CLINICAL RESEARCH

Generalised smooth-muscle disease with defective muscarinic-receptor function

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Abstract

A patient with widespread smooth-muscle disease presented with chronic intestinal pseudo-obstruction but had in addition defects of the bladder, pupils, sweating, and cardiovascular function. There was no evidence of a primary neural lesion, and minor changes in the muscle did not resemble those of a myopathy. In each organ affected muscarinic cholinergic function was at fault, but instead of supersensitivity to cholinergic drugs, which occurs in postganglionic autonomic neuropathies, there was a lack of response to cholinergic drugs and anticholinesterases. It was therefore concluded that the patient had a new type of defect of muscarinic-receptor function. The cause was unknown, but it may have been an autoimmune disease resembling myasthenia, in which there is a postjunctional defect of muscarinic receptors.

In similar cases binding of muscarinic agonists and antagonists should be tested. When antibodies to purified human muscarinic receptors become available different patterns of smooth-muscle defect may be identifiable, enabling the lesion to be defined more precisely.

Introduction

An increasing number of syndromes of autonomic failure have recently been described, both chronic[1] and acute.[2 3] These syndromes may affect both the sympathetic and parasympathetic parts of the autonomic nervous system, centrally and peripherally. We report here on a patient who presented with apparent chronic intestinal pseudo-obstruction[4] and had, in addition to intestinal symptoms, defects of the bladder, pupils, sweating, and cardiovascular control. Studies in this patient suggested a postjunctional defect of muscarinic receptors—that is, a type of smooth-muscle disease with cholinergic autonomic failure not previously described.

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Case report

The patient was a 22-year-old woman, formerly a post-office worker, who had suffered from constipation and recurrent urinary infections since childhood. There was no family history of any similar disorder. In 1974 she was admitted to hospital with the first of a series of increasingly severe attacks of acute abdominal pain and vomiting. Intestinal obstruction was suggested by the clinical features and *x*-ray films of the abdomen, but laparotomy failed to show any evidence of obstruction. The pupils were noted to be dilated and unresponsive to light or convergence. From 1974 onwards she had attacks of abdominal pain every two to three months, often requiring admission to hospital. Between 1974 and 1976 her weight fell from 49 kg to 44 kg and she was unable to work. Her abdominal pain did not respond to oral analgesic drugs, and she was eventually treated with intramuscular pentazocine (30 mg) at times of severe pain. She was not improved by carbachol 2 mg thrice daily by mouth or 0·5 mg by subcutaneous injection. Pyridostigmine 30 mg three-hourly, later increased to 60 mg four times daily and then 120 mg four times daily, did not appreciably reduce the frequency or severity of her attacks. Between the attacks of abdominal pain her bowels were open daily, with the aid of danthron, and she passed pale, foul-smelling stools. A rectal biopsy specimen obtained in 1974 showed no appreciable abnormalities of the ganglion cells or muscle. She was referred for special autonomic investigation in 1977.

On examination she was emaciated and pale. Between attacks there were no abdominal signs but the rectum contained an excessive amount of faeces. The pupils were dilated, and clinical examination showed no perceptible reaction to light or on convergence. There was no abnormality of the reflexes and no other neurological abnormality. Lacrimation and salivation were normal.

After she had lost more weight with increasingly severe attacks of abdominal pain a regimen of parenteral feeding was introduced in the hope of improving her nutrition and reducing her pain. Further laparotomy was necessary, however, to exclude a mechanical intestinal obstruction. This was undertaken after 10 days of exclusively parenteral feeding. At laparotomy dilated coils of small intestine were found matted together with adhesions as a consequence of the previous laparotomy. The adhesions were divided but there were no obstructive bands causing any localised obstruction. A full-thickness

biopsy specimen was taken from the second loop of the jejunum. She made an initial recovery from the operation but died suddenly eight days later, probably from cardiac arrest after inhaling vomit. Permission for necropsy was not obtained.

