Lower oesophageal sphincter response to pentagastrin in chagasic patients with megaesophagus and megacolon

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SUMMARY Intraluminal manometric studies were performed in 14 chagasic patients with megaesophagus, 10 chagasic patients with megacolon, and 15 control subjects. Basal lower oesophageal sphincter pressure was 20.27 ± 1.16 mmHg (mean $\pm$ SEM) in controls as compared with 15.16 ± 1.53 mmHg in chagasics with megaesophagus and 14.38 ± 1.50 mmHg in chagasics with megacolon. Dose-response studies to intravenous pentagastrin showed that the chagasic patients exhibited a lower sensitivity to the stimulant than did the controls, as demonstrated by shifting of the dose-response curve to the right and higher individual values of the dose for half maximal contraction (D_{50}). No difference was noted between the calculated maximal contraction (V_{max}) of oesophageal sphincter of controls and chagasics. These data are compatible with the hypothesis of an interaction between pentagastrin and cholinergic nervous excitation on oesophageal sphincteric smooth muscle.

It has been suggested that lower oesophageal sphincter hypertension plays a major role in the impairment of oesophageal emptying in idiopathic megaesophagus (Cohen, 1965; Cohen and Lipshutz, 1971; Cohen *et al.*, 1971). This hypertension has been ascribed to supersensitivity of the lower oesophageal sphincter to endogenous gastrin. Although conclusive evidence demonstrating loss of myenteric ganglia in the achalasic lower oesophageal sphincter is lacking, it has been suggested that this supersensitivity is due to denervation (Cohen *et al.*, 1971).

In Chagas' disease there is autonomic denervation throughout the whole length of the digestive tube, megaesophagus and/or megacolon being its most frequent consequences. Anatomical evidence of a marked and consistent reduction of myenteric ganglia in chagasic megaesophagus was provided by Koerberle (1956, 1957, 1962).

It is the purpose of this study to use a method of proven validity (Dodds, 1976) quantitatively to assess the effect of pentagastrin on lower oesophageal sphincter pressure of patients with proven denervated megaesophagus.

Methods

Studies were performed in two groups of patients: 15 control subjects and 24 chagasic patients. Patients aged 17 to 45 years (mean 31 years) whose upper gastrointestinal radiographs were normal and whose complement fixation tests for Chagas' disease were negative (de Freitas, 1952) were selected as controls. No patient had undergone surgery of the upper gastrointestinal tract. The chagasic patients, aged 24 to 61 years (mean 45 years), met the following criteria: (1) positive complement fixation test for Chagas' disease, (2) clinical and radiographic evidence of chagasic involvement of the digestive tract—that is, megaesophagus or megacolon, and (3) no history of previous therapeutic procedures for megaesophagus. Fourteen chagasic patients had megaesophagus and 10 had megacolon.

Three water-filled polyvinyl tubes, 1.2 mm internal diameter, transmitted intraluminal pressures to external transducers (P-1000 A, Narco Bio-Systems Inc., USA). Pressures were recorded on a 6-channel Physiograph rectilinear ink-writing recorder (Narco Bio-Systems Inc., USA). The tubes were arranged so that intraluminal pressures were recorded at three points 5 cm apart through lateral orifices

1.2 mm in diameter. Pressures were recorded as millimetres of mercury, mean gastric fundic pressure being used as the zero reference.

All patients were studied while resting quietly in the supine position, after 10 hours of fasting. A belt pneumograph around the chest monitored respiration. The recording assembly was positioned with all orifices in the stomach and after a 15 minute rest period it was moved at 0.5 cm intervals through the full length of the oesophagus. Wet deglutitions were elicited by squeezing 5 to 10 ml of distilled water from a plastic bottle into the open mouth of the patient and signed in the record with the aid of an external event marker. After this manometric evaluation, the tube assembly was positioned and anchored so that intraluminal pressures were recorded simultaneously from the distal oesophagus, the lower oesophageal sphincter, and the fundus of the stomach. The pressure recording tubes were infused with distilled water by a syringe pump (Palmer Injection Apparatus, F 135, England), at a constant rate of 0.8 ml/min. Basal pressure in each subject was evaluated by the mean of the pressures obtained during the pull-through of the three recording orifices.

