

Altered Autonomic Control in Rat Intestine Due to Both Infection with *Anisakis simplex* and Incubation with the Parasite's Crude Extract

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Anisakis simplex IgE may bring on allergic responses such as angioedema, vomiting, and urticaria from eating seafood, but it is not the only etiology. Induced cholinergic hyperreactivity or adrenergic blockade in the target tissue can cause these diseases nonimmunologically also. Here we studied the effects on normal intestinal motility of brief *A. simplex* infections and *in vitro* exposures to the parasite's extract (CE). Each approach was evaluated according to its ability to induce cholinergic hyperreactivity or adrenergic blockade in rat duodenum (RD), jejunum (RJ), and ileum (RI) *in vitro*. Additionally, bolus propulsion in RD, RJ, and RI was evaluated with time in vivo utilizing animals infected 4 h previously with *A. simplex* larvae (L3) vs sham animals. Tissues, after inoculation of 1, 5, 10, and 20 L3, exhibited time- and dose-dependent motility changes after carbachol (Ch) and noradrenaline (NA), justifying our using herein rats from the fourth hour of infection with 20 L3. We observed a persistent, yet differential effect of the infection on RD, RJ, and RI responses to Ch or NA. It caused cholinergic (muscarinic) hyperreactivity in RD only, and adrenergic blockade in all other parts, and consequently increased the transit index in RD, not in RJ or RI. In contrast, exposing RD, RJ, and RI to CE persistently increased both parameters, amplitude of twitches and muscular tone, in all, albeit that, here also, responses to Ch and NA were CE dose dependent. Interestingly, sensitivity to CE was in the order RI > RJ > RD, the reverse situation of that observed during active infection. Thus, previously viable *A. simplex* L3, after digestion, can exert bystander disturbance in autonomic control in the whole intestine. Our findings demonstrate that *A. simplex* L3, alive or dead, can induce cholinergic hyperactivity and adrenergic blockade in the whole small intestine and, as a consequence, gastrointestinal symptoms. Significantly, they may do so long before parasite-specific IgE is detectably induced or despite the occurrence of such IgE.

KEY WORDS: *Anisakis simplex* infection; crude extract; intestinal motility; intestinal autonomic control.

Anisakis simplex (As) is a common gastrointestinal parasite of a wide variety of marine mammals. Infection is acquired and completed in these animals by feeding on

fish and shellfish in which its infective larvae (L3) have encysted. However, knowledge gained from human studies (1) and animal experimentation (2, 3) has shown that As L3 can readily infect land mammals too. The human condition has only been recognized since the early 1960s, when there were reports of humans with a severe, occasionally fatal, syndrome after consuming As-infested seafood. In these patients the L3 were shown to excyst in the gut to thereafter cause either gastrointestinal anisakiosis or, after burrowing through the gut wall and migrating to internal organs, ectopic anisakiosis (4). Infection with

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As, like other nematodes, is accompanied by eosinophilia, mastocytosis, elevated IgE levels, and immune responses associated with the T helper (Th)-2 subtype (5). However, in anisakiasis, unlike in most helminthiasis, infections may result in strong allergic reactions (6, 7).

Most of the above work has been done using animal models or patients with confirmed presence of As L3 and associated damage and responses. It is now clear, however, that many As infections remain unnoticed because there are no overt clinical signs (8). Notwithstanding that, they may be important "triggers" involved in the induction of antibodies capable of causing the aforementioned allergic responses (9). Moreover, a stimulus for Th-2 differentiation in As L3 extracts has been demonstrated (10). In contrast, no attention has been paid to nonimmunologic factors that may be involved in the expression of As-type allergy. This is surprising, as both specific IgE and most of the diseases in which allergy is expressed (anaphylaxis and urticaria–angioedema) can occur in the absence of allergy (7, 11). An important condition causing such side effects is a disturbance in the autonomic control, such as adrenergic blockade and cholinergic hyperreactivity in the target tissue (11, 12). This report presents evidence that infections with As and *in vitro* contacts with the parasite's homogenate alter rat small bowel excitability to two exogenous agonists. In addition, we demonstrate that active infection alters normal small bowel propulsion of ingested material. These results are interesting inasmuch as 70% of symptoms in As allergy in our part of the world are confined to the digestive tract (7).

MATERIALS AND METHODS

Drugs/Reagents

Carbachol (Ch) and noradrenaline (NA), both from Sigma Chemical Co. (St. Louis, MO), where needed, were appropriately diluted and added in 5- μ l aliquots to the bath to make final 10^{-5} to 10^{-7} M solutions. PBS is sterile 10 mM phosphate-buffered saline (pH 7.2). PBS.pen.strep is PBS with penicillin (1 mg/ml) and streptomycin sulfate (2 mg/ml) added. Krebs' solution (KrS) is a buffer composed of 119 mM ClNa, 24.9 mM NaHCO₃, 4.6 mM KCl, 1.2 mM MgCl₂ (6H₂O), 1.4 mM KH₂PO₄, 0.027 mM EDTA (2H₂O), 11 mM glucose, and 1.5 mM CaCl₂. Oxygenated KrS is KrS (as bathing solution) aerated with a mixture of 95% O₂ and 5% CO₂.

