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Effect of substance P and capsaicin on stomach fundus and ileum of streptozotocin-diabetic rats

Christian Pinna, Chiara Bolego, Lina Puglisi *

Institute of Pharmacological Sciences, University of Milan, Via Balzaretti 9, 20133 Milan, Italy

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Abstract

The in vitro responses of longitudinal preparations of rat stomach fundus and ileum to capsaicin at 1, 8, 4, 16 and 26 weeks and to substance P at 1 and 8 weeks from diabetes induction were studied. The results were compared with those obtained in age-matched control rats. The contractile responses to exogenous substance P and capsaicin were not affected in the stomach fundus from diabetic rats. Atropine (1 μM) did not antagonize the substance P-induced response whereas it inhibited about 90% of the capsaicin-induced response in controls and about 60% of the response in diabetic rats. At the resting tone, capsaicin induced a relaxation followed by a contraction in stomach fundus of control rats. Only a contraction was evoked in diabetic rats. In carbachol (0.05–0.1 μM) pre-stimulated strips, a complete restoration of the biphasic response was obtained in the diabetic state. The contractile response elicited by exogenous substance P was not significantly increased in the ileum preparations from diabetic rats; nevertheless the EC_{50} value for substance P was reduced 8 weeks after the onset of diabetes. The response elicited by capsaicin in the ileum of control rats was also biphasic. The capsaicin-induced contraction was greater in tissue from diabetic rats as compared with controls and relaxation was not evident. An age-related decrease of the contraction was also evident in both groups. Atropine (1 μM) partially antagonized the responses to substance P and capsaicin. The inhibition of the responses with atropine was more evident in control than in diabetic rats. These results suggest that the myogenic actions of several agonists in these two tissues are differently modified in experimental diabetes.

Keywords: Diabetes; Sensory fibres; Capsaicin; Stomach fundus; Ileum

1. Introduction

Capsaicin has been shown to induce contraction and/or relaxation in different gastrointestinal preparations (Maggi, 1990). Capsaicin-induced contractions are mediated through the release of substance P and neurokinin A from capsaicin-sensitive neuronal structures (Chahl, 1982; Holzer, 1985; Holzer-Petsche et al., 1989; Huidobro-Toro et al., 1982; Maggi et al., 1986b). Substance P acts both directly on smooth muscle and indirectly by inducing the release of acetylcholine from the myenteric plexus (Barthò and Vizi, 1985; Szolcsànyi and Barthò, 1978; Yau and Youther, 1982) whereas several non-adrenergic non-cholinergic (NANC) inhibitory responses which cannot be ascribed to substance P release can be in fact mediated by calcitonin gene related peptide (Katsoulis and Conlon, 1989; Lefebvre et al., 1991; Maggi et al., 1986a, 1988; Sternini et al., 1987), or by other co-transmitters such as nitric oxide and vasoactive intestinal polypeptide (Smits and Lefebvre, 1992). In the gastrointestinal tract, sensory fibers have also been proposed to play a role in a gastric defense mechanism (Maggi, 1990; Holzer and Sametz, 1986; Holzer et al., 1991; Leung, 1992) and in the activation of inhibitory reflexes regulating gastrointestinal motility (Holzer, 1985; Holzer and Lembeck, 1979; Katsoulis and Conlon, 1989; Maggi and Meli., 1988). These observations have made capsaicin an important tool for investigating neurochemical and functional characteristics of afferent neurons (Holzer, 1988), under physiological conditions and also in pathological conditions such as diabetes. Gastrointestinal disorders such as gastric retention and delayed small intestinal transit are common in diabetes mellitus (Camilleri and

* Corresponding author.

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Malagelada, 1984; Clarke et al., 1979; Feldman and Schiller, 1983).

Several studies have investigated peptide-induced responses in the small intestine as well as the effect of streptozotocin or alloxan treatment on these responses (Ballman and Conlon, 1985; Belai and Burnstock, 1987; Belai et al. 1985, 1991; Dhall et al., 1986; Di Giulio et al., 1989; Feher and Penzes, 1987; Mathison and Davison, 1988), and the influence of age on the responses to different agonists (Smits and Lefebvre, 1992), but only a few studies exist on the relationship between the magnitude of the gastrointestinal response to peptide neurotransmitters and the time of onset of diabetes (Belai et al., 1988).

The purpose of the present study was to characterize gastrointestinal smooth muscle responsiveness to substance P at 1 and 8 weeks and capsaicin at 1, 4, 8, 16 and 26 weeks after induction of diabetes in order to evaluate the progression of damage induced by diabetes in order to evaluate the progression of damage induced by diabetes-neuropathy on sensory fibers innervating rat ileum and stomach fundus.

2. Materials and methods

Male Sprague-Dawley rats weighing 200–225 g (initial age ca. 2 months) were used for all experiments. The animals were assigned at random to the control or to the diabetic group and were housed at a controlled temperature of 23°C, with a 12-h light-dark cycle.

2.1. Diabetes induction

After a period of not less than 5 days following arrival in our laboratory, the rats were made diabetic by a single injection of streptozotocin (65 mg/kg i.v.). Control animals received an equivalent volume of streptozotocin vehicle (0.05 M citrate buffer, pH 4.5) and were housed under identical conditions. During the 24 h following streptozotocin injection, 5% glucose in the drinking water was given to the rats in order to prevent hypoglycemia. The efficacy of the treatment was determined by measuring glycosuria (Glukur Test) 7 days later.