Investigations

GASTROINTESTINAL INVESTIGATIONS

Pentagastrin stimulation tests showed free gastric acid to be present. Faecal fat studies showed a low stool volume and faecal fat excretion at the upper limit of normal. A Schilling test was carried out and 0·4% of the oral dose was excreted in the urine; there was no increase with intrinsic factor or after a course of oral antibiotics. A "breath test" after oral administration of radioactive glycine-1-^{14}C-labelled glycocholate showed excessive excretion of $^{14}CO_2$ in the breath, compatible with intestinal stasis. These results indicated some degree of malabsorption secondary to stasis. Radiological barium studies showed no peristaltic activity in the oesophagus and delayed entry of barium into the stomach at the level of the cardiac sphincter. There was also delay in gastric emptying, some barium remaining for 72 hours in an enormously dilated duodenal loop, which extended towards the right iliac fossa (fig 1). The small bowel was so dilated

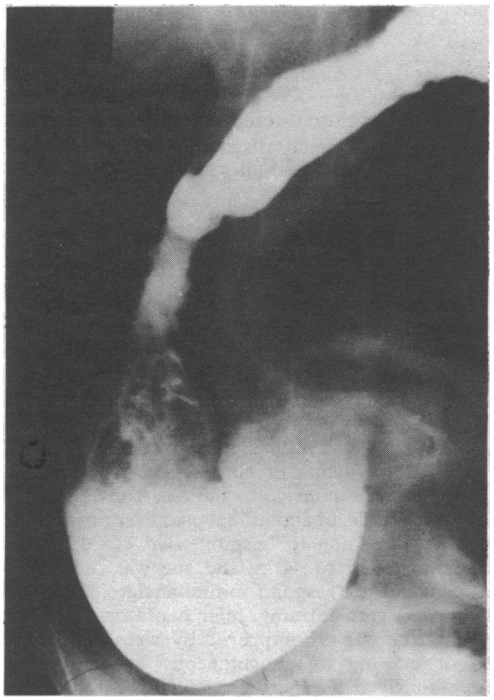

FIG 1—Seventy-two-hour film of barium meal showing dilated duodenal loop extending towards right iliac fossa.

that it resembled large intestine. No barium reached the colon, which was loaded with faeces. On a later examination, undertaken after treatment with pyridostigmine 180 mg three-hourly for 24 hours, barium remained in the duodenum three hours after the meal. A barium-enema examination was normal.

OTHER TESTS OF AUTONOMIC FUNCTION

Sweating—On raising the central body temperature by 1°C there was no trunk or limb sweating. Intradermal 0·5% methacholine chloride caused scanty sweating around the site of the injection of the forearm but none on the leg.

Cardiovascular function—Intra-arterial recording using a radial artery catheter showed no postural hypotension on tilting. Heart-rate responses to stress and tilting were normal, as were blood-pressure and heart-rate responses to the Valsalva manoeuvre. Sinus arrhythmia was absent, however, on beat-to-beat recording. Plasma noradrenaline concentrations, resting and after tilting, were in the low-normal range (mean concentration resting 275 ng/l and after tilting 300 ng/l; normal resting 200-500 ng/l, after tilting 400-800 ng/l).[5] There were normal pressor responses to intravenous infusion of noradrenaline and tyramine without evidence of denervation supersensitivity.[6]

Pupils—The pupils were consistently dilated, being up to 8 mm in diameter in normal illumination, and without perceptible responses to light and accommodation. On slit-lamp examination the iris was noted to be thin. There was no constriction to 2·5% methacholine bromide and only a slight constriction to 1% pilocarpine hydrochloride and 1% physostigmine salicylate. For a dilated pupil these results indicate a lack of both tonic cholinergic innervation and the normal response to cholinergic drugs. The pupillary responses to sympathomimetic drugs were normal, considering the degree of dilatation of the pupil.

Bladder function—A filling cystometrogram was normal, but attempts to void were poor, with an interrupted stream and a large residual volume. The bladder was virtually atonic and did not respond to carbachol 2 mg by mouth or 0·5 mg subcutaneously. An intravenous pyelogram showed dilated atonic ureters and bilateral hydronephrosis (fig 2), which had increased over three years.

