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# EFFECTS OF ARACHIDONIC ACID ON RAT GASTRIC ACID SECRETION IN RESPONSE TO DIFFERENT SECRETOGOGUES; INHIBITION OF THESE EFFECTS BY INDOMETHACIN

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**1** The effects of the prostaglandin precursor, arachidonic acid, and the non-steroidal anti-inflammatory drug, indomethacin, on gastric acid secretion were studied in the rat, *in vivo* and *in vitro*. Gastric mucosal blood flow was also measured *in vivo*.

**2** Arachidonate produced significant inhibition of acid secretion stimulated by pentagastrin or histamine. It did not significantly affect dibutyryl cyclic adenosine 3',5'-monophosphate (db cyclic AMP)-induced acid secretion.

**3** Inhibition of acid secretion by arachidonate was accompanied by a rise in the ratio of mucosal blood flow to acid secretion.

**4** Indomethacin did not significantly alter histamine- or pentagastrin-induced acid secretion.

**5** In the presence of indomethacin, the inhibitory effect of arachidonate was significantly reduced.

**6** These results provide evidence that the rat gastric mucosa is capable of synthesizing, from exogenous precursor, products of cyclo-oxygenase which inhibit gastric acid secretion.

## Introduction

Prostaglandins including prostaglandin $E_2$ ($PGE_2$) and $PGI_2$ are potent inhibitors of gastric acid secretion and cause mucosal vasodilatation (Main & Whittle 1973a; Whittle, Boughton-Smith, Moncada & Vane, 1978), but a role for them as endogenous modulators of secretion has not yet been established. Release of PGE and PGF-like substances from the rat stomach *in vivo* has been demonstrated both during basal acid secretion and secretion stimulated by pentagastrin, histamine or vagal stimulation (Bennett, Friedman & Vane, 1967; Ramwell & Shaw, 1968). Similarly, the rat isolated whole stomach releases prostaglandin-like substances on transmural or vagal stimulation (Bennett *et al.*, 1967; Coceani, Pace-Asciak, Volta & Wolfe 1967). When arachidonic acid, the precursor of the 2-series prostaglandins, is added to homogenates of rat stomach, $PGE_2$ and $PGF_{2\alpha}$ are formed, but also, in greater amounts, 6-oxo-$PGF_{1\alpha}$ (Pace-Asciak, 1976), which is now known to be a major metabolite of $PGI_2$. Formation of $PGI_2$, as well as 6-oxo-$PGF_{1\alpha}$, has recently been demonstrated in the gastric mucosa of several species, including the rat (Moncada, Salmon, Vane & Whittle, 1977). Thus a variety of products of the cyclo-oxygenase enzyme may be candidates as modulators of gastric acid secretion.

We have studied the effects of arachidonic acid and indomethacin, an inhibitor of cyclo-oxygenase, on rat gastric acid secretion *in vivo* and *in vitro*. Our aim was to establish whether products of cyclo-oxygenase can be synthesized from exogenous arachidonic acid during resting and stimulated acid secretion and whether their biosynthesis can subsequently affect acid secretion and mucosal blood flow. The effects of arachidonic acid and indomethacin on erosion formation were also noted.

A preliminary account of this work has been given to the Physiological Society (Frame, Main & Melarange, 1977).

## Methods

### In vivo *experiments*

Female rats weighing 200 to 250 g were deprived of food for 18 h but allowed water. They were then anaesthetized with a 25% urethane solution (1.6 g/kg s.c.) and the trachea was cannulated. Blood pressure was recorded from the left carotid artery and drugs were administered via a cannula inserted into an external jugular vein. Body temperature was maintained at 34 ± 1°C by means of a rectal thermistor probe and a warming blanket.