2.2. Preparation of tissues

At the end of 1, 4, 8, 16 and 26 weeks of diabetes, the animals were killed by a blow on the head. The rats with plasma glucose levels higher than 400 mg/dl were used for further experimentation (9 out of 10 rats). The abdomen was opened and sections of terminal ileum (approximately 3 cm in length, 40 ± 5 mg dry weight tissue) were removed just proximal to the ileocecal valve, and strips of stomach fundus (3×0.5 cm, 30 ± 5 mg dry weight tissue) were prepared according to the method of Vane (1957). Stomach strips were suspended in a 20 ml organ bath perfused with a Krebs solution and ileum was placed in a 10 ml organ bath perfused with a Tyrode solution. Both physiological solutions were continuously bubbled with a 95% O_2 and 5% CO_2 mixture at 37°C (perfusion rate: 60 ml · h^{-1}). The Krebs solution had the following composition (mM): NaCl 113, KCl 4.7, $CaCl_2 \cdot 6H_2O$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25, $MgSO_4 \cdot 7H_2O$ 1.2, Glucose 11.5, pH 7.4; the Tyrode's solution was composed as follows (mM): NaCl 118, KCl 4.7, $CaCl_2$ 1.8, $MgCl_2$ 0.5, NaH_2PO_4 1.0, $NaHCO_3$ 25, glucose 11, pH 7.4).

A resting load of 2 g (19.6 mN) for stomach strips and one of 1 g (9.8 mN) for ileum were applied; the preparation was allowed to equilibrate for 60 min before the start of the experiment under continuous perfusion. Tension was recorded by means of an isometric microdynamometer (2-channel recorder 'Gemini', 7070, Basile, Italy).

2.3. Protocols

After the equilibrium period, the tissues were challenged with two or more doses of acetylcholine (1 μM).

In some experiments the effect of atropine was tested in order to evaluate the contribution of enteric cholinergic neurons to substance P and capsaicin activity. Atropine was added in Krebs or Tyrode solution so as to give a final concentration of 1 μM. Capsaicin was dissolved in ethanol (100%) to give a 50 mM stock solution that was stored at 4°C and diluted in Krebs solution as needed for the experiment. The solvent, at the levels used, proved to be devoid of myogenic activity. Substance P was dissolved in physiological solution just before the experiment. Concentration-response curves for substance P and acetylcholine added noncumulatively to longitudinal strips of stomach fundus and ileum were recorded at different stages of diabetes (1–8 weeks for substance P and 4–26 weeks for acetylcholine). An equilibrium period of 15 min was used between each addition of the substance P in order to avoid tachyphylaxis. Capsaicin was tested only once in each preparation at a concentration giving a maximum response (1 μM). In order to exclude the absence of any unspecific effect of capsaicin (1 μM), an experiment was performed on a strip of stomach fundus and ileum preparation; after the first attempt the response to capsaicin was abolished. In order to avoid cross-desensitization in all preparations, capsaicin was delivered after a 1-h interval from the last addition of substance P.

In some experiments the effect of capsaicin was tested in carbachol precontracted tissues. Carbachol was added in Krebs solution so as to give a final concentration of 0.05 or 0.1 μM.

After the pharmacological evaluation, the tissues were carefully desiccated by means of a vacuum-desiccator and the weight dry was determined. Contractile and relaxant responses were expressed in mN per mg dry weight tissue (mN/mg, d.w.t.).

2.4. Drug

The drugs used were: acetylcholine (Sigma), atropine (Sigma), capsaicin (Sigma), carbachol (Sigma), substance P (Sigma), streptozotocin (Upjohn).

2.5. Statistical analysis

All data are expressed as means ± S.E.M. of four to eight experiments and represent unpaired data. When more than two means were compared i.e. control preparations, diabetic preparations with or without atropine treatment, an analysis of variance with repeated measurements was used. If a significant F value was found, Scheffé's test for multiple comparisons was used to identify differences among groups (Ludbrook, 1991). The concentration-response curves were analyzed by means of the computer program Allfit. For each curve, the program determines four parameters (the lower plateau, the slope, the EC_{50} and the upper plateau). The program also allows comparison of two or more concentration-response curves simultaneously. At the end of the analysis, the statistical significance of the difference was evaluated by means of the F test. A probability value of $P < 0.05$ was considered statistically significant.

3. Results

The effects of experimentally induced diabetes on animal weight and on blood glucose levels are shown in Table 1.

3.1. Effect of acetylcholine on stomach fundus

The contractile responses to acetylcholine (0.001 μM to 1 mM) tended to be higher in tissues from 26-week diabetic rats especially for higher concentrations of the drug, but statistical significance was noy reached (Fig. 1). The maximal response to acetylcholine was: 5.48 ± 0.60 vs. 4.30 ± 0.50 mN/mg ($n = 5$) in diabetic rats and their age-matched controls respectively. The EC_{50} values for acetylcholine were not significantly different in diabetic and control rats: 0.55 ± 0.13 vs. 0.94 ± 0.30 μM. Furthermore the responses to KCl (40 mM) were similar in both 8-week diabetic and control rats: 2.18 ± 0.41 vs. 1.87 ± 0.44 mN/mg, and in tissues from 26-week diabetic rats in comparison with controls (1.17 ± 0.51 vs. 1.80 ± 0.39 mN/mg).

