On the relationship between gastrin, gastric secretion, and adjuvant arthritis in rats

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SUMMARY The elevation of plasma immunoreactive gastrin known to occur during the induction of adjuvant-induced arthritis (Rooney *et al.*, 1973) has been shown to be maximal at 7 days after injection. Gastrin administered exogenously accelerated and exacerbated the inflammatory joint disease. Some evidence has been presented that the endogenous immunoreactive gastrin had biological activity in terms of gastric acid secretion.

Previous studies having indicated significant elevation of plasma immunoreactive gastrin occurring in association with inflammatory joint disease both in human rheumatoid arthritis (Rooney *et al.*, 1976) and in rats with adjuvant-induced arthritis (Rooney *et al.*, 1973). The current study was undertaken to determine the relationship of changes in immunoreactive gastrin to the time course of adjuvant disease and to determine whether these changes in immunoreactive hormone were biologically meaningful in terms of gastric acid output. The effect of exogenously administered synthetic gastrin pentapeptide on the time course and severity of adjuvant disease was also studied.

Materials and methods

The animals used in these studies were adult Sprague-Dawley rats each weighing around 300 g. In all instances induction of adjuvant arthritis was by a standard procedure involving the sub-plantar injection of killed *Mycobacterium butyricum* in heavy mineral oil into the left hind paw. The standard dose was 0.5 mg in 0.1 ml oil, alterations to the dose are discussed below in detail. The progress and severity of the adjuvant disease was assessed by means of weight change and paw scores (Currey and Ziff, 1966) immediately before withdrawal of blood.

Gastric fistula rats were prepared surgically at least 2 weeks before study. The surgery consisted of the formation of a simple gastric fistula at the junction of the main stomach and the rumen. Patency was maintained by means of a titanium steel cannula.

Plasma gastrin was assayed in blood obtained from the rat tail vein. In every instance the rats had been fasted for 15 hours overnight in wire mesh cages designed to prevent coprophagia. The hormone was measured by a sensitive and specific radioimmunoassay. In this assay, the anti-serum is raised in rabbits to synthetic human gastrin 1 (I.C.I.). Labelled hormone is prepared using a modification of the chloramine-T method of Hunter and Greenwood (1962) and separation of free hormone from that bound to antibody is achieved by treatment of the assay incubate with dextran-coated charcoal (Buchanan and McCarroll, 1971). Precision is greatest below 600 pg/ml (ng/ml) and the sensitivity of the assay, that is the lowest concentration of hormone that can be reliably differentiated from zero, is 10 pg/ml (ng/ml). Cross reaction with cholecystokinin-pancreozymin is minimal and preliminary studies show that the antibody recognises not only the heptadecapeptide but also the 'big' gastrin described by Yalow and Berson (1970).

In the first group of 8 intact rats studied, the standard dose of adjuvant was used. These animals were fully assessed on days 0, 1, 7, 14, and 21 after injection. A further 3 groups, each of 16 intact animals, were injected with varying doses of adjuvant. In 1, the standard dosage regime was used (this would be expected to induce arthritis in 90% of animals). In the second group 0.3 mg *M. butyricum* was used and in the third 0.1 mg each in the

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standard volume of oil. In addition in all 3 groups each rat received 5 µg synthetic pentagastrin (I.C.I.) subcutaneously twice daily for 3 weeks following the injection of adjuvant. Paw scores were assessed on days, 7, 14, and 21 after the injection. Gastric acid output was assessed by both volume and concentration over a period of 30 minutes. The titration was carried out against sodium hydroxide using a pH meter.

Results

The time course of the elevation of plasma immunoreactive gastrin following the induction of adjuvant arthritis is shown by Table 1 and Fig. 1. The peak level of immunoreactive gastrin is reached on day 7. This coincides with the onset of the secondary

Fig. 1 Gastrin in adjuvant arthritis. The elevation of mean ($\pm$ SEM) immunoreactive gastrin in rats at 7 days is shown by the black line. The hatched area indicates the course of the inflammatory joint disease with the small initial rise due to the inflammation locally in the injected paw followed by the progressive generalised arthropathy.

inflammatory response to the injected paw. Thereafter as the generalised arthropathy develops immunoreactive gastrin concentration falls.

The effect of exogenously injected gastrin on the course of adjuvant-induced arthritis is shown in Table 2 and Fig. 2. These data indicate that the exhibition of pentagastrin in this way causes earlier onset of generalised joint disease and that this occurs

Fig. 2 Paw scores in rats receiving sub-optimal doses of adjuvant. In each group the rats also receiving gastrin injections (+G) are compared to those receiving adjuvant alone.

Table 1 Immunoreactive gastrin (pg/ml; ng/ml) in intact rats on the day before and on days 1, 7, 14, and 21 following injection of standard adjuvant

Rat	Day				
	0	1	7	14	21
1	60	85	105	250	105
2	115	155	—	165	180
3	165	125	155	165	50
4	145	135	230	135	135
5	210	125	495	105	260
6	75	65	240	180	80
7	135	85	230	250	145
8	125	—	210	260	230
Mean $\pm$ SEM	128.7 $\pm$ 16.9	110.7 $\pm$ 12.3	237.8 $\pm$ 46.7	188.7 $\pm$ 20.3	157.8 $\pm$ 27.3

Table 2 Paw scores in rats receiving varying doses of adjuvant and concomitant administration of pentagastrin (I.C.I.) in a dose of 5 µg per rat twice daily

