile responses to electrical field stimulation (EFS) of atropine-treated detrusor strips were decreased in 4, 8 and 12 week STZ-diabetic rats. The decrease was most marked in 12 week diabetic rats and least in 4 week ones.

3 Concentration-response curves showed no change in sensitivity of the detrusor to acetylcholine (ACh) in diabetic rats. The maximum tension generated by ACh was similar in diabetic and non-diabetic animals.

4 The contractile responses to EFS at frequencies $\geqslant 1$ Hz were not maintained during stimulation. The 'fade' was significantly greater in detrusor strips of diabetic rats.

5 The contractile response of detrusor to EFS was significantly greater in 12 week diabetic rats treated with the aldose reductase inhibitor sorbinil, than in untreated 12 week diabetic rats. The sensitivity to ACh was similar in the two groups.

6 It is concluded that the reduction of the neurogenic non-cholinergic responses of detrusor to EFS in STZ-diabetic rats is probably caused by a reduction in the release of the non-cholinergic motor transmitter. The results are discussed in relation to bladder dysfunction in human diabetes mellitus.

Introduction

The incidence of bladder dysfunction in diabetic patients is reported to be as high as 87% (Faerman *et al.*, 1971). There is a widely held view that the bladder dysfunction originates as a result of diabetic autonomic neuropathy (Buck *et al.*, 1974; Ellenberg, 1980). Although several studies on different aspects of bladder dysfunction have been reported in the streptozotocin (STZ)-induced diabetic rat, an animal model of human diabetes (Lincoln *et al.*, 1984; Longhurst & Belis, 1986; Moss *et al.*, 1987; Kolta *et al.*, 1985; Santicioli *et al.*, 1987), the effect of diabetes on the non-cholinergic motor transmission to the detrusor has remained unclear. The results from some of the studies lead to divergent conclusions regarding the effects of diabetes on the non-cholinergic motor transmission to the detrusor. Thus the findings of Moss *et al.* (1987) have indicated no change in the overall response to non-cholinergic motor nerve stimulation, even though postjunctional sensitivity to a stable purinoceptor agonist ranged from high in 8 week diabetic animals to low in 16 week diabetic animals. Using non-atropinised strip preparations from STZ-diabetic rats, Longhurst & Belis (1986) have reported a drastic reduction in neurogenic responses without any significant alteration in adenosine 5'-triphosphate (ATP) sensitivity. Lincoln *et al.* (1984) found no impairment in the ability of the detrusor muscle to respond to cholinergic nerve stimulation. The results of the latter two investigations, taken together, imply that the reduction in the motor transmission, observed by Longhurst & Belis (1986), was ascribable to the non-cholinergic component of the transmission.

In the present study, isolated strips of rat detrusor have been contracted by acetylcholine (myogenic) and by electrical field stimulation (neurogenic) the latter being performed in the presence of atropine. These myogenic and non-cholinergic neurogenic contractions have been compared in non-diabetic controls and STZ-diabetic rats, in order to determine the effect of STZ-diabetes on the non-cholinergic motor transmission and detrusor contractility.

The accumulation of excess sorbitol within peripheral nerves as a direct consequence of diabetes has been advanced as one of the principal causes of neuropathy (Green *et al.*, 1985) and has been described in human (Mayhew *et al.*, 1983) and experimental (Clements, 1979; Mayer & Tomlinson 1983) diabetes. In the present investigation we have therefore also examined the effect of inhibition of sorbitol formation by an aldose reductase inhibitor, sorbinil, on the damaging effect of STZ-induced diabetes upon non-cholinergic motor transmission in this organ.

Methods

Animals

All experiments were performed on young adult male Wistar rats, 8 weeks old, weighing 180–200 g at the start of the experiment. The animals were randomly allocated to different experimental groups each consisting of 6 animals. The animals were individually weighed and the weight recorded.

Induction of diabetes

The rats to be made diabetic were fasted overnight and their blood glucose levels estimated. Diabetes was induced by administration of a single intraperitoneal injection of streptozotocin (STZ), 75 mg kg^{-1}, to each rat. Three days after the STZ treatment, the animals were fasted for 2 h before obtaining another blood sample from each animal in order to estimate the blood glucose level. This was to ensure that diabetes had been induced. Animals with blood glucose levels $\geqslant 300$ mg 100 ml^{-1} were deemed to be diabetic and therefore suitable for the study. The blood glucose levels of the diabetic rats were thereafter monitored at regular intervals. Age- and weight-matched controls of every diabetic group were treated

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in an identical fashion except that saline was injected instead of STZ.

