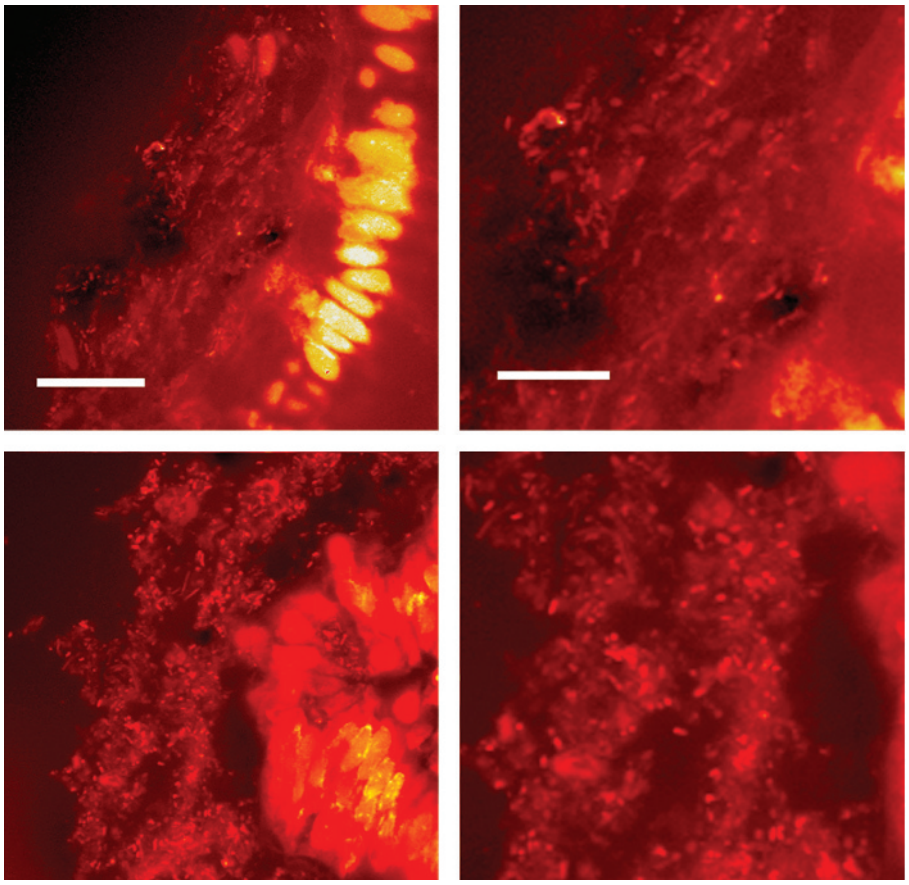

Figure 1 Biofilms adjacent to epithelium in a normal human appendix obtained from a deceased organ donor were observed using a confocal laser microscope following flash freezing, cryosectioning and rapid staining of the tissue with acridine orange as previously described.[6] Two representative sections are shown, and images on the right show an enlarged section of the images on the left. Photos were taken of the areas at the border between the epithelium and the lumen. The smaller fluorescent spots are bacteria within the mucus layer stained with acridine orange, and the larger brightly stained areas are the nuclei of the epithelial cells that also stain with acridine orange. The bars = 30 μm (panels on left) and 15 μm (panels on right).

