Non-adrenergic non-cholinergic inhibitory innervation shown by electrical field stimulation of isolated strips of human gall bladder muscle

M L McKirdy, H C McKirdy, C D Johnson

Abstract
Non-adrenergic non-cholinergic (NANC) inhibitory nerves have been described in all regions of the gastrointestinal tract, but have not been shown previously in the human gall bladder. Electrical field stimulation was used in the presence of various agonists and antagonists to show NANC inhibitory innervation in strips of human gall bladder muscle. Gall bladder strips were set up isometrically in an organ bath containing oxygenated Krebs's solution. Electrical field stimulation was applied at 10 Hz, pulse width 0-3 ms and supramaximal voltage at intervals of 3 to 5 minutes. Of 60 strips that contracted in response to electrical field stimulation, 30 first time a NANC inhibitory response on electrical field stimulation in the presence of either carbachol (5-10 μM) or else atropine (0-5-2 μM) plus cholecystokinin octapeptide (0-01-0-1 μM) or caerulein (0-1 nM) or histamine (5-10 μM). In 22 strips this relaxation was not abolished by guanethidine (2-5 μM) showing the NANC nature of this response. The NANC relaxation was abolished by L-nitroarginine (100 μM) and this effect was partly reversible by L arginine (200 μM). All responses to electrical field stimulation were abolished by tetrodotoxin (0-2-2 μM). These results show for the first time a NANC inhibitory innervation in human gall bladder muscle. The probable neurotransmitter is nitric oxide.

(Gut 1994; 35: 412–416)

Non-adrenergic non-cholinergic (NANC) inhibitory nerves supply the smooth muscle of the upper, middle, and lower regions of the digestive tract of all mammalian species studied to date. These nerves have been shown to particpate in relaxation of sphincters, in the descending inhibitory component of the peristaltic reflexes, and in receptive relaxation and accommodation of the stomach. Similar to the stomach, the gall bladder accommodates varying volumes with little change in pressure. It is unknown how much of this adaptation is a result of passive physical properties of the viscus and how much a result of active mechanisms reducing muscle tone.

In the only previous study using electrical field stimulation in human gall bladder, no evidence for such NANC innervation was found. Evidence for mediation of muscle relaxation, however, by NANC inhibitory nerve activity has been found in guinea pig gall bladder. We have reinvestigated the innervation of human gall bladder muscle strips using electrical field stimulation. Our findings suggest a sparse NANC inhibitory innervation. Preliminary findings have already been reported.6

Methods
COLLECTION OF TISSUE
Strips of gall bladder wall were taken from 138 surgically removed gall bladders. One hundred and thirty four gall bladders were removed for cholelithiasis and four normal gall bladders were removed during partial hepatectomy or pancreatic surgery. Macroscopically, inflamed, or fibrosed gall bladders, and those severely distended by a mucousce were not accepted for the study. The gall bladder was removed within 15 minutes of ligation of the cystic artery. Immediately after removal, the gall bladder was opened longitudinally and a segment (about 2-3 cm) was taken from the body of the gall bladder and placed in cooled oxygenated Krebs's solution in a vacuum flask for transport.

Segments of gall bladder wall were then pinned out flat in a Petri dish under cooled oxygenated Krebs's solution. The serosa and tunica adventitia were removed to leave the tunica muscularis with attached mucosa. In 10 specimens the mucosal layer was removed by scraping with a scalpel blade. Several strips (usually four) 10×2-3 mm in size were obtained from each gall bladder and the remaining tissue was used for histological study. It was found that at 2°C, the gall bladder muscle deteriorated within a few hours, unlike muscle strips from other regions of the digestive tract. Experiments were therefore started within one hour of removal of the gall bladder.

RECORDING
Each strip was suspended between parallel platinum electrodes in one of four identical 5 ml organ baths. The bathing fluid was Krebs's solution at 37°C and gassed with 95% oxygen and 5% carbon dioxide. Isometric tension was monitored with a strain gauge transducer. The tension was displayed on a pen recorder (Devices, Letchworth Garden City, Herts). Each preparation was subjected to a maximum initial tension of 0-1 g.

STIMULATION
Electrical field stimulation was performed using rectangular pulses produced by a programmable digital stimulator. Tissues were stimulated at supramaximal voltage using 5-10 second trains

University Surgical Unit, Southampton General Hospital
M L McKirdy
C D Johnson

Department of Surgery, University of Wales College of Medicine, Cardiff
H C McKirdy

Correspondence to: Mr C D Johnson, University Surgical Unit, F Level, Centre Block, Southampton General Hospital, Tremona Road, Southampton SO9 4XY.