Gastrin pentapeptide (Peptavlon, ICI) was given intravenously as 30 second injections through an indwelling antecubital catheter. Multiple injections were given on a single day, the doses ranging from 25 to 500 ng/kg and successive injections were separated by an interval after which lower oesophageal sphincter pressure had returned to basal values (20 minutes). These studies were carried out in all controls and in 20 chagasic patients (11 with megaesophagus and nine with megacolon). Individual dose-response curves were constructed taking the highest value of pressure recorded in response to each dose administered. Regression analysis according to Dowd and Riggs (1965) was applied to individual dose-response curves and permitted the calculation of individual values of the theoretical maximal contraction (V_{max}) and the dose for half maximal contraction (D_{50}). In four additional chagasic patients (three with megaesophagus and one with megacolon) dose-response studies were performed with the following doses of pentagastrin: 50, 150, 250, 750 and 1000 (1500 in one patient) ng/kg.

Student's *t* test for unpaired data was used in the statistical analysis of the data. Differences were regarded as significant if $P < 0.05$.

Results

In Fig. 1 are shown the basal pressures recorded from 15 controls, 10 chagasics with megacolon and

Fig. 1 Basal lower oesophageal sphincter pressures recorded in 15 controls (○), 10 chagasics with megacolon (▲) and 14 chagasics with megaesophagus (●). Each point represents the mean of three measurements in an individual. Horizontal bars show the respective means.

14 chagasics with megaesophagus. Mean sphincter pressure in the control group was 20.27 ± 1.16 mmHg (mean $\pm$ SE) as compared with 14.38 ± 1.50 mmHg in patients with megacolon ($P < 0.005$) and 15.16 ± 1.53 mmHg in patients with megaesophagus ($P < 0.025$).

In Fig. 2, dose-response curves of the lower oesophageal sphincter pressure to intravenous injections of pentagastrin are shown for 15 controls, nine chagasics with megacolon and 11 chagasics with megaesophagus. Qualitatively the response of the lower oesophageal sphincter was identical in the three groups, but quantitatively the response of controls differed from that of chagasics. No difference in quantitative response was noted between chagasics with megacolon and with megaesophagus. The dose-response curve in the patients with megacolon and in those with megaesophagus were shifted to the right, which indicates a lower sensitivity of the sphincter to pentagastrin, compared with the controls. This finding is also illustrated in Fig. 3, where individual values of D_{50} for all controls and chagasics are shown. Mean D_{50} in the control group was 35.5 ± 5.7 ng/kg (mean $\pm$ SE) as compared with 69.8 ± 14.8 ng/kg in patients with megacolon and 172.2 ± 71.5 ng/kg in patients with megaesophagus.

Fig. 2 Dose-response curves of change in lower oesophageal sphincter pressure against log dose of pentagastrin. At each point is the mean $\pm$ ISE. Data are from responses obtained in 15 controls ($\circ$), nine chagasics with megacolon ($\blacktriangle$) and 11 chagasics with megaesophagus ($\bullet$). * $P < 0.005$; ** $P < 0.01$; *** $P < 0.05$.

Fig. 3 Individual values of D_{50} in 15 controls ($\circ$), nine chagasics with megacolon ($\blacktriangle$) and 11 chagasics with megaesophagus ($\bullet$). Horizontal bars show the respective means.

The most striking feature, though, was the shape of the dose-response curves: for controls it started 'bending' at the dose of 150 ng/kg and then followed to a plateau, while those for chagasics proceeded as straight lines up to the highest dose used. Another interesting finding is that the highest pressures developed by the chagasic sphincter (30.09 ± 5.47 mm Hg and 29.27 ± 4.55 mm Hg at the dose of 500 ng/kg in patients with megacolon and megaesophagus respectively) were lower than that of controls at the dose of 150 ng/kg (35.55 ± 3.48 mm Hg), though not statistically significant ($P > 0.25$). The hypothesis was raised that the chagasic dose-response curve would start 'bending' at the same pressure levels of the control one. In accordance with this is the finding that the calculated maximal response (V_{max}) in the control group (42.54 ± 3.94 mm Hg) was not significantly different from that of chagasics with megacolon (32.48 ± 6.51 mm Hg; $P > 0.1$) and that of chagasics with megaesophagus (35.40 ± 6.17 mm Hg; $P > 0.25$ (Fig. 4)). To test this hypothesis, dose-response studies using higher doses of pentagastrin were carried out in four additional chagasic patients. Individual dose-response curves for these patients are shown in Fig. 5. In all these patients 'bending' of the dose-response curve

Fig. 4 Individual values of V_{max} in 15 controls (○), nine chagasic with megacolon (▲) and 11 chagasic with megaesophagus (●). Horizontal bars show the respective means.

occurred at a dose of pentagastrin higher than 150 ng/kg.