Table 1
Body weight and glycemia in normal and diabetic rats. Each value is mean ± S.E.M. of five determinations.

	Body weight (g)		Blood glucose level (mg/dl)	
	Control	Diabetic	Control	Diabetic
Initial	215 ± 10		98 ± 6.0	
1 week	270 ± 5.0	170 ± 4.0	110 ± 7.0	405 ± 20
4 weeks	420 ± 16	260 ± 10	106 ± 6.0	471 ± 18
16 weeks	540 ± 20	270 ± 10		480 ± 20
26 weeks	600 ± 12	265 ± 7.0		464 ± 23

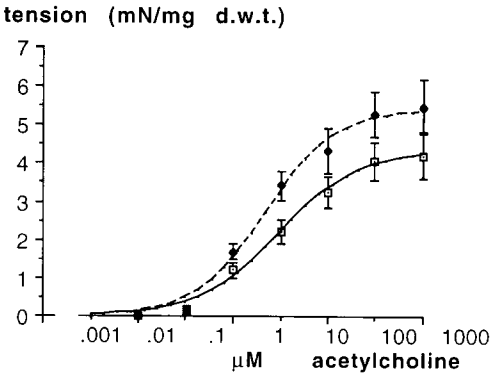

Fig. 1. Concentration-response curves for acetylcholine added non-cumulatively to stomach fundus strips from control (solid lines) and diabetic rats (dashed lines). Diabetes was induced 26 weeks earlier with streptozotocin. Each value is mean ± S.E.M. of five determinations.

3.2. Effect of substance P and capsaicin on stomach fundus

Substance P added non-cumulatively induced a concentration-related contraction in smooth muscle strips of stomach fundus, which was similar in both groups of diabetic rats (1 week and 8 weeks after induction) and in their age-matched controls (Fig. 2). No change in the sensitivity and in the response to substance P (10 μM) was detected: 4.68 ± 0.28 vs. 4.26 ± 0.04 mN/mg in 1-week diabetes and control rats; 4.33 ± 0.32 vs. 4.11 ± 0.35 mN/mg in 8-week diabetes and control rats. The response to substance P did not reach the plateau even at the maximal tested concentration, therefore EC_{50} values were not determineted.

Fig. 3 shows a typical tracing of substance P (20 nM) and capsaicin (1 μM) on resting tone of stomach fundus form control and 8-week diabetic rats. Substance P induced a rapid and transient contraction, whereas capsaicin in 80% of control preparations showed a biphasic response: an immediate relaxation followed by a short-lived contraction. In the presence of atropine (1 μM) the contractile component of the response evoked by capsaicin was almost completely

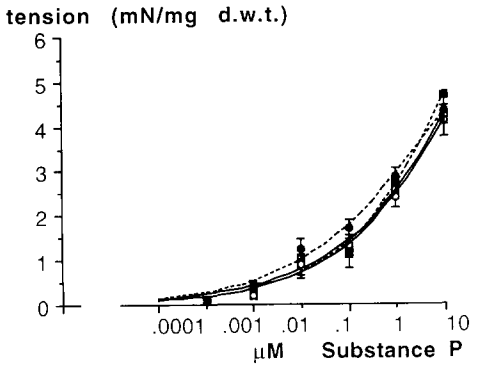

Fig. 2. Concentration-response curves for substance P added non-cumulatively to stomach fundus strips. Dashed lines show data from diabetic animals at 1 week (■) and 8 weeks (●) after diabetes induction, solid lines data from age-matched controls (□) at 1 week and (○) at 8 weeks. Each value is mean ± S.E.M. of five determinations.

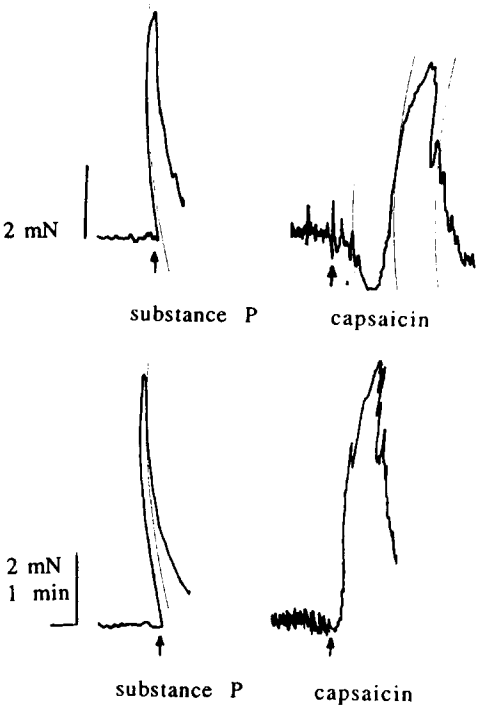

Fig. 3. Typical tracing of the response of the longitudinal strips of stomach fundus from control rats (top panel) and diabetic rats (bottom panel) to substance P (20 nM) and capsaicin (1 μM). Diabetes was induced 8 weeks earlier with streptozotocin.