Parasite and Whole-Parasite Extracts

Encysted As L3 were collected from naturally infected fresh blue whiting (*Micromesistius poutassou*). The L3 larvae were stripped of all adherent membranes and then washed (by gentle agitation) 10 $\times$ with sterile saline solution and once with PBS.pen.strep.

Crude extracts (CE) were prepared from a pelleted As L3 by homogenization in PBS using a glass–Teflon homogenizer. After

centrifugation at 100,000g for 30 min at 4°C, the supernatant (CE) was removed. The CE was assayed for total protein using the Bradford (Bio-Rad) or Lowry et al. assay (13). Aliquots of the CE (capable of giving final doses of 0.5 to 6 mg by the addition of 5- μ l aliquots to the organ bath) were either used immediately after preparation or stored at –80°C until used.

Study Animals

Wistar rats of both sexes and weighing between 180 and 200 g were used in the study. Animals were housed in separate cages and were given water and food *ad libitum*. There were two experimental groups: uninfected controls and As L3-infected animals (i.e., with varying numbers of the parasite). Animals were anesthetized (with chloroform) where required, and killing was done by cervical dislocation.

Intestinal Contractility Tests

Tissue Preparation. Intestinal tissues were prepared as described below to detect changes in tone, amplitude, and frequency of spontaneous contractions and adrenergic–cholinergic irritability *in vitro* of rat intestinal segments after active parasitization by As L3 or incubation with As L3 CE. Experiments involving measurements of the above contractility parameters were done with at least five separate sets of intestinal segments (duodenum, jejunum, or ileum) from five rats. Small intestines from infected/uninfected rats were excised and placed in oxygenated KrS at 37°C. They were then cut at 1–5 cm from the pylorus, 1–5 cm from the Treitz ligament, and 1–5 cm from the caecum, thus providing a proximal duodenal, a jejunal, and an ileal segment, respectively. The segments were cleaned out by gentle perfusion with KrS and then prepared for isometric muscle contraction measurements.

Measurement of Isometric Muscle Contractions. The above intestinal segments were each cut in to pieces 2 mm long and suspended vertically in an organ bath (10 ml) using two silk wires, one attached to the bath's bottom and the other to a force transducer (Dioden Controls Ltd., Dynamometer UF1). The organ bath and its attached reservoir were kept at 37°C and filled with oxygenated KrS. The signal of the force transducer was amplified and recorded continuously on a Powerlab program. Tissues were equilibrated for 30 min at 37°C in KrS at a resting tension of 1 g prior to assay.

Experimental Protocol

Experiment 1. Determination of Ch and NA concentration–response curves: Intestinal segments from uninfected rats were tested before and after increasing (10^{-7} to 10^{-5}) noncumulative doses of the muscarinic (Ch) or exogenous α -adrenergic agonist (NA). After each observation, the tissues were rinsed three times and equilibrated in fresh KrS during 30 min before the addition of the next dose. The Ch or NA concentration that gave an evident response was used, where appropriate, in further tests.

Experiment 2. Stimulatory/inhibitory effects of Ch or NA on intestines from rats previously infected with variable numbers of As L3: Rats were inoculated orally with 1, 5, 10, or 20 As L3. Control rats ($n = 5$) were sham infected with PBS. Intestinal segments isolated 30, 60, 120, or 240 min after inoculation (p.i.) were exposed to Ch or NA (10^{-6} M) to assess responses. Agonists were applied 5 min before any observation to give sufficient time for the agonist delivered to reach a steady-state level. To estimate infection-induced "injury" the excitatory responses of infected

rat intestines to Ch or NA were compared with those of controls. Successful infections were confirmed by recovering As L3 during postmortem examinations.

Experiment 3. The first studies realized were the effect of As L3 extracts on intestine contractions. Uninfected rats were used as tissue donors and tests were repeated with tissues from five rats each. The first experiments involved the addition of increasing (noncumulative) doses (0.5, 1, 2, 3, 4, 5, or 6 mg) of CE to the tissues to determine dose–response curves. Recordings were made during 5 min and parameters analyzed were basal tone, amplitude, and frequency of the spontaneous contractions. After each observation, the applied dose of CE was washed out (by repeated washings of 20 min).

In the second series of experiments we evaluated effects of As L3 CE on intestinal stimulation or relaxation. To this end, Ch- or NA (10^{-6} M)-induced activity after a 5-min incubation period was examined both with and without adding CE. Doses of CE used were the first that, as in the preceding section, showed activation responses (6, 4, and 2 mg of CE in duodenum, jejunum, and ileum, respectively). The responses found during pretrial or pretreatment (no CE added) were compared with those posttreatment to indicate As L3-specific cholinergic or adrenergic stimulatory/inhibitory effects. We also examined the effect of adding multiple noncumulative doses of the CE. This was intended to analyze the adverse effects (if any) of the compound on intestinal contractions on the excitability of intestinal cells responsive to Ch or NA. The parameters analyzed were the same as described before. Each intestinal segment was allowed to stabilize for 45 min before assay. Then, after finding stable contractile activity in these tissues for 15 min, six (6-, 4-, or 2-mg) consecutive doses of CE were added to the organ baths (noncumulatively) to determine whether, compared to the single-dose exposures (see above), additional changes in test parameters occurred. In parallel control experiments, duodenal, jejunal, and ileal segments were left with no CE added. Exposure period per CE dose was 20 min, and between steps the intestinal lumens were flushed for 20 min with KrS to eliminate any residual effects of previous CE doses. Ch or NA (10^{-6} M) was added to the test segments 20 min after each CE exposure step. Contractile activity in the above studies, where required, was recorded for 15 min; analysis of the data, however, was performed on the last 5-min (or 1-min) recordings (the control period).