Experimental organisation

The diabetic animals were divided into four groups of 6 rats each. The first three groups were kept for 4, 8 and 12 weeks respectively after the induction of diabetes. The fourth group of 6 diabetic animals was maintained for 12 weeks while receiving treatment with sorbinil (see below). Each of the aforementioned groups except the last one, had a corresponding group of age- and weight-matched controls which were treated in an identical fashion. The group of diabetic animals maintained for 12 weeks served as control for the sorbinil-treated group.

Sorbinil-treatment

Treatment with the aldose reductase inhibitor, sorbinil (Pfizer research, Pfizer corporation, Groton, Connecticut, U.S.A.) was begun on the day of injection of STZ. The sorbinil was administered by gavage in a freshly made daily dose of 20 mg kg^{-1} suspended in 0.1 M NaOH titrated down to pH 7 with HCl. This treatment was maintained for 12 weeks.

Maintenance of animals

Each group of 6 animals was caged separately and kept under precisely the same conditions as the other groups. All groups were kept in a temperature controlled room (22 ± 2°C), artificially lit from 06 h 00 min–18 h 00 min each day. The animals were fed with standard laboratory diet and provided with water *ad libitum*. After the initial weights had been noted, each rat was routinely weighted once a week and the weight recorded up to the end of the study.

Estimation of blood glucose levels

Periodic routine estimation was carried out by taking a drop of blood from the tail vein of the rat and estimating its glucose level with BM-Test-Glycemie strips (Boehringer-Manheim).

The final estimate of blood glucose was carried out after the animal had been killed. Blood samples and glucose standards (50 μl) were collected into ice cold 0.016% uranyl acetate (1 ml). The mixture was agitated, centrifuged at 800 *g* and the supernatant assayed for glucose spectrophotometrically by the standard glucose oxidase method using the GOD-PAP assay kit (Boehringer-Ingelheim, London).

Experimental procedures

At the end of the allocated period for maintenance (4, 8 or 12 weeks) the animals were killed by concussion and decapitation. Final blood samples were collected for estimation of their glucose contents.

(a) *Preparation of the detrusor strip* The strip was prepared according to the procedure of Ambache & Zar (1970), as subsequently modified by Zar *et al.* (1990). After the animal had been killed the lower abdomen was opened and the bladder exposed. The urine present in the bladder was withdrawn into a 5 ml syringe and its volume recorded. The bladder was then held at its apex slightly stretched and the investing layers of serosal coat, connective tissue and accompanying blood vessels were cut away as close as possible from the outer surface of the bladder wall. The bladder was excised by a cut above the trigone and rapidly weighed. The bladder was then placed in a Petri dish and washed with several changes of Krebs-Henseleit solution. The bladder was opened by two lateral incisions and then unfolded to give a rectangular sheet of tissue. The unfolded tissue was laid on Krebs-soaked tissue paper and strips of bladder 1–1.5 cm long and 0.2 cm wide were cut with a pair of fine scissors. The strip preparation of the detrusor was suspended in a 1 ml jacketed organ bath between built-in vertical platinum electrodes, at a resting tension of 0.5 g. The resting tension was kept constant at 0.5 g throughout the experiment by appropriate adjustments whenever needed. The preparation was maintained at 37°C in Krebs-Henseleit solution gassed in the reservoir and in the organ bath with 95% O_2 plus 5% CO_2 mixture. Indomethacin 10 μM was present in the Krebs solution throughout the duration of the experiment in order to minimize the spontaneous activity of the isolated preparation (Zar *et al.*, 1990). For recording the tension of the detrusor muscle, the preparation was connected to an isometric transducer and a potentiometric recorder. An equilibration period of 30 min was allowed before the start of further experimentation (see below); during this period the preparation was repeatedly washed with fresh Krebs solution.

(b) *Experiments on isolated detrusor strips* In each experiment, isolated detrusor strips were used to obtain contractile responses to two types of stimuli: electrical field stimulation and acetylcholine. The two types of stimuli were not delivered to the same strip. One, or if necessary, more strips were reserved for each type of stimulus.