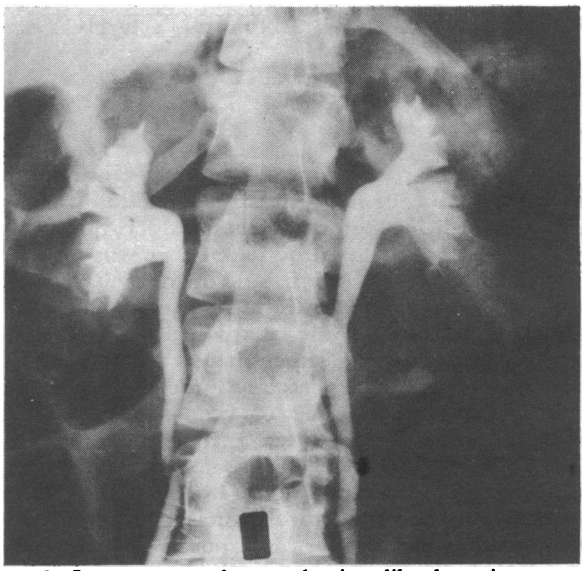

FIG 2—Intravenous pyelogram showing dilated atonic ureters and bilateral hydronephrosis.

JEJUNAL BIOPSY

No abnormality of the villous architecture or epithelium was seen. Silver staining showed that a few of the neurones in the myenteric plexus had thick processes such as have been reported in drug overuse[7] but that the plexus was otherwise morphologically normal (Dr B F Smith, St Bartholomew's Hospital, London). There was no evidence of the type of neural defect described in two cases of intestinal pseudo-obstruction.[8] In material processed for ultrastructural examination neuronal perikaryon was identified in both the submucous (Meissner's) and myenteric (Auerbach's) plexuses. There was no structural abnormality in the perikaryon and dendrites of neurones in the submucous and myenteric plexuses. Axons containing accumulations of small clear vesicles or numerous large dense-cored vesicles were also observed, both in the plexuses and in the nerves in the muscularis mucosa and muscularis externa (fig 3).

MUSCLE CELLS

The overall morphology of the muscle appeared normal. Some large eosinophilic bodies, however, were seen in the paranuclear cytoplasm of some of the muscle cells. With periodic-acid Schiff

these structures appeared as vacuoles and some contained small granules that had taken up the stain. At the ultrastructural level these bodies consisted of finely granular or fibrillar electron-dense material (fig 4) not bounded by membranes. The presence in some cells of deposits of dense material between the paranuclear organelles also suggested that the bodies were formed by cytoplasmic condensation of material rather than by autophagocytosis.

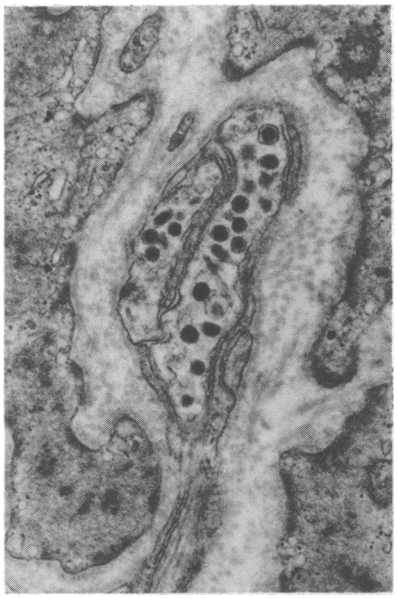

FIG 3—Axonal terminals containing mainly large, dense-cored vesicles in small nerve in muscularis externa. ×27 000 (original magnification).

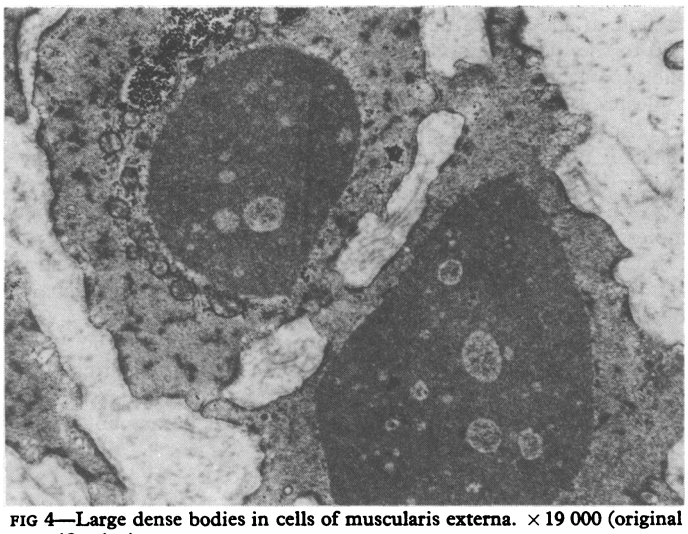

FIG 4—Large dense bodies in cells of muscularis externa. ×19 000 (original magnification).