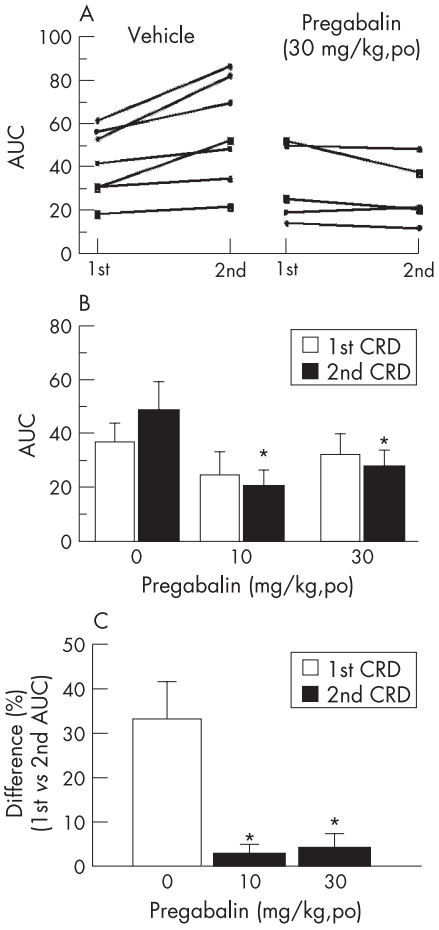

Figure 1 Oral pregabalin decreased the area under the curve of contraction (AUC) of the abdominal electromyogram to two successive tonic colorectal distensions in rats. (A) Individual rat's response to the first and second colorectal distension (60 mm Hg, 10 minutes each and a 10 minute interval) one hour after administration of vehicle (water) or pregabalin (30 mg/kg orally). (B) Group mean of the AUC in rats given vehicle or pregabalin 10 or 30 mg/kg. (C) Per cent difference in AUC between the second and first colorectal distensions in vehicle and pregabalin (10 and 30 mg/kg) treated rats. Values are mean, error bars = SEM. Differences within and between groups were analysed using one way analysis of variance (ANOVA) or a two way repeated measures ANOVA (one factor repetition). *$p<0.05$ *vs* the corresponding vehicle treated rats. CRD, colorectal distension; po, oral administration.

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Pregabalin decreases visceral pain and prevents spinal neuronal activation in rats

We read the recent article by Houghton *et al* (*Gut* 2007, Apr 19 [Epub ahead of print]), reporting that pregabalin, a new generation of $\alpha_2\delta$ ligand, increased sensory thresholds to normal levels in 26 patients with irritable bowel syndrome (IBS) and baseline rectal hypersensitivity, in a randomised double blind, placebo controlled, parallel group study. The authors concluded that $\alpha_2\delta$ ligands are worthy of further physiological and clinical investigations for diseases affecting gut sensory function. Experimental studies to date indicate that pregabalin prevents colorectal allodynia and hyperalgesia in rats exposed to intracolonic trinitrobenzene-sulphonic acid[1] or septic shock.[2] Visceral hyperalgesia and symptoms in IBS are, however, characterised by the absence of overt colonic damage or mucosal abnormality. In the study we describe here, pregabalin given orally in a rat non-inflammatory model