(i) *Electrical field stimulation* The strips reserved for electrical field stimulation (EFS) were treated with atropine 3 μM. EFS was started after 30 min exposure to atropine and during its continued presence for the rest of the experiment. The parameters of EFS were as follows for trains of pulses: pulse duration = 0.1 ms; train duration = 10 s; frequency = 1, 2, 4, 6, 8 and 10 Hz; interval between two trains = 60 s; voltage = supramaximal for evoking maximal contraction at each frequency. The chosen concentration of atropine (3 μM) was based upon observations in several experiments that the responses to EFS were maximally depressed by atropine 0.5 μM and atropine in higher concentrations was no more effective than atropine 0.5 μM. It was therefore considered prudent to employ atropine in a concentration (3 μM) which would be supramaximal for blocking the cholinergic component of the EFS-evoked response but yet be sufficiently low not to exert any non-specific depressent effect on the EFS-evoked response. The choice of parameters of EFS was based upon the satisfaction of two criteria: first, reproducibility of the response at each frequency of stimulation and second, the ability of tetrodotoxin, 0.5 μM to abolish the response. Fulfilment of both criteria was deemed to imply that the EFS evoked responses were neurogenic and supramaximal.

(ii) *Construction of dose-response curves to acetylcholine* The object of the experiments was to assess the ability of the detrusor to generate tension in response to a spasmogen. The spasmogen chosen for this purpose was ACh which was used in the bath concentration range of 10^{-8}–10^{-3} M, in a non-cumulative manner. Exposure to a dose of ACh was maintained until the maximal response to that concentration of ACh was obtained. The preparation was then repeatedly washed with several changes of Krebs solution. The preparation was allowed to relax fully before exposing it to the next higher concentration of ACh.

Statistics

At the end of each experiment, the bladder strip was detached from the recording set up, blotted dry and weighed. In order to render the results comparable from one strip to another and from strips of different animals, the tension generated by the strip was then used to calculate the tension/100 mg bladder tissue. All values are expressed as mean ± s.e.mean and the statistical significances were calculated by Student's *t* test.

Materials

The drugs used and their sources were: acetylcholine chloride, atropine sulphate, indomethacin, streptozotocin (Sigma), tet-

Table 1 Body weight (g) and blood glucose concentration (mg 100 ml^{-1}) in streptozotocin-diabetic and control rats

	Duration of diabetes (weeks)						
	4		8		12		
	Control	*Diabetic*	*Control*	*Diabetic*	*Control*	*Diabetic*	*Diabetic + sorbinil*
Weight (g)							
Initial	220 ± 5	219 ± 3	203 ± 5	201 ± 3	210 ± 5	203 ± 4	199 ± 5
Final	340 ± 8	195 ± 7***	419 ± 12	162 ± 8***	480 ± 11	172 ± 8***	165 ± 11
Blood glucose (mg 100 ml)$^{-1}$	63 ± 7	410 ± 29***	58 ± 2	446 ± 17***	70 ± 3	468 ± 37***	520 ± 45

All values are means ± s.e.mean ($n = 6$ in each group). The values in diabetic animals have been compared with their corresponding controls and the level of significant difference is indicated by *** $P < 0.001$. There was no significant difference between the values from 12 week diabetic animals and 12 week sorbinil-treated diabetic animals.

rodotoxin (Sankyo) and sorbinil (Pfizer). Solutions of drugs were made fresh on the day of their use with the exception of tetrodotoxin the stock solution of which was stored at −20°C.

Preparation of drug solutions

Streptozotocin: this was dissolved immediately prior to its injection in 50 mM sodium citrate buffer and titrated to pH 4.5 with HCl. The drug was kept on ice at all times before its use.

Sorbinil: this drug was dissolved in freshly prepared 0.1 M NaOH on the day of treatment. The resulting alkaline solution was titrated down to a neutral pH with HCl.

Indomethacin: absolute ethanol was used to dissolve this drug to make a solution of 10^{-2} M.

All other drugs were dissolved in distilled water.

Composition of Krebs-Henseleit solution

The composition (in mM) was: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2 and glucose 11.

Results

Body-weight and blood glucose level

Diabetic animals in all groups (4, 8 and 12 weeks) with or without sorbinil-treatment did not gain weight over the course of the investigation. By contrast, non-diabetic controls acquired significant weight gains during this period. The % gain in mean body weight of non-diabetic animals was 55, 106 and 128 after 4, 8 and 12 weeks respectively (Table 1). As was to be expected, the blood glucose levels in all diabetic animals were high and treatment with sorbinil had no significant effects on either the blood glucose levels or on loss of body weight (Table 1).