IMMUNOLOGICAL STUDIES

Plasma electrophoresis showed a mild increase in α_2-globulin, but routine organ-specific and non-specific antibody screening tests were negative. An assay for the antiacetylcholine-receptor antibodies found in myasthenia was negative. Two-dimensional electrophoresis showed a lack of recognisable Gc globulin and an extremely high concentration of antithrombin III (Dr N Anderson, Argonne, USA). The patient's serum did not affect the binding in vitro of antibody to muscarinic receptors (Dr N J M Birdsall, Medical Research Council, London).

Discussion

The disturbance of bowel function in this patient was consistent with the syndrome of idiopathic intestinal pseudo-obstruction.[4] In this condition gross intestinal malfunction is associated with recurrent attacks of abdominal pain similar to that seen in acute obstruction. Patients with the condition usually die eventually of malnutrition or intercurrent infection or after operation, having undergone multiple laparotomies to exclude organic obstruction. Idiopathic intestinal pseudo-obstruction can be diagnosed only by excluding other diseases, drug abuse, and obstructive lesions. In the present case there was no evidence that the abnormality of gastrointestinal function was secondary to other diseases; drug overuse followed but did not precede the bowel symptoms, and organic obstruction was excluded by laparoscopy.

Idiopathic intestinal pseudo-obstruction may be due to defects of the neural or myogenic control system. Pathological changes in the myenteric plexus have been reported in a few cases,[8] and the possibility that the condition is due to a defect of the neural mechanisms controlling gastrointestinal function has been proposed on the basis of in-vivo neurophysiological studies.[9–11] A response of the intestine to neostigmine may, however, be preserved. Cases of a familial smooth-muscle myopathy principally affecting the intestine but occasionally affecting the bladder have also been described[12–14] in which some response to cholinergic and anticholinesterase drugs was preserved. Mydriasis with normal responses to cholinergic drugs, and defective sweating, have also been reported in a few cases. In the present case light and electron microscopical examination of the jejunal biopsy specimen removed at laparotomy showed no degenerative changes in the neurones or axons in the enteric plexuses. There was also no evidence of intercellular deposition of connective tissue or of the membrane disruption and myofilaments in the muscle cells found in other cases of familial smooth-muscle myopathy.[12–14]

The abnormalities in the function of the gut and other organs observed in the present case may most reasonably be attributed to defects in cholinergic muscarinic excitation of smooth muscle. It is generally accepted that muscle-cell contraction in the gut is mediated mainly by cholinergic axons, though other types of neurones probably play some part in controlling contractile activity in both this and other viscera, such as the bladder. Pupillary constriction and sweating are also controlled by cholinergic axons, though the latter are sympathetic not parasympathetic. The reflex mechanism responsible for sinus arrhythmia depends on the innervation of the cardiac pacemaker cells by efferent cholinergic fibres of vagal origin and, like other muscarinic muscle function, is abolished by atropine. The evidence therefore points to a selective cholinergic muscarinic postganglionic lesion. The unique feature of this case was not merely the absence of denervation supersensitivity to cholinergic drugs, which has been present in the few described cases of postganglionic cholinergic neuropathy,[2 3] but a lack of response to cholinergic or anticholinesterase drugs. This points to a postjunctional defect of the muscarinic receptor or its connections with the contractile mechanisms within the cell. The disease was not, however, a myopathy in the accepted sense, and the changes in muscle-cell structure may have been secondary to such a defect or the use of drugs.