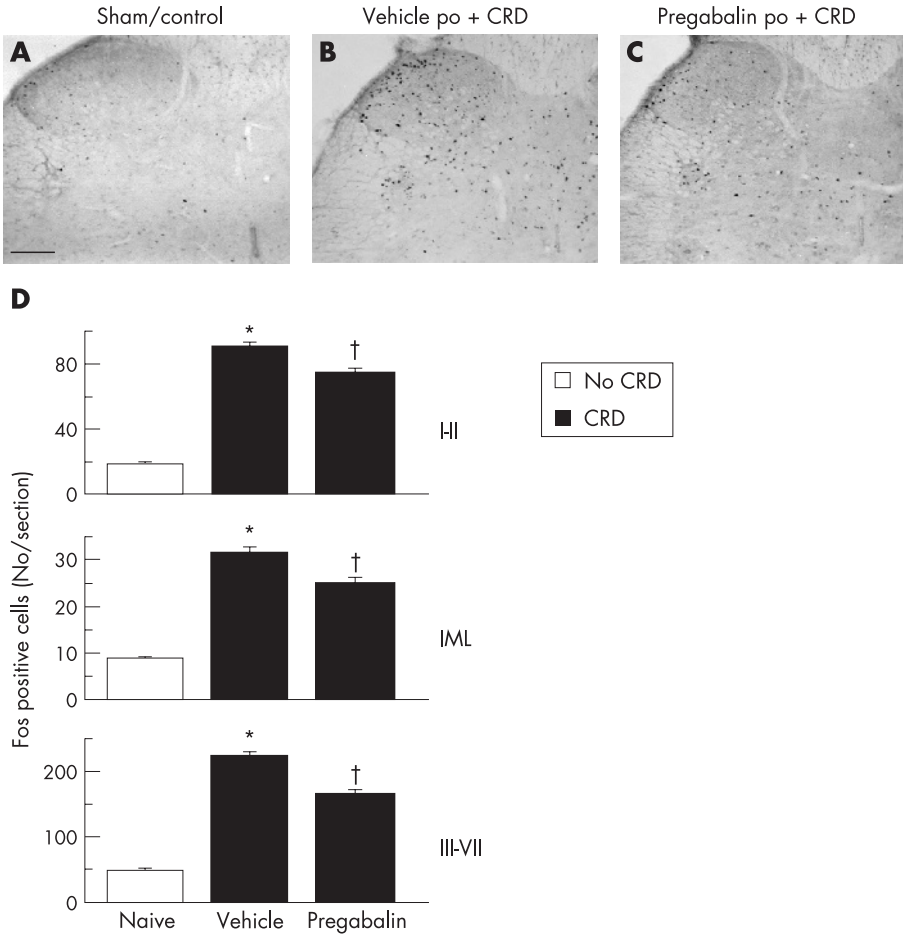

Figure 2 Oral pregabalin blunted spinal activation of dorsal horn neurones induced by two colorectal distensions (60 mm Hg, 10 minutes each with a 10 minute interval) in rats. Photomicrographs of rat L6–S1 spinal cord section showing Fos expression 60 minutes after the last colorectal distension. Scale bar = 100 μm. (A) Sham/control rats. (B) Vehicle + colorectal distension. (C) Pregabalin (30 mg/kg, orally) + colorectal distension. (D) Number of Fos expressing cells in laminae I–II, the intermediolateral column (IML), and laminae III–VII. Fos positive cells were counted bilaterally in the superficial laminae I and II, in the deep layers III–VII, and in the IML in 50 sections of the L6–S1 spinal cord. Values are mean, error bars = SEM. *$p<0.05$ *vs* sham and pregabalin; †$p<0.05$ *vs* sham and vehicle groups; one way analysis of variance followed by Dunn's multiple comparison test.

of repeated tonic colorectal-distension-induced hypersensitivity[3] prevented visceral hyperalgesia and blunted lumbosacral spinal neurone activation.

Adult male Sprague–Dawley rats with or without electrodes implanted in the abdominal muscles were given orally (po) either vehicle (water, 1 ml/rat) or pregabalin (10 or 30 mg/kg; Parke Davis, Fresnes, France) and placed individually in Bollman cages. After a 60 minute stabilisation period, basal abdominal contractions were monitored for 10 minutes, then isobaric colorectal distension (60 mm Hg for 10 minutes twice, with a 10 minute rest interval) was carried out as before.[3] The visceromotor response was quantified either visually (contractions/10 min) or as the area under the curve of contraction (AUC) of the abdominal muscle electromyogram, as previously described.[3] For the spinal neuronal activation study, naive rats received water (1 ml/rat, po) or pregabalin (30 mg/kg, po), or no treatment/no distension (sham control), and were placed in a Bollman cage. Sixty minutes later, colorectal distension was applied as above. At 60 minutes after the end of the second colorectal distension, or at the corresponding time for the sham control, the rats were killed and the lumbosacral spinal cord processed for Fos immunohistochemistry, as previously reported.[4]