Days after adjuvant	Dose					
	100 µg		300 µg		1000 µg	
	Without gastrin	With gastrin	Without gastrin	With gastrin	Without gastrin	With gastrin
10						
Mean ± SEM	0	0	0	0.5 ± 0.19	0.62 ± 0.18	1.0 ± 0.19
t						1.43
P						NS
12						
Mean ± SEM	0	0	0.87 ± 0.39	2.12 ± 0.44	2.13 ± 0.44	3.63 ± 0.3
t				2.10		2.74
P			0.005 >	P > 0.025	0.01 >	P > 0.005
17						
Mean ± SEM	0.75 ± 0.37	0.87 ± 0.23	2.62 ± 0.53	4.0 ± 0.46	4.12 ± 0.35	4.5 ± 0.19
t		0.29		1.95		0.94
P		NS	0.01 >	P > 0.005		NS

at lower doses of injected adjuvant than is usually required, except in those rats receiving 100 g of the adjuvant. The mean paw scores at 12 days in those rats not receiving gastrin were significantly lower than those in the injected animals where both the full adjuvant dose as well as the 0.3 mg dose had been given. A similar effect was seen in those rats receiving 0.3 mg adjuvant at 17 days but in view of the severity of disease in both the penta-gastrin-treated and untreated groups this effect is no longer apparent in those rats given full doses of adjuvant.

The effect of adjuvant-induced arthritis on the basal gastric acid secretion is summarised in Table 3. It is clear that at 7 days after adjuvant injection fall in gastric acid output occurred. However, although some rise in immunoreactive gastrin is noted at this time in the fistula rats, this failed to reach levels of statistical significance. It may be significant that the severity of the arthritis in the rats with fistulae was much less than that observed in the other experiments although the weight changes expected to accompany the inflammatory joint diseases were still readily apparent.

Discussion

Significant elevation of fasting immunoreactive gastrin occurs during the induction of adjuvant arthritis (Rooney *et al.*, 1973). The mechanism of this effect is unclear, but if it reflects the same phenomenon as has been reported in some patients with rheumatoid arthritis (Rooney *et al.*, 1976), it is clear that drug therapy and specific rheumatoid factors are not implicated in its production. It seems likely that it will prove to be part of the cascade of phenomena associated with the inflammatory responses (McQueen, 1973; Ward, 1974). Certainly in these studies the peak increase in the concentration of plasma immunoreactive gastrin coincides with the severe inflammatory disease characteristically induced by adjuvant injection (Pearson and Wood 1963).

The action of gastrin in this model appears to be a phlogistic one as evidenced by the effect of exogenous gastrin on the onset and severity of joint disease. This finding suggests that gastrin is either an active mediator of inflammation or that its secretion parallels that of another agent with this

Table 3 Mean values (± SEM) for weight volume of gastric juice, total titratable activity (TTA), and plasma immunoreactive gastrin concentration in rats with gastric fistulae after induction of adjuvant arthritis

Day		Weight (g)	Gastric juice (ml)	TTA (m-equiv)	Gastrin (pg/ml)
0	Control	286.3 ± 3.8	0.57 ± 0.09	90.03 ± 24.98	37.9 ± 10.2
	Arthritis	290.5 ± 4.2	0.56 ± 0.13	94.36 ± 28.04	56.4 ± 7.8
7	Control	322.8 ± 5.1	0.48 ± 0.05*	89.60 ± 15.0*	74.3 ± 12.6
	Arthritis	305.1 ± 4.3	0.25 ± 0.04	37.43 ± 6.34	82.9 ± 8.7
14	Control	341.5 ± 4.9*	0.58 ± 0.06	93.72 ± 10.62	45.8 ± 3.0
	Arthritis	302.7 ± 5.1	0.39 ± 0.11	74.19 ± 27.47	62.1 ± 10.3
21	Control	368.4 ± 8.3*	0.56 ± 0.06	96.86 ± 17.50	62.9 ± 15.5
	Arthritis	298.5 ± 8.2	0.59 ± 0.05	66.98 ± 15.37	61.4 ± 8.1
28	Control	403.1 ± 9.4*	0.73 ± 0.10	90.96 ± 14.95	—
	Arthritis	310.4 ± 8.2	0.83 ± 0.08	68.96 ± 11.31	—

* t Test shows significant difference.

effect. In the human disease situation, the absence of the normal biological effect of the hormone, the secretion of gastric acid, in the face of a normal target organ (Rooney *et al.*, 1976) tends to suggest that gastrin may not be the primary agent in the effect. Prostaglandins are known to be involved in inflammatory responses (Greaves *et al.*, 1971) and prostaglandin E_2 and its methylated analogues have been shown to inhibit gastric acid secretion whether the stimulus to this be gastrin, histamine, or vagal activity (Karim *et al.*, 1973). It is conceivable, therefore, that the involvement of prostaglandins or related mediators in the inflammatory cascade results in inhibition of gastric acid secretion with resultant biofeedback leading to hypergastrinaemia as occurs in atrophic gastritis (Ganguli *et al.*, 1971).

It is interesting, in these experiments, that, although some biological effects on the target organ for gastrin were observed, in that changes in gastric secretion occurred in the fistula rats, no marked elevation of plasma immunoreactive gastrin was observed. Whether this reflects the reduction in the severity of the arthropathy observed or whether the presence of the fistula interferes in some way with the stimulus to gastrin secretion is not known. It is also interesting to speculate whether the presence of this fistula contributed in some way to protecting the animals from the inflammatory joint disease.

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