Accepted for publication 26 July 1993
of stimuli at 10 Hz and pulse width 0·3 ms. Stimulation was performed once every three to five minutes.

DRUGS AND HORMONES
Drugs and hormones were added in various concentrations to the organ bath. Values in the text show final bath concentration.

Drugs used were as follows: L-arginine (Sigma, St Louis, USA), atropine sulphate (Macfarlan Smith, Edinburgh, UK), caerulein (Sigma), carbachol (Macartphys Medical, Romford, UK), cholecystokinin octapeptide (Sigma), guanethidine sulphate (Ciba, Hershford, UK), histamine acid phosphate (BDH, Poole, UK), L-Ng-nitroarginine (Sigma), pentagastrin (ICI, Wilmslow, UK), and tetrodotoxin (Sigma). Adequate time was permitted after adding each agent for equilibration of the response before further testing.

ANALYSIS OF DATA
Tone, spontaneous contractions, and response (contraction or relaxation) to electrical field stimulation or to added agents were expressed in g tension or frequency/minute. Values are presented as mean (SD), but the important feature of these responses is their presence or absence as neurally mediated responses are generally held to be all or none phenomena. Statistical analysis was performed using Student’s t test.

Results

SPONTANEOUS CONTRACTIONS
Of 138 gall bladders studied, strips from 32 showed little or no spontaneous activity and little or no response to added agonists. These were considered non-viable and were excluded from the study. Of the remaining 106 viable specimens, strips from 92 showed spontaneous contractions at some stage during the experiment.

Spontaneous contractions were recorded as two different forms (Fig 1). Firstly, as low amplitude (0·18 (0·12) g, n=153) high frequency (6·23 (4·7) per min, n=111) rhythmic contractions, and secondly as high amplitude (0·42 (0·2) g, n=15) low frequency (0·69 (0·51) per min, n=15) phasic contractions. These last phasic contractions were recorded in only 15 of the viable specimens and when present had the low amplitude contractions superimposed (Fig 1).

TONE
Tone (sustained resting tension) was consistently low (0·66 (0·32) g, n=153). A greater value of spontaneous tension was attained only during phasic contractions (Fig 1) recorded in strips from 15 of 106 specimens.

ELECTRICAL FIELD STIMULATION
The response of gall bladder strips to electrical field stimulation was a contraction in 60 of 106 specimens. The remainder either did not respond or the response deteriorated rapidly. The response to electrical field stimulation was small (0·32 (0·15) g, n=60) compared with the tension after 10 minutes exposure to carbachol 5 μM (1·05 (0·4) g, n=21) or to cholecystokinin 0·15 μM (1·22 (0·61) g, n=26) in the same set of strips. Larger responses to electrical field stimulation could be obtained by using a longer pulse width, but such responses may not be rapidly abolished by tetrodotoxin.1

EFFECT OF ADDED AGENTS ON THE RESPONSE TO ELECTRICAL FIELD STIMULATION
Of the 60 specimens that showed repeatable contractile responses to electrical field stimulation, strips from 30 showed relaxation of 0·23 (0·08) g to electrical field stimulation when the tone was raised by either firstly reducing (by 45–95%) the response in contraction to electrical field stimulation with atropine (0·5–2 μM) then adding cholecystokinin (0·01–0·1 μM) (Fig 2) or caerulein (0·1 μM) or histamine (5–10 μM); or secondly by using carbachol 5–10 μM (Fig 3) to raise the tone; it has been assumed that

Figure 1: This shows tone and spontaneous activity. (A) The activity of a gall bladder strip approximately 10 minutes after setting up and during equilibration. Low amplitude high frequency rhythmic contractions are superimposed on high amplitude low frequency phasic contractions: (B) a different strip after 45 minutes equilibration to show the interaction between high amplitude low frequency phasic contractions and the response to electrical field stimulation shown by the filled circles. Tension shown in grams.