reduced in control tissue: 0.68 ± 0.23 vs. 0.10 ± 0.01 mN/mg ($n = 5$; $P < 0.01$) leaving the unaltered relaxant component only (-0.21 ± 0.03 vs. -0.21 ± 0.03 mN/mg). Substance P-induced response was not significantly affected by atropine. In stomach strips of 8-week diabetic rats the contraction elicited by capsaicin was not preceded by relaxation and it was partially reduced by atropine: 0.71 ± 0.20 vs. 0.30 ± 0.10 mN/mg. The contractile responses induced by capsaicin (1 μM) at different stages of diabetes are shown in Fig. 4. At 1 week after the onset of diabetes, no significant difference was detectable in both the relaxant and contracting components of the response to capsaicin. In the subsequent stages of diabetes (4, 16 and 26 weeks) the contractile component was not significantly affected, whereas the relaxant component disappeared. A restoration of the biphasic response to capsaicin in stomach preparations of diabetic rats could be obtained with (0.05 μM) carbachol-prestimulated tissues (Fig. 5), whereas at 0.1 μM carbachol concentration, capsaicin only produced a sustained monophasic relaxant response.

3.3. Effect of acetylcholine on ileum

Fig. 6 shows the concentration-response curves for acetylcholine (0.001 μM to 100 μM) added non-cumu-

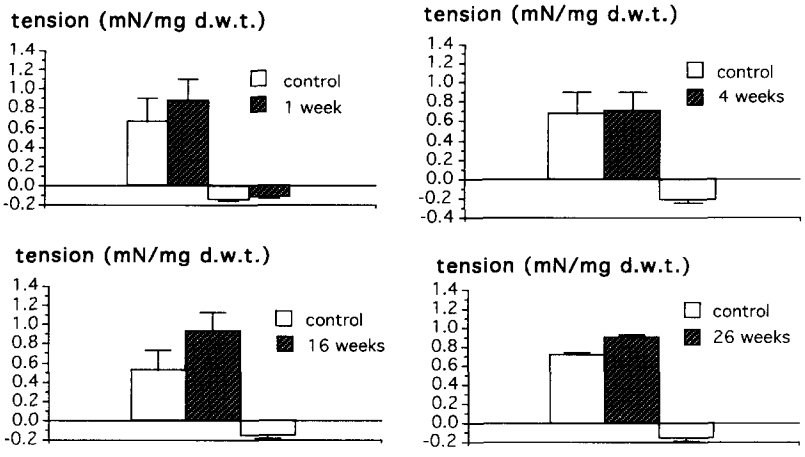

Fig. 4. Contractile response of the rat stomach fundus preparations to capsaicin (1 μM) at different stages of diabetes. Each value is mean ± S.E.M. of five determinations.

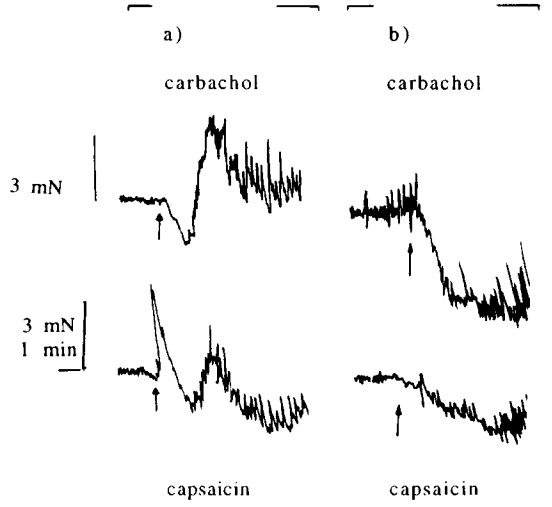

Fig. 5. Original tracing showing the effect of capsaicin (1 μM) on carbachol-stimulated stomach fundus strips of control (top panel) and diabetic rats (8 weeks from induction; bottom panel). (a) 0.05 μM; (b) 0.1 μM carbachol concentration.

latively to ileal preparations from 26-week diabetic rats and age-matched controls. The contractile responses tended to be higher in preparations from diabetic rats reaching statistical significance at the maximal tested concentration: 2.20 ± 0.20 mN/mg vs. 1.24 ± 0.20 (n = 5; $P < 0.05$). The EC_{50} values for acetylcholine were not significantly different in diabetic and control rats: 0.30 ± 0.08 vs. 0.48 ± 0.15 μM. Moreover the responses to KCl (40 mM) were already increased in tissues from 8-week diabetic rats 1.48 ± 0.12 vs. 0.88 ± 0.08 mN/mg (n = 5; $P < 0.01$) as well as in tissues from 26-weeks diabetic rats in comparison with controls (1.15 ± 0.10 vs. 0.80 ± 0.08 mN/mg).

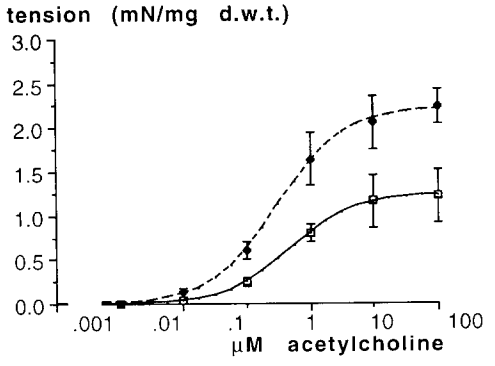

Fig. 6. Concentration-response curves for acetylcholine added non-cumulatively to ileum preparations from control (solid lines) and diabetic rats (dashed lines). Diabetes was induced 26 weeks earlier with streptozotocin. Each value is mean ± S.E.M. of five determinations.