Bladder weight and residual urine

The weight of the whole urinary bladder increased only very modestly with increasing age in control (non-diabetic) rats (mean weights in mg after 4, 8 and 12 weeks = 63, 65 and 75 respectively). In sharp contrast to the control animals, the weight of urinary bladder rose sharply in diabetic animals and there appeared to be a direct relationship between the duration of diabetes and the weight of the bladder (mean weights of bladder in mg 4, 8 and 12 weeks following the induction of diabetes = 287, 710 and 820 respectively). Sorbinil-treatment of diabetic rats did not affect the weight-gain of bladder and no significant difference in the weights of the urinary bladder was seen in 12 week diabetic animals treated or untreated with sorbinil (Table 2).

The volume of residual urine (VRU) i.e. the volume of urine present in the bladder after the death of the animal was considerably higher in diabetic animals compared to their controls (Table 2). The VRU increased with increasing age both in controls and diabetic animals. Mean VRU in sorbinil-treated diabetic rats was 2.8 ml compared to 3.8 ml in the corresponding sorbinil-untreated diabetic controls, a difference that was statistically significant at the 5% level (Table 2).

Contractile responses to electrical field stimulation (EFS)

The experiments were conducted in the presence of atropine (3 μM) in order to eliminate the cholinergic contribution to the transmission and limit the motor transmission to its non-cholinergic component alone. EFS, at every frequency used (1, 2, 4, 6, 8 and 10 Hz), evoked contractions of the detrusor in all groups of animals. Three phases in the contractile response to EFS were distinguishable.

Phase 1: during the first 2 s of the onset of stimulation, the detrusor strip contracted sharply and the attainment of the peak tension signalled the end of phase 1.

Phase 2 was the plateau phase, following the termination of phase 1. Its duration was inversely proportional to the frequency of stimulus burst. At the lowest frequency of 1 Hz it was very prolonged but at the highest frequency (10 Hz), it was extremely brief (Figure 1).

Phase 3 was the phase of relaxation during which the strip lost tension despite continuing stimulation. This phase was virtually non-existent at 1 Hz but pronounced at higher frequencies, specially at 10 Hz (Figure 1).

Table 2 Weight of bladder and volume of residual urine in streptozotocin-diabetic and control rats

	Duration of diabetes (weeks)						
	4		8		12		
	Control	*Diabetic*	*Control*	*Diabetic*	*Control*	*Diabetic*	*Diabetic + sorbinil*
Bladder weight (mg)	58 ± 6	287 ± 35***	65 ± 5	710 ± 33***	75 ± 4	820 ± 29***	790 ± 19
Residual urine (ml)	0.2 ± 0.05	1.1 ± 0.3***	0.3 ± 0.05	2.2 ± 0.2***	0.3 ± 0.04	3.8 ± 0.16***	2.8 ± 0.12

All values are means ± s.e.mean ($n = 6$ in each group). The values in diabetic animals have been compared with their corresponding controls and the level of significant difference is indicated by *** $P < 0.001$. There was no significant difference between the values from 12 week diabetic animals and 12 week sorbinil-treated diabetic animals. Volume of residual urine represents the volume of urine present in the bladder immediately after exsanguination of the animal.

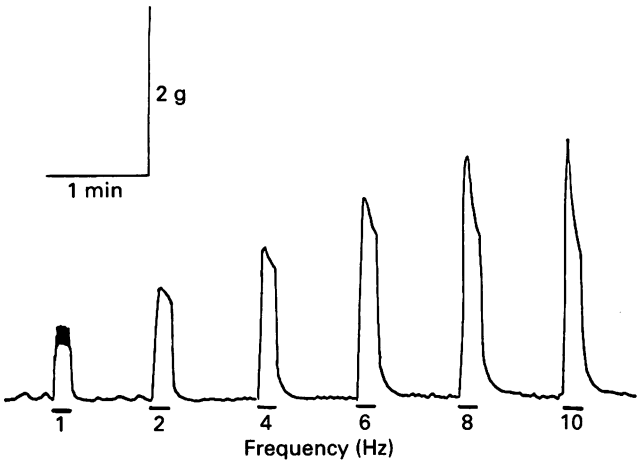

Figure 1 Rat isolated detrusor: a typical experiment showing contractile responses to electrical field stimulation in the presence of atropine 3 μM. The frequency of pulses within each train is given by the subscripts (1–10 Hz) and the duration of each stimulus burst (10 s) is indicated by a horizontal bar. Note that the peak tension was maintained only at 1 Hz and the 'non-sustainability' of the peak tension increased with increasing stimulation-frequency.