Intestinal Transit Index Determination

Ten rats were infected per os with 20 As L3/rat and 10 other rats were sham-infected with 1 ml PBS as control. Four hours after each rat received a 1-ml oral dose of a mixture composed of 10% charcoal, 10% arabic gum, and 80% water. At 10, 20, 30, 60, and 120 min post-mixture inoculation, infected and control rats were killed, the small intestine was removed, and gastrointestinal transit was characterized by the transit index (TI). TI was defined as the ratio between the distance from the pylorus to the front of the charcoal and the total length of the small intestine multiplied by 100.

Data and Statistical Analysis

Data are mostly presented as means. For contractile activity data means are expressed as the values obtained during a set period of time (mostly 5 min) pre- and posttreatment to standardize the data. Amplitude data are the tension values (in grams) through analyses of 25–30 peaks. Tone changes were evaluated in terms

of the change (in grams) from the basal, the initial tension being 1 g. Frequency data are the numbers of peaks observed during 1 min. Study As L3 effects on the excitability of intestinal cells responsive to Ch are expressed as mean percentage increases in tone and/or amplitude of the twitches for tests performed on intestines (i) without CE added, (ii) with CE added, and (iii) from previously infected rats. NA-induced effects are percentage decreases in amplitude of contractions due to tests performed on intestine sources as described for Ch testing. An ANOVA was used to analyze data before and after As L3 contacts. Student's *t*-test was used to determine the statistical significance of differences between means, with $P < 0.05$ considered statistically significant.

RESULTS

Determination of Ch and NA Dose–Response Curves

Ch and NA altered both the tone and the amplitude of spontaneous twitches of all intestinal segments in a dose-dependent manner. For Ch and NA reproducible composite dose–response curves for five successive assays were obtained (data not shown). The optimal effect of both agonists in the different intestinal segments was obtained at 10^{-6} M, so this final concentration was used where required in (below) further experiments.

Intestinal Motility Effects of As Infection

The tissues of rats infected with 1–10 As L3 did not react differently from controls at any time after Ch or NA. In contrast, a dose of 20 As L3 resulted in several rats having altered responses to Ch and NA by 120 min, and all rats by 240 min post-infection. These data indicate time and dose dependency of events and effects on all rats 240 min p.i. As shown in Table 1 the normal tone and amplitude of contractions changed significantly ($P < 0.05$ to $P < 0.001$) in all infected animal tissues after Ch. A differential stimulatory or inhibitory effect of the infection on intestinal excitability was observed. While in infected rat jejunum and ileum Ch induced a 50% lower tone response than in control rats, it increased twofold in infected rat duodenum compared to controls. And the amplitude of twitches increased in all intestinal segments after Ch stimulus with respect to control rats. As L3 infection also modified the α -adrenergic response. So the amplitude of twitches of infected rats showed a lower decrease after NA incubation than that of control rats, being more evident in the jejunum (Table 1).

To test whether the above changed excitatory responses we ascribed to the As infection were not transient effects, we repeated the measurements after six consecutive Ch or NA stimulations. Figures 1 and 2 illustrate examples of recordings in the different intestinal portions as well as changes in tone and amplitude, respectively, ex-

TABLE 1. EFFECT OF AS L3 CONTACTS ON EXCITATORY RESPONSES TO EITHER CH OR NA OF ISOLATED INTESTINAL SEGMENTS

Tissue	Responses to Ch after Treatments ^a			Responses to NA after Treatments ^b		
	NI	Expt 1	Expt 2	NI	Expt 1	Expt 2
Duodenum						
Tone	0.35	0.77 ²	1.29 ²	ND	ND	ND
Amplitude	2.2	21.5 ²	32.02 ²	64.23	54.15 ¹	25.59 ²
Jejunum						
Tone	1.64	0.90 ¹	2.55 ²	ND	ND	ND
Amplitude	9.65	16.32 ¹	10.05	60.00	33.01 ²	25.47 ²
Ileum						
Tone	3.8	1.97 ²	1.4 ²	ND	ND	ND
Amplitude	265.35	344.57 ²	223.21 ²	91.12	82.63 ¹	75.53 ¹

Expt 1: rats infected per os with 20 As L3. Expt 2: duodenum, jejunum, and ileum exposed to 2, 4, and 6 mg of CE, respectively. NI, not infected or sham-infected; ND, not done. Data are expressed as mean of increment (a) or decrement (b) percentages from basal tone and/or initial amplitudes of twitches. ¹ $P < 0.05$. ² $P < 0.001$.

pressed as mean percentage changes of the Ch and NA response in control and As L3-infected rats. Note that infection changes the cholinergic response significantly during the six Ch stimulations. Moreover, the tone variations at the duodenum increased during the experiment, and jejunal and ileal tone showed similar variations. On the other hand, As L3 infection also modified the NA response, but these changes were not uniform during the 6X NA, except at the jejunum. In general, the NA response variations were lower than those determined for the Ch response.