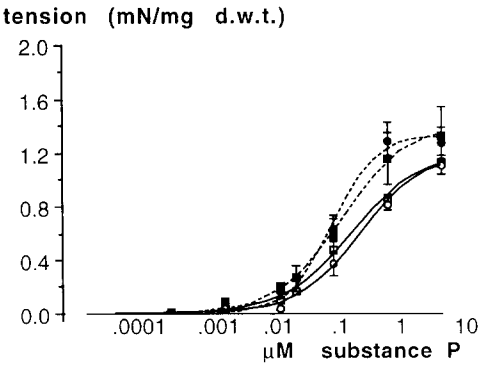

Fig. 7. Concentration-response curves for substance P added non-cumulatively to ileum preparations. Dashed lines show data for diabetic animals at 1 week (■) and 8 weeks (●) after diabetes induction, solid lines data from age-matched controls (□) at 1 week and (○) at 8 weeks. Each value is mean ± S.E.M. of five determinations.

3.4. Effect of substance P and capsaicin on ileum

The concentration-response curves for substance P in ileal preparations of control and diabetic rats (1 week and 8 weeks after diabetes induction) are shown in Fig. 7. No change in the sensitivity to substance P was detected after 1 week of diabetes: the EC_{50} values for substance P were: 146 ± 27 vs. 232 ± 60 nM in tissues from diabetic rats and controls respectively. The responses induced at the maximal tested concentration of the drug were similar in diabetic and control rats: 1.40 ± 0.06 vs. 1.20 ± 0.07 mN/mg at 1 week and 1.30 ± 0.04 vs. 1.20 ± 0.08 mN/mg at 8 weeks. Nevertheless the EC_{50} values for substance P were significantly decreased in tissues from 8-week diabetic rats in comparison with their age-matched controls: 98 ± 13 vs. 326 ± 97 nM (n = 5; $P < 0.05$). Fig. 8 shows an original tracing of the response to substance P (20 nM) and to capsaicin (1 μM) in ileal preparations from 8-week diabetic and control rats. Substance P produced a phasic contraction in all tissues; moreover a series of rhythmic contractions superimposed on the response was also evident. Atropine (1 μM) was able to significantly reduce contractile responses to exogenous substance P (20 nM) in control preparations only: 0.10 ± 0.01 vs. 0.05 ± 0.01 mN/mg (n = 5; $P < 0.05$). The substance P-induced response was not significantly affected by atropine in tissues from 8-week diabetic rats: 0.30 ± 0.06 vs. 0.23 ± 0.04 mN/mg. Capsaicin (1 μM) produced a transient relaxation followed by a contraction in control tissue at resting tone. In tissues excised from 8-week diabetic rats, capsaicin only produced a contractile response which was significantly enhanced as compared with that in controls: 0.52 ± 0.07 vs. 0.30 ± 0.03 mN/mg (n = 5; $P < 0.05$). In the presence of atropine, capsaicin-induced relaxation was not modified whereas the contractile component was signifi-

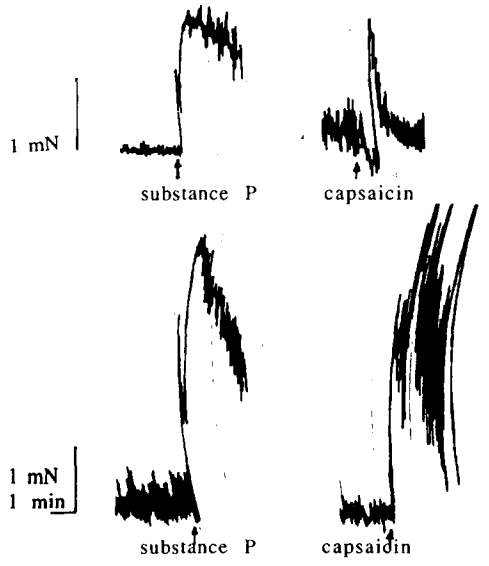

Fig. 8. Original tracing showing the effect of substance P (20 nM) and capsaicin (1 μM) on ileal preparations from control rats (top panel) and diabetic rats (bottom panel). Diabetes was induced 8 weeks earlier with streptozotocin.

cantly inhibited in tissues from control rats: 0.30 ± 0.03 vs. 0.13 ± 0.10 mN/mg ($n = 5$; $P < 0.05$) but not in tissues from diabetic rats: 0.52 ± 0.07 vs. 0.40 ± 0.08. Fig. 9 shows the capsaicin-induced response of ileal preparations at 1, 4, 16 and 26 weeks after the onset of diabetes. At 1 week, as already observed in stomach fundus, no significant difference was found in either relaxant or contractile components. In the later stages of the disease, the amplitude of the response to capsaicin was greater in tissues from diabetic rats as compared with controls, whereas the relaxant component was not present. In both groups an age-related decrease of the response was also evident.

4. Discussion

Several disorders of the gastrointestinal system that affect diabetic patients (Katz and Spiro, 1966; Feldman and Schiller, 1983), such as gastric retention, delayed small intestinal transit, diarrhea or constipation, megacolon, can be reproduced in animals by chemically induced diabetes.