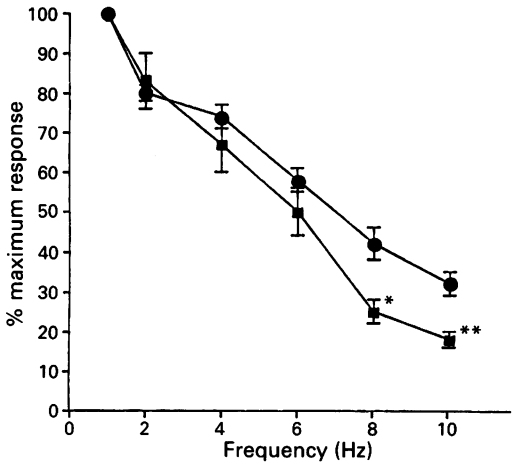

Figure 2 Comparison of the degree of 'non-sustainability' of the peak tension in response to electrical field stimulation at 1–10 Hz between detrusors from 12 week streptozotocin-diabetic (■) and 12 week non-diabetic controls (●). Each value is the mean of $n = 6$; vertical bars show s.e.mean. In each experiment the value was calculated by expressing the tension of the strip, during the delivery of the last pulse in the train, as % of the maximum tension to the same train. Note that the degree of 'non-sustainability' of the peak tension increases with increasing frequency in both diabetic and non-diabetic controls but is greater in diabetic detrusor. * $P \leq 0.05$, ** $P \leq 0.01$ indicate a significant difference from the non-diabetic control value.

Figure 1 is a record of part of a typical experiment on a detrusor strip from a non-diabetic control. The preparation responded to each stimulus burst by a contraction and the peak tension was reached approximately within the first 2 s of the 10 s bursts. The tension was not maintained at any frequency of stimulation except 1 Hz and the strip partially relaxed during the remaining period of EFS. The degree of relaxation after reaching the peak tension was directly reciprocal to the frequency of pulses within the stimulus burst, being least at 2 Hz and highest at 10 Hz (Figures 1 and 2). The responses to EFS were qualitatively and in general similar in strips from diabetic and non-diabetic animals. But there were significant quantitative differences in the responses to EFS between diabetic and non-diabetic detrusor strips. Diabetic detrusor developed a significantly lower peak tension in response to EFS compared to the non-diabetic controls. Peak tensions developed by 4, 8 and 12 week diabetic rat strips and their respective controls are presented in Figure 3 as % of the maximum tension. At every frequency of stimulation, the peak tension generated by diabetic detrusor was lower than the corresponding control value and the difference was statistically significant at every frequency used. The adverse effect on the generation of peak tension in response to EFS appeared to be related to the duration of diabetes. It was most pronounced in 12 week diabetic rats and least in 4 week diabetic ones. It has already been shown that the peak tension in response to EFS at all frequencies except 1 Hz was not sustained and the preparation started to relax during maintained stimulation (Figure 1). The degree of non-sustainability of peak tension was compared in 12 week diabetic rats and their non-diabetic controls (Figure 2). The final tension values (tension of the strip when the last pulse of the stimulus was delivered) are plotted in Figure 2 as a percentage of the peak tension and therefore serve as inverse indices of the degree of relaxation. These values are significantly lower in diabetic rats compared to the controls at 8 and 10 Hz, thus indicating an acceleration of the non-sustainability of the peak tension in diabetic animals.

Sorbinil-treated diabetic rats

The tension generated in response to EFS by detrusor strips from sorbinil-treated diabetic rats was markedly greater in comparison to that generated by the untreated diabetic ones. This was particularly noticeable at 4, 6, 8 and 10 Hz. Figure 4 allows a direct comparison to be made of the tension generated by the two groups of diabetic animals in response to EFS, at different frequencies. The peak tension developed by sorbinil-treated diabetic rats was greater at every frequency of stimulation, being 144%, 142%, 167%, 187%, 190%, 191% of the control tension, at 1, 2, 4, 6, 8 and 10 Hz respectively.