The data show that the first colorectal distension caused 23.6 (2.5) (mean (SEM)) abdominal contractions/10 min in the vehicle group ($n = 17$), while none or few were observed under basal conditions. Pregabalin (10 and 30 mg/kg) lowered colorectal-distension-induced contractions/10 min to 18.3 (1.3) ($p>0.05$, $n = 7$) and 12.4 (2.6) ($p<0.05$, $n = 17$), respectively. During the second distension, the abdominal contractions/10 min increased to 29.5 (2.4) in the vehicle group but not in the pregabalin group at both 10 mg/kg (19.1 (1.5)) and 30 mg/kg (11.3 (2.2)). The response to the second distension in the vehicle group was 25.1 (2.3)% greater than the first, suggesting occurrence of sensitisation. Pregabalin at both doses abolished the second distension induced sensitisation. Likewise, in rats fitted with abdominal electrodes and treated with vehicle, the AUC response to the second colorectal distension was increased by 33.2 (8.3)% compared with the first, while in rats treated with pregabalin (10 and 30 mg/kg), this hypersensitivity was blocked (29.5 (3.8) and 30.6 (6.5), respectively, *vs* vehicle 48.6 (10.8), $p<0.05$) (fig 1, panels A and D).

Sham control rats had no or little spinal neuronal activation (fig 2, panels A and C). Colorectal distension induced Fos activation in laminae I–II and the intermediolateral column (IML) (90.4 (2.8) and 31.6 (1.2) cells/section, respectively) in the vehicle group (fig 2, panels B and D). Pregabalin (30 mg/kg, po) significantly reduced the number of Fos positive cells in laminae I, II, and IML to 74.6 (2.6) and 25.1 (1.1), respectively (fig 2, panels C and D). Similarly, in laminae III–VII, the number of Fos positive cells/section induced by colorectal distension was reduced from 223.5 (5.6) in the vehicle group to 164.9 (7.7) with pregabalin ($p<0.05$) (fig 2, panels B to D).

These data show that a single oral dose of pregabalin prevents visceral sensitisation and dampens spinal neuronal activation induced by repeated episodes of colorectal distension in a conscious rat model of visceral hyperalgesia with no underlying colonic pathology.[3] Colorectal distension in non-inflamed rat colon activates neurones in the lumbosacral spinal cord where the nociceptive visceral inputs mainly reach the superficial lamina I, outer II, and deeper V and X, as shown by Fos expression and ERK 1/2 phosphorylation.[3] The inhibitory effect of pregabalin on neurotransmitter release[5] may contribute to the decreased spinal neuronal activation observed in the present study.

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Single aetiology and dual associations of cardiac cancer

With interest we read the article by Hansen *et al.* (*Gut* 2007;**56**:918–25). The authors have to be congratulated on their contribution which profoundly adds to our understanding of the pathophysiology leading to oesophageal and gastric cancer. In addition, the paper points out the difficulties we have to correctly assign tumours of the oesophago-gastric junction (oesophageal vs gastric?). Cardiac cancers were subtyped for their associations with serum anti-*Helicobacter pylori* IgG antibody titer and biochemical markers of loss of gastric secretory function associated with atrophy (pepsinogen I/II ratio and serum gastrin concentration). However, the anatomic criterium to define cardiac carcinoma, that is, tumours centred within 2 cm distal to the oesophago-gastric junction, is inaccurate. Interpretation of the data should be conducted with the inclusion of clear, anatomical and histopathological criteria.

It is well accepted that cardiac cancer and adenocarcinoma of the oesophagus share epidemiological and pathogenetic features.[1 2] After birth the oesophagus is lined by squamous epithelium, whereas the stomach is lined by gastric oxyntic mucosa (with parietal and chief cells).[1 3] Due to gastro-oesophageal reflux, squamous epithelium is damaged and replaced by cardiac mucosa (CM). Thus CM is interposed between squamous and oxyntic mucosa. Via intestinal metaplasia and dysplasia CM may progress towards adenocarcinoma of the oesophagus.[1–3] Anatomy proves that CM is oesophageal: unlike the stomach, the oesophagus has submucosal glands. Histopathology studies of full-thickness oesophago-gastric specimens revealed that CM is only present above submucosal glands, whereas gastric oxyntic mucosa is not underlined by submucosal glands.[1] CM is columnar-lined oesophagus and not stomach.[1] Using multilevel biopsy sampling around the endoscopic visible oesophago-gastric junction in patients with gastro-oesophageal reflux disease, Ringhofer *et al*.[4] showed that CM cannot be differentiated from gastric oxyntic mucosa by endoscopy. Reflux-damaged columnar-lined oesophagus was mistaken as proximal stomach in 51% of the patients.[4] Based on histopathology, tumours arising within CM are of oesophageal origin, while those arising within oxyntic mucosa are of gastric origin.[1 3]