The gastrointestinal dysfunction in diabetes may be due to diabetic neuropathy affecting the sensory nerves of these organs. In order to study the development of diabetic neuropathy in motor and sensory nerves, the action of acetylcholine, substance P and capsaicin was tested in stomach fundus and in ileum of control and streptozotocin-induced diabetic rats at different stages of the disease.

In rat stomach fundus strips the responses elicited by acetylcholine and substance P were never affected by chemically induced diabetes even at 26 weeks. In fact the sensitivity to both agonists was not significantly different between tissues from diabetic rats and tissues from controls, indicating integrity of acetylcholine and peptide receptors. Furthermore the smooth muscles responded similarly to KCl challenge. The effect of capsaicin appears to be different in tissues from diabetic rats in comparison with control preparations. Capsaicin elicited an immediate relaxation followed by a short-lived contraction in control conditions whereas in tissues from diabetic rats the contraction was not preceded by relaxation.

The lack of relaxation already observed 4 weeks after the onset of diabetes in stomach fundus in vitro,

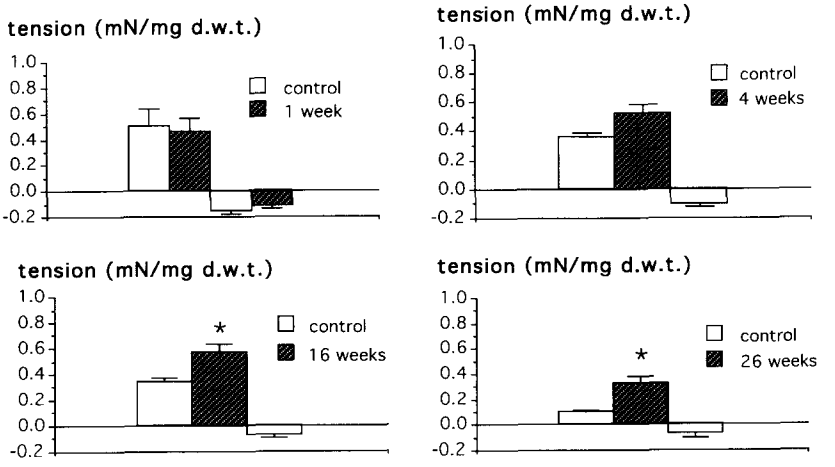

Fig. 9. Contractile response of the rat ileum preparations to capsaicin (1 μM) at different stages of diabetes. Each value is mean ± S.E.M. of five experiments. Statistical significance: * $P < 0.05$.

is at least partially dependent on the reduced tone of the smooth muscle provoked by the development of the disease, rather than by an impairment of all NANC inhibitory systems.

A reduced tone in the gastrointestinal tract has also been observed by Lefebvre et al. (1991). Several neurotransmitters such as vasointestinal polypeptide, calcitonin gene related peptide, nitric oxide and purines can activate inhibitory reflexes regulating gastrointestinal motility. Even if one or two of these inhibitory systems are impaired by diabetes – calcitonin gene-related peptide is reduced and vasointestinal polypeptide release is decreased in gastrointestinal tract after 8 weeks of diabetes (Belai and Burnstock, 1987) – other systems such a purinergic one could increase its activity and partially restore in vivo the inhibitory response regulating the motility of the gut.

In our experiments the strips excised from diabetic rats did not maintain the resting tone (2 g for stomach strips and 1 g for ileum) and from time to time regulation of the baseline was need. Nevertheless a biphasic response could be partially restored in tissue from diabetic rats, when the tone of the strips was increased by carbachol.

Therefore capsaicin may induce contraction or relaxation depending upon the tone of the smooth muscle preparation (Maggi, 1990). The contractile activity elicited by capsaicin in rat stomach fundus seems to be partially cholinergically mediated since it was antagonized by atropine. In contrast the contraction elicited by exogenous substance P was not affected by atropine, probably because, as already shown by Bertaccini and Coruzzi (1977) cholinergic enteric neurons activated by substance P project predominantly on circular rather than on longitudinal layers.

The contractions elicited by acetylcholine in ilea preparations obtained from 26-week diabetic rats were higher than in their aged-matched controls. Nevertheless no significant change in the sensitivity or in EC_{50} value was detected.

An increased responsiveness of ileum smooth muscle was also observed after KCl stimulation, at 8–26 weeks after diabetes induction, suggesting an alteration provoked by diabetes in the contractile apparatus of myocytes. Similar results were obtained by Carrier and Aronstam (1990) in ileum, 10–12 weeks after diabetes induction.

The response to exogenous substance P in ileum tended to be higher in tissues from diabetic rats especially for higher concentrations of the drug, but did not reach statistical significance at 1 week or at 8 weeks from the onset of diabetes. In contrast, the EC_{50} value for substance P was significantly decreased after 8 weeks of diabetes, indicating an increased sensitivity to the agonist, as also demonstrated in rat jejunum (Mathison and Davison, 1988).

The increased sensitivity of smooth muscle to substance P during diabetes could be explained by an attempt to counteract the decreased content of this neuropeptide in enteric fibers. In fact, in intestinal fibers the content of substance P 14 weeks after diabetes induction is decreased by 60% (Di Giulio et al., 1989).