To analyze whether food passage was changed, we determined TI in sham and infected rats. As can be seen in Table 2, infected rats, at test time points of 10 and 20 min, had higher mean TIs.

Intestinal Motility Effects of As L3-Derived CE

Parasite CE increased both tones and amplitudes of twitches of all test tissues in a dose-dependent manner (Figura 3). Sensitivity to the CE was in the order ileum > jejunum > duodenum. So significantly increased responses by these tissues were detected using 2, 4, and 6 mg of CE, respectively, in the assay. Moreover, we demonstrated that CE had no cumulative effect on intestinal motility, because these effects were also determined after six noncumulative CE incubations, showing identical increments between doses (data not shown).

We have also evaluated intestine irritability using cholinergic and α -adrenergic stimulations in test specimens after exposure to As CE (duodenum, jejunum, and ileum were stimulated with the above CE doses). The responses to a once-only dose of Ch or NA of tissues previously stimulated with CE are summarized in Table 1 and responses after 6X CE are shown in Figure 4. After a single dose, the tissues (similar to p.i. tissues) had

significantly changed responses to both Ch and NA. It is clear that in duodenum, a previous CE exposure enhances cholinergic excitability so as to cause the tones to increase above control values after Ch ($P < 0.001$). And the ileum with CE induces a state that opposes normal Ch effects, so the tone increase thereafter was down by $\approx 50\%$ compared to controls. Amplitudes of twitches after Ch were increased above normal values only in duodenal segments pretreated with CE ($P < 0.001$). In contrast, all tissues had a significantly decreased response to NA vs controls after a once-only dose of CE. In summary, as Table 1 indicates, tissues previously exposed to CE behaved in a similar fashion to those of infected rats.

On the other hand, Figure 4 shows that the effects of the CE on responses in duodenum, jejunum, and ileum to Ch (A) and NA (B) persisted during at least six incubations. Note, also, that as determined with infected tissues, the magnitude of the tone changes was similar during 6X CE in the different intestine portions and all of them were statistically significant ($P < 0.05$ – 0.001). On the other hand, NA response change showed clear differences (quantitatives and/or qualitatives) between doses in the duodenum and jejunum, while the same changes were determined at the jejunum throughout the experiment.

DISCUSSION

Mechanisms underlying the expression of clinical allergy to As are incompletely understood. Although early studies justify an allergy consistent with (past or active) anisakiasis, others authors are disagreement (7, 8, 14). These studies, together with others indicating the diseases in which allergy is expressed to occur also in the absence of allergy (11, 15), suggest a role for nonimmune factors also in the pathogenesis