Discussion

These studies indicate that the lower oesophageal sphincter in chronic chagasic patients with megaesophagus and with megacolon is hypotensive and shows lower sensitivity to pentagastrin compared with that in control individuals. These findings do not agree with those reported by Cohen *et al.* (1971) in idiopathic megaesophagus—that is, hypertension and hypersensitivity of the sphincter to intravenous gastrin I. These authors have suggested that the high basal pressure in patients with achalasia is due to supersensitivity to endogenous gastrin and have also proposed that the sphincter hypersensitivity to gastrin is due to denervation of the oesophagus, although conclusive evidence of denervation of the achalasic sphincter is not available (Trounce *et al.*, 1957; Cassella *et al.*, 1964).

In Brazil, Koeberle (1962) has counted the myenteric ganglion cells in 39 different levels of the oesophagus from upper to the lower sphincter in specimens from chagasic patients and age-matched controls who died in accidents. The chagasic patients showed a striking reduction of myenteric ganglion cells, those with megaesophagus disclosing a

Fig. 5 Individual dose-response curves of change in lower oesophageal sphincter pressure against log dose of pentagastrin in three chagasic with megaesophagus (●) and one with megacolon (▲).

reduction of more than 90%. Neural involvement in Chagas' disease is predominantly parasympathetic because of the special preference of the parasite for smooth and cardiac muscles, but sympathetic involvement also occurs, though less intense.

Gastrin has been proposed to stimulate the postganglionic segment of cholinergic nerves to the sphincter (Bennett, 1965; Lipshutz *et al.*, 1971; Vizi *et al.*, 1973) and so to increase its pressure. Cannon (1939) has shown that denervation leads to supersensitivity not only to natural transmitters but also, non-specifically, to other chemical stimulants.

In the truly denervated chagasic sphincter a lower sensitivity to pentagastrin was established by showing a rightward shift of the dose-response curve, a higher D_{50} , and the same calculated maximal contraction (V_{max}) compared with controls. These findings indicate that the full stimulatory effect of pentagastrin on the lower oesophageal sphincter depends on vagal completeness and also raise some questions about the mechanism of action of pentagastrin on sphincter muscle.

There seems to be no doubt that the lower oesophageal sphincter function is controlled by the complex interaction of sphincteric smooth muscle, autonomic innervation, and gastrointestinal hormones (Castell, 1975). Despite extensive investigation (Crispin *et al.*, 1967; Lind *et al.*, 1968; Mann *et al.*, 1968; Blackman *et al.*, 1971; Dodds *et al.*, 1975; Roling *et al.*, 1972; Mazur *et al.*, 1973; Behar and Kastendieck, 1974; Rattan and Goyal, 1974; Higgs and Castell, 1975), the specific role of cholinergic control of sphincter pressure still remains to be clarified (Castell, 1975). In the past decade, considerable controversy has arisen regarding the importance of gastrin in physiological control of sphincter pressure (Cohen and Harris, 1972; Nebel and Castell, 1972; Grossman, 1973; Goyal, 1974; Farrell *et al.*, 1974); however, the recent observations of Freeland *et al.* (1975) give support to such a role for gastrin. Of particular interest are the observations made by Zwick *et al.* (1976), who showed that in the dog the stimulatory effect of pentagastrin is mainly due to a direct action on the sphincter muscle. In addition, the possibility of an interaction between hormonal and nervous mechanisms on gastrointestinal smooth muscle, like the synergism between gastrin and cholinergic nervous excitation on the gastric oxyntic glands, has been raised by Davison (1974) who also presented some more encouraging data on this possibility.

Should the observations of Zwick *et al.* and Davison be valid for the human gastro-oesophageal sphincter they would explain the present findings in the chagasic sphincter. The parasympathetic denervation in Chagas' disease, mainly at the post-

ganglionic level, would result in loss of the tonic vagal activity to the muscle fibres of the sphincter, so decreasing its responsiveness to pentagastrin. A similar explanation has been given for the decreased responsiveness of the gastric pepsin- and acid-secreting cells to histamine and to pentagastrin in Chagas' disease (Padovan *et al.*, 1977, 1979). If gastrin plays an important role in physiological control of sphincter pressure, then the above interpretation would explain the lower levels of basal pressure found in chagasic patients as well.

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