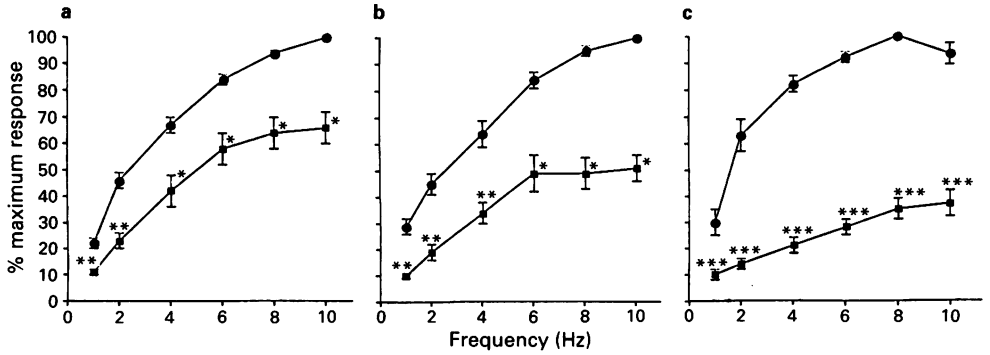

Figure 3 Non-cholinergic contractile responses of detrusor from streptozotocin (STZ)-diabetic (■) and non-diabetic control (●) rats to electrical field stimulation (EFS), 4 (a), 8 (b) and 12 (c) weeks following the induction of STZ-diabetes. All values are means (s.e.mean shown by vertical bars) and have been plotted as % of the maximum responses in their responsive group, (a), (b) or (c). Atropine (3 μM) was present throughout the experiment. Note that the degree of reduction of the EFS-evoked response increases with the increase in the duration of diabetes. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

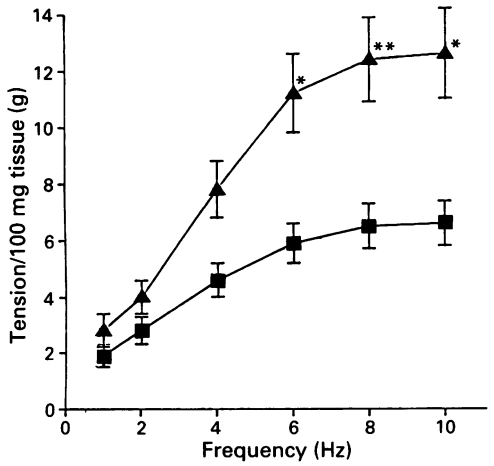

Figure 4 Contractile responses of detrusor from sorbinil-treated (▲) and nontreated (■) 12 week diabetic rats to electrical field stimulation. Atropine, (3 μM) was present throughout the experiment. All values are means (s.e.mean shown by vertical bars) ($n = 6$) of the tension (g) developed by 100 mg bladder tissue (further details are given in the methods). Note the greater tension developed by the detrusor from sorbinil-treated rats. * $P \leqslant 0.05$; ** $P \leqslant 0.01$.

Contractile responses to acetylcholine

ACh in concentrations ranging from 10^{-7} M to 10^{-3} M caused contractions of the detrusor. Dose-response curves to ACh in 12 week diabetic, non-diabetic and sorbinil-treated diabetic animals were very similar (Figure 5). Threshold concentration of ACh for evoking a contractile response in all 3 groups was 10^{-7} M and 50% of the maximal response was obtained with ACh concentrations ranging between 10^{-5} M and 3×10^{-5} M. Compared to normal, the peak tension achieved was somewhat greater in the two diabetic groups (Figure 5) but the differences were not significant.

Discussion

It is now widely recognised that the motor transmission in the rat urinary bladder, in common with other mammalian species, contains a large non-cholinergic component (Ambache & Zar, 1970; Taira, 1972). Impairment of this component of the motor transmission in streptozotocin-diabetic animals and the partial prevention of the impairment by an aldose reductase inhibitor, sorbinil, were the most striking

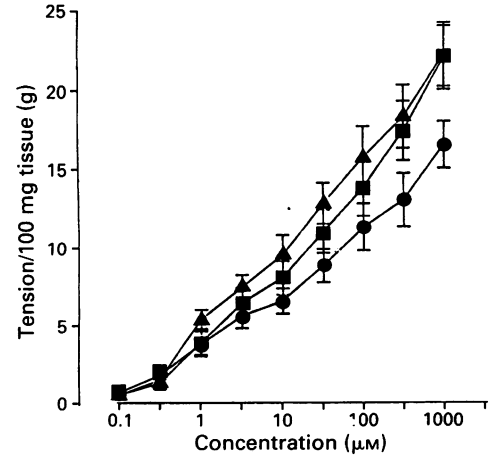

Figure 5 Concentration-response curves to acetylcholine (ACh) in detrusor strips from 12 week sorbinil-treated diabetic (▲), untreated diabetic (■) and non-diabetic control (●) rats. All values are means ($n = 6$) and represent the tension developed by 100 mg of bladder tissue. There was no significant difference between the three curves at any concentration of ACh.