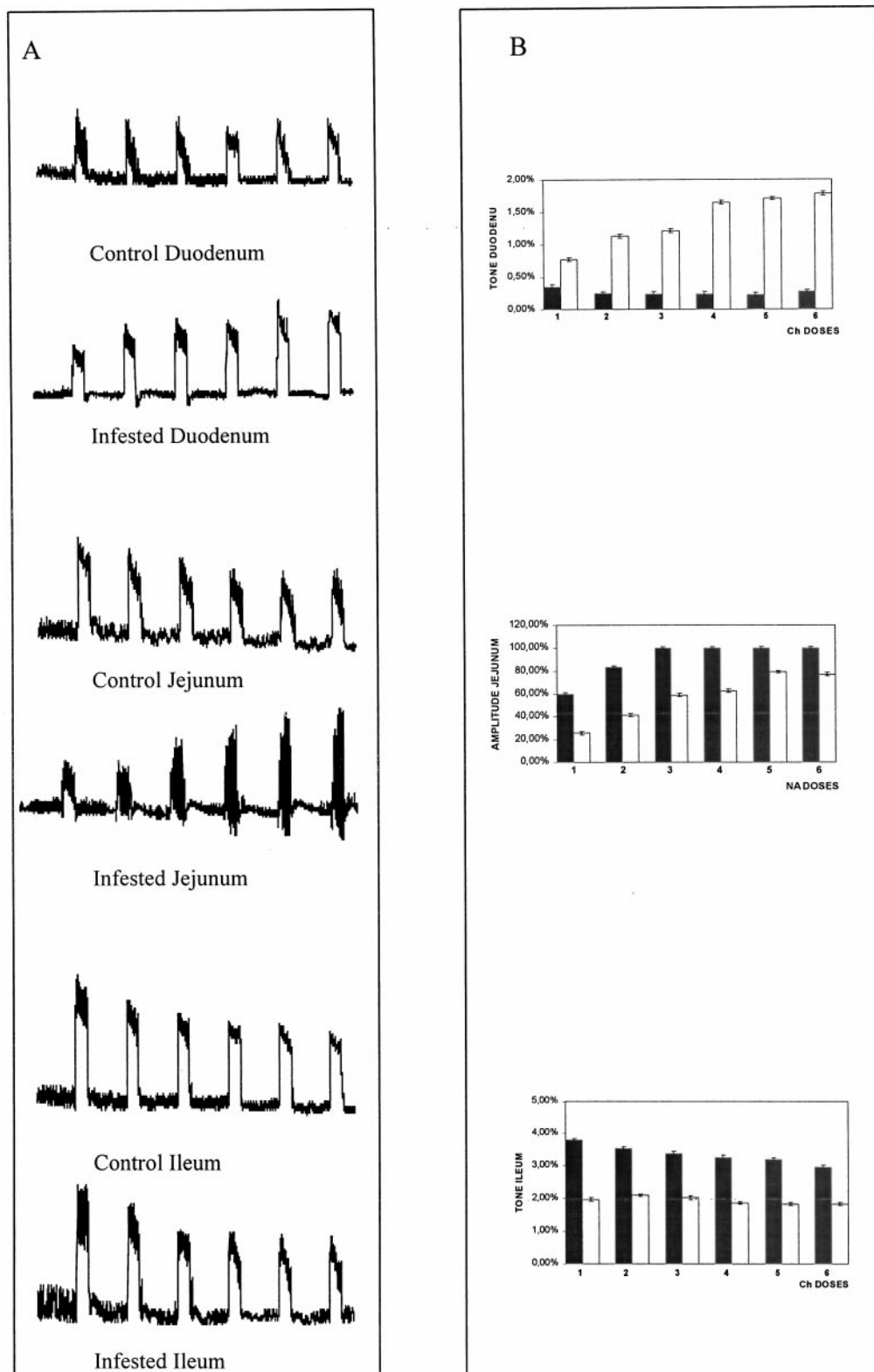


Fig 1. *In vitro* responses by duodenum, jejunum, and ileum of infected rats (20 *Anisakis simplex*, [As] L3/rat) or controls to six successive and noncumulative exposures to carbachol (Ch) assessed by test-induced changes in muscular tone. (A) Representative example. (B) Mean of incremental percentages from basal tone. The black columns represent the control intestinal response and the white columns represent the As L3-infected intestinal response to Ch. Basal tone (in grams) was normalized to 0%. $N = 5$.