Several authors suggest that substance P acts both directly and indirectly on the smooth muscle, by activating myenteric cholinergic neurons (Barthò and Vizi, 1985; Szolcsànyi and Barthò, 1978; Yau and Youther, 1982). The above-mentioned indirect response seems to be affected by diabetes, as atropine was able to reduce 45% of the substance P response in control rats but only by 20% of the response in diabetic rats.

These results suggest that the reduced availability of substance P can be overcome either by an increased sensitivity of myocytes to substance P or by an increased responsiveness of the contractile apparatus itself. The activity of capsaicin on the ileum smooth muscle is biphasic, as in gastric smooth muscle. The relaxant component of capsaicin, which is not present in ileum preparations of diabetic rats, has been correlated with the release of calcitonin gene-related peptide from afferent fibers. The content of calcitonin gene related peptide in rat ileum is significantly reduced after 8 weeks of diabetes (Belai and Burnstock, 1987; Belai et al., 1987) and this reduction could explain our results.

The age-related decrease of the capsaicin-induced contraction in ileum from both control and diabetic rats could be explained by a reduced content and/or release of neuropeptides, as shown by Andersson et al. (1992) in the urinary bladder and urethra.

In conclusion these data suggest that in the stomach fundus we have no evidence in vitro for an impairment of afferent fibers in diabetes, since the contractile responses of this tissue to capsaicin were not significantly altered at different stages of diabetes. Nevertheless, there are indications suggesting a loss of tone in the gastrointestinal system due to diabetes, as already shown by Lefebvre et al. (1991). In contrast, rat ileum appears to be more rapidly affected by the disease, since 8 weeks after the onset of diabetes a higher responsiveness to different drugs was demonstrated. Furthermore the capsaicin-sensitive sensory fibers seem to be damaged gradually with the progression of diabetes.

Our data indicate that in the gastrointestinal system the alteration in capsaicin-sensitive afferent fibres provoked by diabetes proceeds along the intestine in a caudal to oral direction, similarly to the alterations in cholinergic neurotransmission (Mathison and Davison, 1988). The differential effects of diabetes on stomach fundus in comparison with ileum could be related with differences in the innervation.

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References

Andersson, P.O., J. Fahrenkrug, A. Malmgren and B. Uvelius, 1992, Effects of age and streptozotocin-induced diabetes on contents and effects of substance P and vasoactive intestinal polypeptide in the lower urinary tract of the rat, Acta Physiol. Scand. 144, 361.

Ballman, M. and J.M. Conlon, 1985, Changes in somatostatin, substance P and vasoactive intestinal polypeptide content of the gastrointestinal tract following streptozotocin-induced diabetes in the rat, Diabetologia 28, 355.

Barthò, L. and E.S. Vizi, 1985, Neurochemical evidence for the release of acetylcholine from the guinea-pig ileum myenteric plexus by capsaicin, Eur. J. Pharmacol. 110, 125.

Belai, A., J. Lincoln, P. Milner, R. Crowe, A. Loesch and G. Burnstock, 1985, Enteric nerves in diabetic rats: increase in vasoactive intestinal polypeptide but not substance P, Gastroenterology 89, 967.

Belai, A. and G. Burnstock, 1987, Selective damage of intrinsic calcitonin gene-related peptide-like immunoreactive enteric nerve fibers in streptozotocin-induced diabetic rats, Gastroenterology 92, 730.

Belai, A., J. Lincoln and G. Burnstock, 1987, Lack of release of Vasoactive intestinal polypeptide and calcitonin gene-related peptide during electrical stimulation of enteric nerves in streptozotocin-diabetic rats. Gastroenterology 93, 1034.

Belai, A., J. Lincoln, P. Milner and G. Burnstock, 1988, Progressive changes in adrenergic serotonergic and peptidergic nerves in proximal colon of streptozotocin-diabetic rats, Gastroenterology 95, 1234.

Belai, A., J. Lincoln, P. Milner and G. Burnstock, 1991, Differential effect of streptozotocin-induced diabetes on the innervation of the ileum and distal colon, Gastroenterology 100, 1024.

Bertaccini, G. and G. Coruzzi, 1977, Action of some natural peptides on the stomach of the anaesthetized rat, Arch. Pharmacol. 298, 163.

Camilleri, M. and J.R. Malagelada, 1984, Abnormal intestinal motility in diabetics with the gastroparesis syndrome, Eur. J. Clin. Inv. 14, 420.

Carrier, G.O. and R.S. Aronstam, 1990, Increased muscarinic responsiveness and decreased muscarinic receptor content in ileal smooth muscle in diabetes, J. Pharmacol. Exp. Ther. 254 No. 2, 445.

Chahl, L.A., 1982, Evidence that the contractile response of the guinea-pig ileum to capsaicin is due to substance P release, Arch. Pharmacol. 319, 212.

Clarke, B.F., D.J. Ewing and I.W. Campbell, 1979, Diabetic autonomic neuropathy, Diabetologia 17, 195.

Dhall, U., T. Cowent, A.J. Haven and G. Burnstock, 1986, Perivascular noradrenergic and peptide-containing nerves show different patterns of changes during development and ageing in the guinea-pig, J. Auton. Ner. 16 No. 2, 109.

Di Giulio A.M., B. Tenconi, R. La Croix, P. Mantegazza, F. Cattabeni and A. Gorio, 1989, Denervation and hyperinnervation in the nervous system of diabetic animals. I. The autonomic neuronal dystrophy of the gut, J. Neurosci. Res. 24, 355.