Because the morphology and neurophysiology of smooth-muscle muscarinic receptors and the intracellular contractile mechanisms are highly complex[15] we cannot locate the lesion more precisely at this stage. The precise cause of the lesion in this case likewise remains unknown. While the possibility of an autoimmune disease was considered, there was no abnormality of the patient's serum on muscarinic binding in vitro. If fresh tissue from similar patients becomes available, however, the binding of muscarinic agonists and antagonists should clearly be tested. There appear to be different types of muscarinic receptors in different organs, which raises the possibility of being able to identify different patterns of smooth-muscle

defect when antibodies to purified human muscarinic receptors become available. With these techniques it may be possible to define more precisely the lesion in cases of idiopathic intestinal pseudo-obstruction and other smooth-muscle disorders. If immunosuppression or other treatment proved effective then death, which so often eventually follows the diagnosis of idiopathic intestinal pseudo-obstruction, might also be prevented.

We thank Dr J G Walker for helpful advice and Mrs P Barker for skilled technical help.

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Do colonic bacteria contribute to cholesterol gall-stone formation? Effects of lactulose on bile

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Abstract

Ten healthy middle-aged women volunteered for a study to test the effect of lactulose—a synthetic, non-absorbable disaccharide—on the colonic metabolism of bile acids and on bile lipid composition. Lactulose (60 g daily in eight cases, 39 g daily in two) was taken as a proprietary syrup for six weeks, and bile was collected by duodenal intubation before and immediately after the six weeks.

All subjects showed a fall in the percentage of the 7-alpha-dehydroxylated bile acid deoxycholic acid (mean 28·4±SEM 3·7 to 15·6±2·4; $p<0.002$) and a rise in the percentage of the primary bile acid chenodeoxycholic acid (mean 33·2±3·2 to 42·9±2·9; $p<0.001$). The percentage of cholic acid rose in eight subjects but mean values did not differ significantly. Bile was initially supersaturated with cholesterol in most subjects and became less saturated with cholesterol in all but one (mean saturation index 1·40±0·11 to 1·19±0·07; $p<0.005$).

These data support the theory that colonic bacteria contribute to cholesterol gall-stone formation.

Introduction

Bile acids which escape reabsorption in the terminal ileum pass into the colon. There, bacterial 7α-dehydroxylation converts primary bile acids into the secondary forms, cholic acid becoming deoxycholic acid, chenodeoxycholic acid becoming lithocholic acid. These secondary bile acids are partially absorbed and enter the bile acid pool. Feeding small amounts of deoxycholic acid reportedly raised bile cholesterol saturation, leading to the suggestion that increased return of deoxycholic acid to the liver alters its metabolism to favour the secretion of bile supersaturated with cholesterol[1] and hence apt to precipitate cholesterol gall stones. Feeding larger amounts of deoxycholic acid, however, did not produce this effect.[2-4] Administration of metronidazole, an antimicrobial agent active against many colonic bacteria, reduced the proportion of deoxycholic acid in bile, raised that of chenodeoxycholic acid, and lowered bile cholesterol saturation.[5]

In vitro, 7α-dehydroxylation of bile acids is inhibited below pH 6·0-6·5.[6-8] Lactulose, a synthetic, non-absorbable disaccharide, is metabolised to organic acids by the colonic flora and reduces the pH in the right colon to less than 5·0.[9] We therefore predicted that lactulose would reduce 7α-dehydroxylation. To test this hypothesis and look for associated improvement in bile cholesterol saturation we have analysed bile before and after administration of lactulose.

Subjects and methods

Ten apparently healthy women aged 42-51 years (mean 46) volunteered for the study. Their mean body weight was 118% of ideal (range 104-135%). Six subjects were chosen as being known from other studies to have bile supersaturated with cholesterol. For ethical reasons oral cholecystograms were not performed, but no subject had symptoms of gall-bladder disease. As some subjects had highly supersaturated bile, however, a few may have had asymptomatic gall stones. Standard liver function values were normal, and no subject was taking any medication, including oral contraceptives. Subjects maintained their usual diet and activities throughout the study and kept a daily record of bowel movements.

The women were given a proprietary syrup containing 670 g lactulose, 60 g lactose, and 110 g galactose per l). They were instructed

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