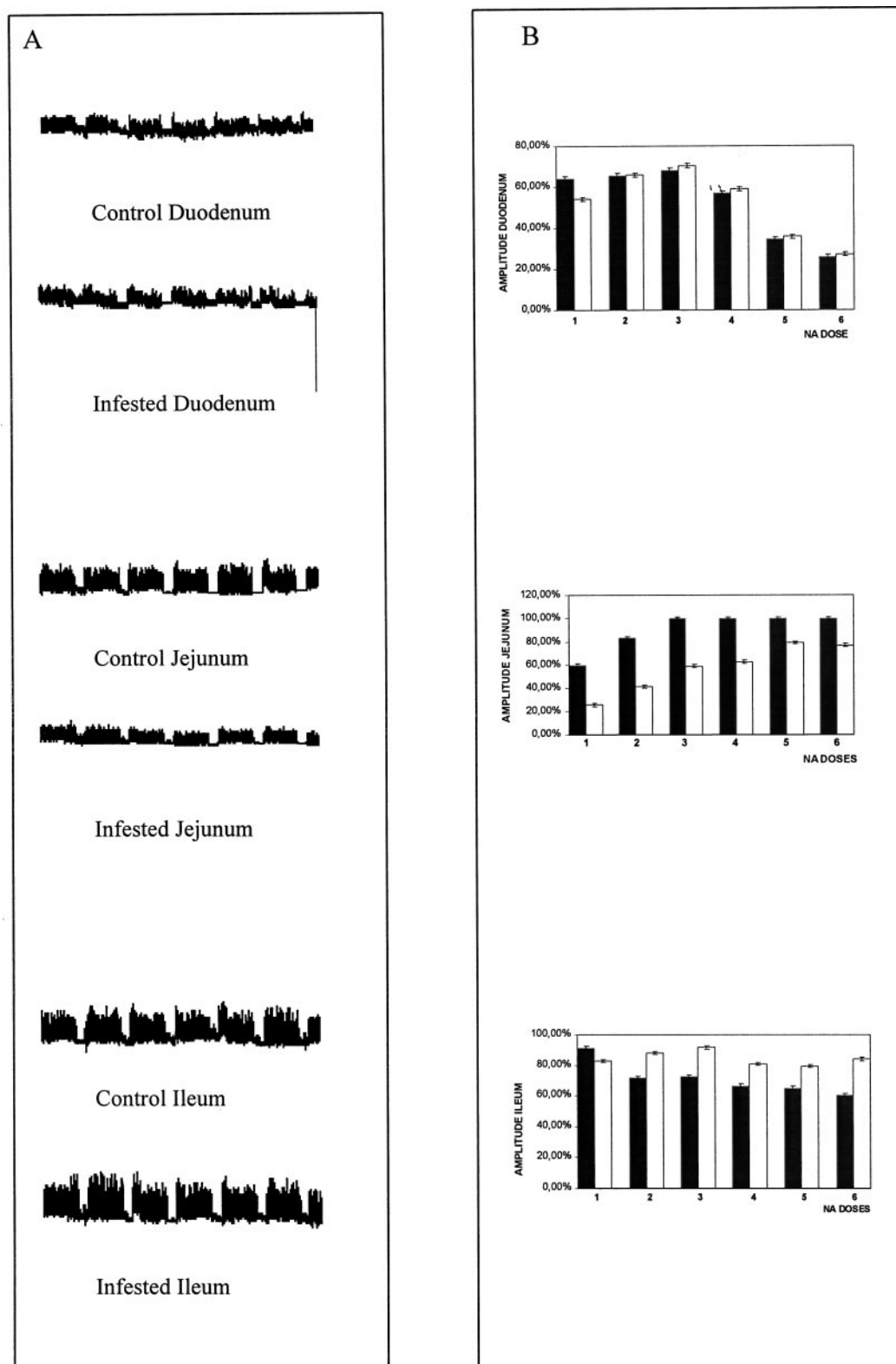


Fig 2. *In vitro* responses by duodenum, jejunum, and ileum of infected rats (20 *Anisakis simplex* L3/rat) or controls to six successive and noncumulative exposures to noradrenaline (NA) assessed by test-induced changes in amplitude of twitches. (A) Representative example. (B) Mean of decremental percentages from amplitude of initial twitches. The black columns represent the control intestinal response and the white columns represent the *As* L3-infected intestinal response to NA. Amplitude of initial twitches (in grams) was normalized to 100% $N = 5$.

TABLE 2. INTESTINAL TRANSIT INDICES (TI)*

Treatment†	Test time point				
	10 min	20 min	30 min	60 min	120 min
Sham	53.29	61.51	74.63	81.62	98.60
Infection	59.61	66.99	76.29	83.54	96.00

*Data are mean values for TI determination performed individually on 10 rats per group at the times indicated.

†Rats infected (20 *As* L3) or sham infected (PBS) per os 4 hr prior to testing

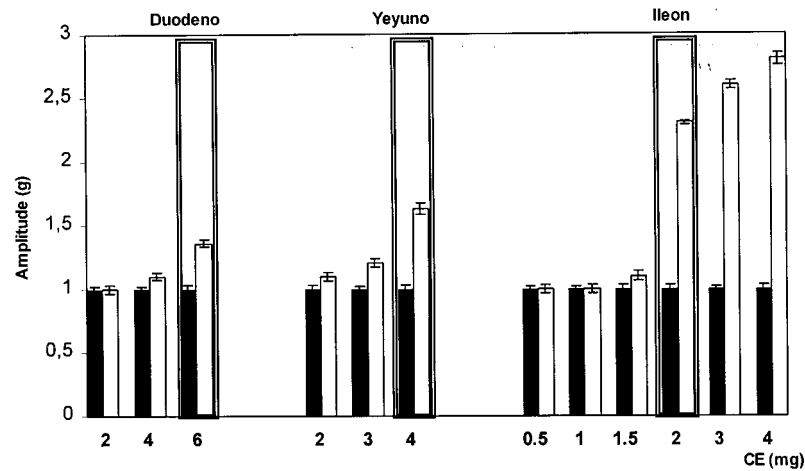
of allergy to *As*. The factors involved in this process are not yet known, although a disturbance in the target tissue's autonomic control has been postulated. In particular, adrenergic blockades and/or cholinergic hyperreactivities may induce such side effects (11, 12). To approach the gastrointestinal allergy problem (7), we examined infection-induced changes in bolus propulsion and responses to Ch and NA in rat intestine. We also included the incubations with *As* CE described herein, to determine if *As* was dangerous both dead and alive. Previous studies of ours demonstrated that rat ileum, after oral CE from another fish parasite, *Gymnorhynchus gigas*, had altered autonomic control functions (16). Similar effects of parasitic helminths or products that elicit specific IgE but no allergic disease have also been described (17, 18). Evocation of a disturbance in autonomic control such as cholinergic hyperreactivity in the intestine is, thus, a general feature in hosts with helminth infections. The present work both reconfirms this fact and extends previous descriptions of the above-type changes in, e.g., ileum alone, to all small bowel segments. More importantly, this study expands all these previous findings by considering responses concurring in the three main intestinal parts. Our data indicate that *As* L3 induce intestinal hyperreactivity long before any allergy would develop. The effect of the parasite could be observed when rats received ≥ 10 *As* L3, indicating it to be a dose-dependent process. Significantly, our results also demonstrate that modulation here comprised cholinergic, i.e., muscarinic, hyperreactivity in duodenum and α -adrenergic blockade in all segments. That these were not transient effects, or a casual finding, was indicated by the tests giving consistently repeatable patterns of excitability to Ch or NA. The concurrent increased and minimized excitatory responses to Ch and NA, respectively, very likely underlie the increased TI found in *As*-infected rat duodenum. The return to normal TI values across the intestinal tract can be explained by the fact that their potentiating cholinergic effects were gradually being lost in the rats' jejunum and ileum.