Feher, E. and L. Penzes, 1987, Density of substance P, vasoactive intestinal polypeptide and somatostatin-containing nerve fibers in ageing small intestine of the rat, Gerontology 33, 341.

Feldman, M. and L.R. Schiller, 1983, Disorders of gastrointestinal motility associated with diabetes mellitus, Ann. Int. Med. 98, 378.

Holzer, P., 1985, Stimulation and inhibition of gastrointestinal propulsion induced by substance P and substance K in the rat, Br. J. Pharmacol. 86, 305.

Holzer, P., 1988, Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides, Neuroscience 24, 739.

Holzer, P., 1991, Capsaicin: cellular targets, mechanisms of action, and selectivity for thin sensory neurons, Pharmacol. Rev. 43, No. 2, 143.

Holzer, P. and F. Lembeck, 1979, Effect of neuropeptides on the efficiency of the peristaltic reflex, Arch. Pharmacol. 307, 257.

Holzer, P. and F. Lembeck, 1980, Neurally mediated contraction of ileal longitudinal muscle by substance P, Neurosc. Lett. 17, 101.

Holzer, P. and W. Sametz, 1986, Gastric mucosal protection against ulcerogenic factors in the rat mediated by capsaicin-sensitive afferent neurons, Gastroenterology 91, 975.

Holzer, P., E.H. Livingston, A. Saria and P.H. Guth, 1991, Sensory neurons mediate protective vasodilatation in rat gastric mucosa, Am. J. Physiol. 260 (Gastrointest. Liver Physiol. 23) G363.

Holzer-Petsche, U., H. Seitz and F. Lembeck, 1989, Effect of capsaicin on gastric corpus smooth muscle of the rat in vitro, Eur. J. Pharmacol. 162, 29.

Huidobro-Toro, J.P., C.A. Chelala, S. Bahouth, R. Nodar and J.M. Musacchio, 1982, Fading and tachyphylaxis to the contractile effects of substance P in the guinea-pig ileum, Eur. J. Pharmacol. 81, 21.

Katsoulis, S. and J.M. Conlon, 1989, Calcitonin gene related peptides relax guinea pig and rat gastric smooth muscle, Eur. J. Pharmacol. 161, 129.

Katz, L.A. and H. Spiro, 1966, Gastrointestinal manifestations of diabetes, New Engl. J. Med. 275, 1350.

Lefebvre, R.A., F.A. De Beurme and S. Sas, 1991, Relaxant effect of capsaicin in the rat gastric fundus, Eur. J. Pharmacol. 195, 131.

Leung, F.W., 1992, Role of capsaicin-sensitive afferent nerves in mucosal injury and injury-induced hyperemia in rat colon, Am. J. Physiol. 262 (Gastrointest. Liver Physiol. 25), G332.

Ludbrook, J., 1991, On making multiple comparisons in clinical and experimental pharmacology and physiology, Clin. Exp. Pharmacol. Physiol. 18, 379.

Maggi, C.A., 1990, Capsaicin-sensitive nerves in the gastrointestinal tract, Arch. Int. Pharmacodyn. 303, 157.

Maggi, C.A. and A. Meli, 1988, The sensory-efferent function of capsaicin-sensitive sensory neurons, Gen. Pharmacol. 19, No. 1, 1.

Maggi, C.A., P. Santicioli, S. Manzini and A. Meli, 1986a, Capsaicin activates neurogenic non-adrenergic non-cholinergic relaxations of the isolated rat duodenum, Eur. J. Pharmacol. 120, 367.

Maggi, C.A., S. Giuliani, S. Manzini, P. Santicioli and A. Meli, 1986b, Motor effect of neurokinins on the rat duodenum: evidence for the involvement of substance K and substance P receptors, J. Pharmacol. Exp. Ther. 238 No. 1, 341.

Maggi, C.A., R. Patacchini, F. Santicioli, E. Theodorsson and A. Meli, 1988, Several neuropeptides determine the visceromotor response to capsaicin in the guinea-pig isolated ileal longitudinal muscle, Eur. J. Pharmacol. 148, 43.

Mathison, R. and J.S. Davison, 1988, Modified smooth muscle response of jejunum in streptozotocin-diabetic rats, J. Pharmacol. Exp. Ther. 244 No. 3, 1045.

Smits, G.J.M. and R.A. Lefebvre, 1992, Influence of age on responsiveness of rat gastric fundus to agonists and to stimulation of intrinsic nerves, Eur. J. Pharmacol. 223, 97.

Sternini, C., J.R. Reeve, Jr. and N. Brecha, 1987, Distribution and

characterization of calcitonin gene related peptide immunoreactivity in the digestive system of normal and capsaicin-treated rats, Gastroenterology 93, 852.

Szolcsànyi, J. and L. Barthò, 1978, New type of nerve-mediated cholinergic contractions of the guinea-pig small intestine and its selective blockade by capsaicin, Arch. Pharmacol. 305, 83.

Vane, J.R., 1957, A sensitive method for the assay of 5-hydroxytryptamine, Br. J. Pharmacol. 12, 344.

Yau, W.M. and M.L. Youther, 1982, Direct evidence for a release of acetylcholine from the myenteric plexus of guinea-pig small intestine by substance P, Eur. J. Pharmacol. 81, 665.