findings of the present investigation. There are sound reasons to believe that the observed impairment of the non-cholinergic motor transmission in the diabetic bladder might have a prejunctional mechanism, as opposed to a postjunctional disturbed state of detrusor contractility. Our results indicate that the sensitivity of the detrusor to the spasmogenic action of ACh, a physiological spasmogen in this tissue, was not diminished in diabetic animals (ED_{50} for ACh was 4.02×10^{-5} M in controls and 3.51×10^{-5} M in diabetics). Nor can the impaired response to EFS in diabetic animals be attributed to a reduction in the tension generating capacity of the detrusor. The peak tension generated by the detrusor in response to ACh was greater in the diabetic animals. The impression that the STZ-induced diabetes does not adversely affect the contractility of the detrusor is also consistent with the observations of other workers in this field; Lincoln *et al.* (1984), Kolta *et al.* (1985) and Moss *et al.* (1987) also found no impairment in the ability of the detrusor from the STZ-diabetic rats to respond to ACh. A large body of evidence has accumulated over the years, in support of the original proposal by Burnstock and his colleagues that ATP might be the non-cholinergic motor transmitter in the mammalian urinary bladder (Burnstock *et al.*, 1972; 1978; Downie & Dean, 1977; Moss *et al.*, 1985). It is of considerable importance to recall that Longhurst & Belis (1986) found no statistically significant difference in the contractile responses of bladder strips to ATP between control and STZ-diabetic rats. If ATP is indeed the non-cholinergic transmitter, it would seem reasonable to conclude that the impairment of the non-cholinergic transmission observed in this investigation was prejunctional in origin and reflects a diminished release of the non-cholinergic (purinergic) transmitter in diabetic animals. A close examination of the time course of the response to EFS also appears to provide further corroborative evidence for this conclusion. The atropine-resistant response of the detrusor to 10 s bursts of EFS consisted of 3 phases: phase 1 was a sharp rise in tension lasting about 2 s; phase 2 was the plateau phase during which the tension remained stable. Its duration was dependent on the stimulation-frequency and was most prolonged at 1 Hz and least at 10 Hz. Phase 3 followed the plateau phase and was characterized by a gradual decline in tension despite continuing stimulation. EFS at 1 Hz produced a response in which phase 3 was missing while phase 2 was very prolonged. With increasing frequency the duration of phase 2 declined while phase 3 became more prominent. Thus the peak tension could be maintained at 100% throughout the duration of stimulation at 1 Hz whereas the tension towards the end of the stimulation period at 10 Hz was less than 35% of the peak value (Figure 2). Since the duration of the stimulus period remained constant at 10 s for both 1 Hz and 10 Hz stimulation schedules, the main difference between the two schedules lay in the total number of pulses delivered i.e. 10 at 1 Hz and 100 at 10 Hz. Although in theory it is possible that the failure to sustain the tension at the higher frequencies of EFS was in part, or wholly attributable to a decline in postjunctional sensitivity to the non-cholinergic transmitter, the complete absence of the occurrence of the fade at 1 Hz, indicates the improbability of this explanation being correct. It therefore seems attractive to suggest that the fade at the higher frequencies was caused by the high demand on releasable transmitter stores leading to a prejunctional failure to maintain an undiminished non-cholinergic transmitter release. Given this scenario, it is highly relevant to find that the failure to maintain tension at higher frequencies was significantly greater in diabetic animals, thus supporting the notion that a prejunctional mechanism was involved in the transmission failure following STZ-induced diabetes (Figure 2). This conclusion is in agreement with that of Santicioli *et al.* (1987) who also noted the reduced responses to field stimulation in STZ pretreated rats and ascribed this reduction, at least in part, to the ongoing diabetic neuropathy. They also made the important observation that unlike the diabetic animals, the bladder strips of sucrose-fed animals, having normal blood glucose

levels but a similar increase in urine production responded to EFS with normal or above normal contractions. Their results clearly show that prejunctional failure of transmission in diabetic animals is not a consequence of a saccharide (glucose)-led polyuria since another saccharide (sucrose)-led polyuria of comparable magnitude exerted no such effect.