What the authors believe to be a ''dual'' aetiology are in fact ''dual associations''. Their data prove that oesophageal adenocarcinoma arising within CM may be present with or without gastric atrophy and *H pylori* infection of the stomach.[1] *H pylori* may also infect columnar-lined oesophagus (ie, CM)[1 3] and be protective against adenocarcinoma, possibly due to atrophy-induced changes in the composition of the refluate.[1]

Taken together, the aetiology of cancers at the oesophago-gastric junction can only be defined by histopathological criteria: tumours arising within cardiac and oxyntic mucosa are of oesophageal and gastric origin, respectively.[1 3] Consequently, cardiac carcinomas have to be treated as oesophageal cancers and tumours arising from oxyntic mucosa as gastric malignancies. Cardiac cancer has a single aetiology, gastro-oesophageal reflux, but may have associations with *H pylori* infection and gastric atrophy. The authors are kindly asked to address this issue.

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Authors' reply

We are grateful to Dr Lenglinger and colleagues for their interest in our paper and thoughtful comments. However, we disagree with their contention that cardia cancer has a single aetiology and is always a consequence of gastro-oesophageal reflux.

We agree that cardia cancer shares epidemiological similarities with oesophageal adenocarcinoma but would also point out that there are clear epidemiological differences between these two cancers.[1 2] Indeed, the epidemiology of cardia cancer tends to share epidemiological features with both oesophageal adenocarcinoma and non-cardia gastric cancer consistent with these cancers being a mixture of two aetiological subtypes – one being similar to non-cardia cancer and one being similar to oesophageal adenocarcinoma.

We disagree that cardia mucosa always represents a metaplastic response. Indeed, cardia mucosa at, or proximal to, the angle of His has been shown to be established during gestation and present at birth.[3] Whether this transition zone between the squamous epithelium of the oesophagus and the oxyntic mucosa of the stomach is oesophageal or gastric may in the end be a matter of definition.

We also disagree that the only aetiology of carditis and columnar intestinal metaplasia at the gastro-oesophageal junction is gastro-oesophageal reflux. *Helicobacter pylori*-induced atrophic gastritis may also produce inflammation, loss of oxyntic glands and intestinal metaplasia at the gastric cardia.[4–6] Whereas reflux disease causes intestinal metaplasia extending proximally to the original squamocolumnar junction, *H pylori* atrophic gastritis causes intestinal metaplasia extending distally from the original squamocolumnar junction.[7 8] In practice, it is very difficult to determine by purely examining the columnar mucosa at the gastro-oesophageal junction whether it has developed from original oesophageal or original gastric mucosa. Contrary to Dr Lenglinger and colleagues' statement that all cardia tumours are oesophageal in origin, the latest version of the International Classification of Diseases classifies cardia tumours as gastric tumours (C 16.0).[9]

We agree that in the Western world where *H pylori* and gastric atrophy are relatively rare, the majority of cancers at the gastro-oesophageal junction will have arisen as a consequence of gastro-oesophageal reflux. However, in China and Japan, such cancers are mainly of the other aetiological subtype being a consequence of *H pylori*-induced atrophic gastritis.[10 11] Consequently, at a global level, we believe that cancer of the gastro-oesophageal junction and cardia can be of two aetiological subtypes. As proposed in our paper, examination of the gastric mucosa distant from the gastro-oesophageal junction is likely to be helpful in determining the particular aetiology of gastro-oesophageal junction cancer in individual patients.

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