Gut: first published as 10.1136/gut.35.3.412 on 1 March 1994. Downloaded from http://gut.bmj.com/ on October 21, 2023 by guest. Protected by copyright.
cholinergic receptors are desensitised at this concentration of carbachol.1 In strips from 22 of these gall bladders a relaxation response to electrical field stimulation remained (0·19 (0·09) g) in the presence of guanethidine 2–5 μM, showing the presence of a non-adrenergic non-cholinergic response. In some of these strips the relaxation to electrical field stimulation seemed initially to have been abolished but with repeated stimulation a small relaxation that could be recorded at higher sensitivity developed over a period of up to 60 minutes in the presence of guanethidine (2–5 μM) (Fig 4). All the responses to electrical field stimulation were abolished by tetrodotoxin (0·2–2 μM, n=5) (Fig 2), or by lignocaine (1 μM, n=5).

ROLE OF NITRIC OXIDE
The potent nitric oxide synthase inhibitor L-nitroarginine (L-NOARG) was used at two concentrations in eight specimens. At 10 μM the NANC relaxation response to electrical field stimulation was abolished in one, reduced in three, and unaffected in four.

At 100 μM L-NOARG the NANC response was abolished in all four strips tested. The effect of L-NOARG thus seems to be a dose dependent response. The effect of L-NOARG was partly reversible by exposure to L-arginine (200 μM) (Fig 4).

Discussion

TONE, SPONTANEOUS CONTRACTIONS, AND RESPONSE TO ADDED AGENTS
Tone (spontaneous resting tension) in these studies was low in contrast with the finding of Mack and Todd.16 This may reflect the difference between a lightly loaded isotonic recording system and the isometric system16 that we used. Otherwise the results of these and previous studies11 are in substantial agreement with those of other workers15 regarding spontaneous activity and response to added agents. There is,
Figure 4: Relaxant response to electrical field stimulation developing in the presence of atropine (Atr 1 μM), caracrin (Caer 0·1 μM), and guanethidine (Guan 5 μM). L-nitroarginine (L-NOARG 10 μM) has no effect on the developing response. The response is abolished, however, by L-NOARG 100 μM, and recovery of the response is rapid in L-arginine (L-ARG).

that NANC responses often develop during exposure to guanethidine. We have confirmed that it may be necessary to wait some time to see this phenomenon. Finally the high concentration of propranolol used by Feeley et al would be expected to have a powerful local anaesthetic activity. This might well have impaired the ability of the NANC conducting nerve fibres to respond to electrical field stimulation. This view is reinforced by the finding that D-propranolol, which has almost exclusively local anaesthetic activity, reduced the relaxation to electrical field stimulation though not as effectively as DL-propranolol (100 μM) which has beta blocking and local anaesthetic activity.

We conclude from our findings that the human gall bladder seems to have a sparse NANC inhibitory innervation as well as a cholinergic excitatory innervation and a potentially functional inhibitory noradrenergic innervation.

NITRIC OXIDE
L-nitroarginine reduced or abolished the NANC relaxation in a dose dependent manner, which suggests that nitric oxide participates in NANC transmission in the human gall bladder. This confirms a preliminary report of nitric oxide participation in the relaxation of biliary tract smooth muscle, and is consistent with recent histochemical evidence showing NADPH diaphorase containing neurones in gall bladders of several species including humans. It is also possible that nitric oxide might participate in the NANC relaxation of the sphincter of Oddi. The participation of nitric oxide in the NANC relaxation of other human sphincters and non-sphincter muscle has already been shown.

It is concluded that the human gall bladder may receive a sparse NANC inhibitory innervation. Further studies are required to elucidate the NANC inhibitory response and the possible role of nitric oxide in mediating this response in biliary tract smooth muscle.

We are grateful to Dr R W Marshall for the use of apparatus, to Dr M J Lewis for use of drugs and for constructive suggestions, and to Mr B A Taylor for his help during early experiments. Presented in part at the British Society for Gastroenterology September 1991, and the Physiological Society in December 1991.


5 Al-Hassani MH, Davidson JS. The role of the non-cholinergic, non-adrenergic inhibitory nerves in the regulation of the guinea-pig gallbladder. *J Physiol (Lond)* 1979; 292: 48P.


7 McKirdy ML, Johnson CD. Non-adrenergic, non-cholinergic (NANC) inhibitory innervation of the isolated human gallbladder. *J Physiol (Lond)* 1990; 424: 71P.


10 Talmage EK, Mawe GM. NADPH-diaphorase and VIP are co-localised in neurons of gallbladder ganglia. *J Auton Nerv Syst* 1993; 43: 83–90.