Two further aspects of the transmission failure in diabetic rat detrusor merit some comments. First the reduced response to EFS was seen even at the lowest frequency of stimulation. Given that the failure is prejunctional, this finding implies that the transmitter output in response to even very short trains (10 pulses, 1 Hz) is reduced in diabetic animals. Secondly the degree of transmission failure bears a direct relationship to the duration of diabetes. Responses to field stimulation were most reduced in 12 week diabetic rats and least but still substantially in 4 week ones, suggesting that the process has a rapid onset and is progressive. Our findings of a reduced non-cholinergic neurogenic response in STZ-diabetic rats seem at first glance to run counter to those of Moss *et al.* (1987) who concluded that the atropine-resistant neurogenic response was increased in 8 week diabetic animals and was not significantly reduced in 16 week diabetic animals. Although no obvious reason for this discrepancy is evident, it may perhaps be attributable to the different experimental methodologies in the two investigations. Unlike the present investigation which was conducted on isolated detrusor strips, Moss *et al.* (1987) used *in vitro* whole bladder preparations. Another possible explanation may lie in the development of hypersensitivity of the postjunctional ATP receptors, noted by Moss *et al.*; ATP being the prime candidate for the role of the non-cholinergic transmitter in this tissue, the postjunctional hypersensitivity to ATP, developed as a consequence of diabetes-induced neuronal damage, might have served to mask the prejunctional transmission failure in the investigation of Moss *et al.* (1987).

The biochemical basis of diabetic autonomic neuropathy remains uncertain. There is considerable evidence for the suggestion that enhanced sorbitol concentration within nerves, secondary to diabetes, constitutes the prime factor in the induction of diabetic autonomic neuropathy. It has been known that peripheral nerves contain aldose reductase, the enzyme responsible for the conversion of glucose into sorbitol (Gabbay *et al.*, 1966). The activity of aldose reductase is dependent upon blood glucose concentration and is enhanced by hyperglycaemia (Gabbay, 1973). It has been hypothesised that hyperglycaemia-induced enhanced activity of aldose reductase in diabetes leads to accumulation of sorbitol within nerves which in turn directly or indirectly causes structural damage of the nerves and produces the neuropathy (Green *et al.*, 1985). If indeed excessive sorbitol accumulation within nerves on account of diabetes, is detrimental to nerve function, the inhibition of aldose reductase in diabetic subjects should result in an improvement of neuropathy by effecting a fall in intraneuronal sorbitol concentration. In diabetic rats, the aldose reductase inhibitor, sorbinil has been shown to enhance axonal transport (Tomlinson *et al.*, 1984) and to improve motor nerve conduction velocity (Yue *et al.*, 1982). In human diabetics, sorbinil has been found to improve motor and sensory nerve conduction velocities (Judzewitsch *et al.*, 1983) and to ameliorate painful diabetic neuropathy (Jaspan *et al.*, 1983). In our experiments the tension generated by the detrusor from the sorbinil-treated diabetic animals in response to EFS was significantly greater than the corresponding value from tissues obtained from diabetic rats not treated with sorbinil. Since the response to ACh was not affected by sorbinil treatment, indicating a lack of effect on detrusor contractility, the finding that treatment of diabetic animals with sorbinil partially prevented the failure of non-cholinergic motor transmission, is consistent with a prejunctional (neuronal) basis of the failure and lends support to the sorbitol accumulation hypothesis of diabetic autonomic neuropathy.

An intriguing question arising out of the results of the present investigation concerns the applicability of these findings to human diabetes mellitus. Occurrence of bladder dysfunction in human diabetes is widespread and its incidence high (Faerman *et al.*, 1971). Also, an excellent correlation exists between the incidence of bladder dysfunction and of peripheral neuropathy in diabetic patients indicating a neuronal origin of the dysfunction in man as well (Frimodt-Moller, 1980). The findings of the present investigation relate solely to the non-cholinergic motor transmission in diabetic and non-diabetic rats. Although early reports have cast doubt on the presence of a non-cholinergic component in the motor transmission of the human bladder (Nergardh & Kinn, 1983; Sibley, 1984; Kinder & Mundy, 1985), two more recent studies have provided strong evidence for its presence in human detrusor (Hoyle *et al.*, 1989; Luheshi & Zar, 1990). Hoyle *et al.* (1989) have demonstrated not only the presence of a non-adrenergic, non-cholinergic element of neuromuscular transmission in the human detrusor but also that this component is purinergic. Luheshi & Zar (1990) have additionally given a valid explanation for the negative findings of the earlier workers. In the light of the foregoing observations, it seems reasonable to expect that the bladder dysfunction in human diabetics may in part be attributable to impaired non-cholinergic motor transmission. It would be interesting to know whether administration of an aldose reductase inhibitor to human diabetics exerts a beneficial effect on bladder function as it does in diabetic rats.

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