In addition to nonimmune factors, immune factors may also be involved in the "allergy" to *As* in individuals with

no history of anisakiasis. Like in this study, *As* infections in human can go unnoticed and yet be a trigger for specific IgE induction. In this regard, some authors have shown that infections with one *As* L3 induce a T cell response (10), a prerequisite for subsequent IgE and other Ig synthesis, is of considerable interest. It has been suggested that parasitic helminths' proteases may help these invade tissues (19–21). There they counteract host-protective (macrophage-activating) Th 1 responses and stimulate Th2 responses. It has been shown that the excretory–secretory (ES) products from *As* can prevent macrophages from producing nitric oxide, which upregulates Th1 (22, 23). Moreover, ES and CE from *As* contain a molecule like IL-4, a potent stimulus of Th2 differentiation (10). Thus, *As* may, through both its own IL-4 and induction of IL-4 and IL-10 (by activated Th2), move the immune response away from type 1 toward type 2. However, despite the evidence that present-day allergies to seafood mainly involve such sensitization, we would caution that, e.g., nonimmune factors, could produce similar effects. Moreover, seafood is listed with the many specific agents that activate inflammatory mediators nonimmunologically (11). These findings together with those of the present study suggest that *As*-reactive IgE in "allergic" subjects may reflect previous contacts rather than being the inducer of the allergic response on hand. Further studies are required to clarify this issue.

The present study demonstrated that exposure of rat intestine to *As* CE was also associated with a disturbance in its autonomic control. Several findings here deserve mention. First, *As* CE, after just the first contact, changed the intestine's pattern of spontaneous twitches; it increased both the tone and the amplitude of twitches in the entire intestine. This provides evidence that the acute problems arising from eating *As*-containing seafood do not require the activity of living parasites. On the other hand, it may also explain why in some patients, despite their being serologically *As*-positive, no parasite is ever found. Second, the studies reconfirm the dose dependency of the inducible changes and, as a consequence, the (non)occurrence of the associated effects *in vivo*. Significantly, our results also show that the described modulation, in each intestinal part, required exposure to another dose of *As* CE. In this regard, sensitivity in the test parts was in the order ileum > jejunum > duodenum. The occurrence in the ileum of the biggest changes using the lowest dose of CE was reproducible and is surprising in view of the reversed situation noted after infection. We do not have an explanation for this other than it may reflect complex differences in sensitivity to living *As* L3 vs solubilized parasites. Considering that the ileum and the final portion of the jejunum are the preeminent sites for immune response induction (6), their

A



B

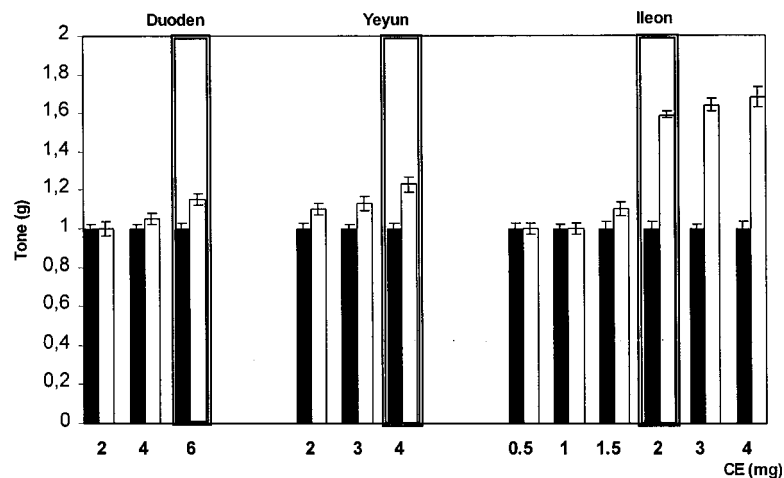
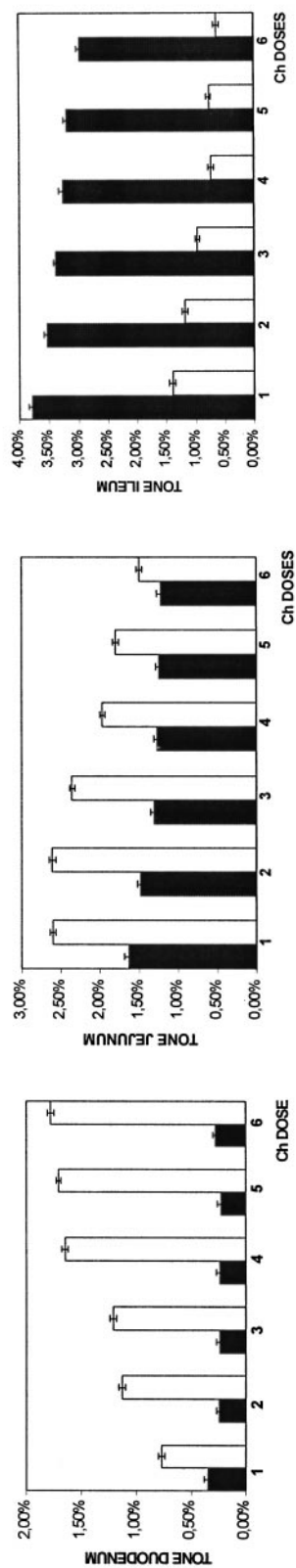


Fig 3. *In vitro* excitatory responses by rat duodenum, jejunum, and ileum after incubation to increasing (noncumulative) doses of *Anisakis simplex* crude extract (0.5, 1, 2, 3, 4, 5, and 6 mg). Effect (A) on the amplitude of the twitches and (B) on the muscular tone. The black columns represent the basal tone and the initial amplitude of the different portions of the intestine (control) and the white columns represent the tone and the amplitude after exposure to *As* CE doses. Data are expressed as grams and represent the mean value. $N = 5$.

highest sensitivity to soluble *As* is not unexpected. Clearly, this propensity will allow motility disturbers present in *As* CE also to reach regulatory centers in the ileum more easily than in the duodenum or jejunum. Our data indicate the ileum, vs the duodenum and jejunum, to contain either the most or the most sensitive autonomic control receptors. Taken together with our infection data, these results are remarkable and may have important public health im-

plications. It would seem that ingestion of both living and nonviable *As* L3 may be associated with significant acute side effects for the seafood consumer. Thus, while viable *As* L3, first, incite problems in the duodenum, and, then, after becoming solubilized, also in the rest of the intestine, already soluble *As* L3 products do so in the whole small bowel. Our findings justify larger studies to determine the minimum (i) numbers of living *As* L3 and (ii) dose of *As*

A



B

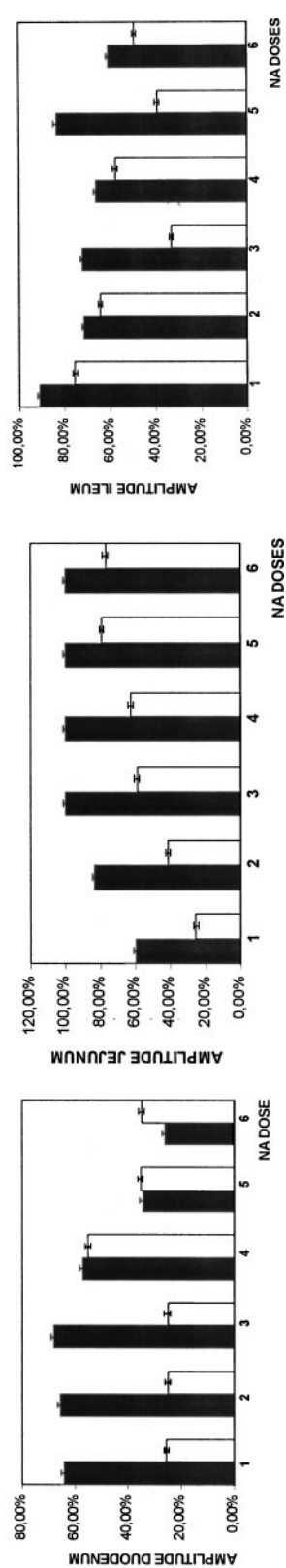


Fig 4. Effect of six successive noncumulative doses of *Anisakis simplex* crude extract on carbachol (A) and noradrenaline (B) responses in the different intestinal portions. Cholinergic response changes are expressed as mean incremental percentages from the basal tone, and α -adrenergic response changes as mean decremental percentages from the amplitude of the initial twitches. Basal tone and amplitude of initial twitches (in grams) were normalized to 0 and 100%, respectively. Black columns represent the response of the control intestine and white columns represent the responses after As CE incubation. $N = 5$.

CE that may be consumed without causing unwanted side effects.

Unexpectedly, in the present study, unlike in our *G. gigas* study (16), we observed no accumulative effects of the CE on test responses. We have no explanation for this other than it may reflect a difference between test *G. gigas* and As CEs in proteolytic capacity. In our experiments intestines are washed extensively between reexposures to CE. Using this protocol *G. gigas* CE (at 20 mg/ml) was found to increase excitatory responses to the agonists after each CE step. Thus *G. gigas* CE, through its contained enzymes, at each subsequent step seemed to destroy more tissue, causing the cumulative effects noted. In view of the fact that such results were obtained using CE at 20 mg/ml, it was not surprising that consonant effects were not observed in this study.

In conclusion, our results demonstrate that eating both living and dead As L3 can cause acute gastrointestinal problems. As well as rapidly (in 4 hr) and consistently increasing the amplitude of twitches in duodenum, As infection inhibited relaxation of tone in all segments. We observed these responses without a likely prior sensitization (by IgE), suggesting that As-specific IgE may have a secondary role only (if any) in acute symptom induction *in vivo*. Interestingly, the disturbance in autonomic control in the target tissue we describe can cause anaphylaxis and urticaria–angioedema, in the absence of allergy (11). Our finding the above effects of As CE in jejunum and ileum also is interesting, as it provides evidence that As L3 killed and digested *in vivo* can exert such bystander effects in jejunum and ileum. True enough, we have not established in our study whether the changes caused by As CE would, in the end, result in the host presenting an allergy-like disease. However, in this regard, it may be significant that oral CE from the fish parasite *G. gigas* was found to cause the above effects in intestine *in vitro* and immediate-type discomfort *in vivo* (12, 16). These results suggest that, despite the current opinion that eating a few As L3 or physically destroyed As L3 involves no health risks, there is a wider public health problem that has yet to be recognized by both the general public and scientists.

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