The gastric lumen was perfused with 0.9% w/v NaCl solution (saline, 0.2 ml/min) via a soft rubber tube inserted down the oesophagus and a cannula inserted through the pyloric sphincter via the duodenum (Main & Whittle, 1973b). Samples of perfusate were collected at 20 min intervals and the acid con-

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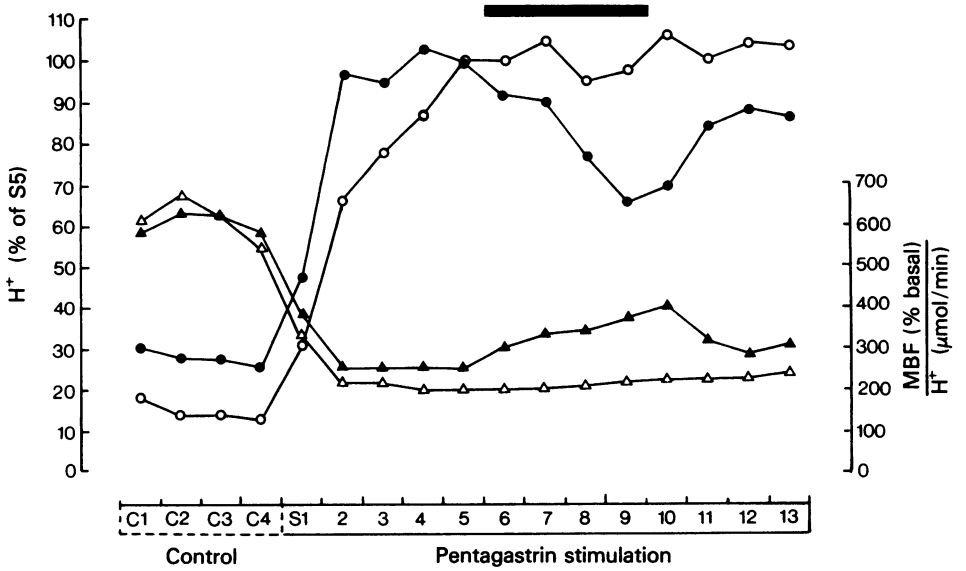


**Figure 1** Effects of arachidonate (80 μg $kg^{-1}$ $min^{-1}$ i.v. at bar; $n = 10$) on gastric acid secretion (●; % of values at S5) and the ratio of mucosal blood (MBF) flow to acid ($H^+$) secretion (▲; % basal MBF/μmol $H^+$ per min) during pentagastrin stimulation (0.33 μg $kg^{-1}$ $kg^{-1}$ min from S1) in the anaesthetized rat. Control pentagastrin curves ($n = 5$) are represented by open symbols.

tent determined by titration to pH 7 with $10^{-2}$ M NaOH. Three or four basal samples were collected before secretogogue administration.

Mucosal blood flow (MBF) was measured by the [$^{14}$C]-aniline clearance technique (Main & Whittle, 1973b). One hour after surgery, a loading dose of [$^{14}$C]-aniline, 2.0 μCi/kg, was injected intravenously followed by a continuous infusion of 0.033 μCi $kg^{-1}$ $min^{-1}$ to maintain a steady plasma concentration. The [$^{14}$C]-aniline content of the perfusate was determined by counting 0.5 ml aliquots in 10 ml Aquasol, using the channels ratio method.

At the end of each experiment, the stomach was removed and the mucosa examined for erosion formation.

### In vitro *experiments*

Experiments were carried out with a rat isolated gastric mucosal preparation (Main & Pearce, 1978). Mucosae were dissected from rats weighing 70 to 120 g, two preparations being obtained from each rat (except in the histamine-treated group). One preparation from each pair was treated as a control and drug treatments were randomized. Drugs were added to the serosal bathing solution and acid secretion from the mucosal surface was monitored by a pH electrode or recorded by a pH-stat system.

### *Drugs*

The following were used: pentagastrin (Peptavlon, ICI); $N^6$-2′-O dibutyryl cyclic adenosine-3′,5′-monophosphate (Boehringer); [$^{14}$C]-aniline (The Radiochemical Centre, Amersham); Aquasol (New England Nuclear). Indomethacin (Merck, Sharpe and Dohme) was dissolved in a small volume of 5% sodium bicarbonate solution and diluted to concentrations up to 10 mg/ml with saline. Arachidonic acid (99%, porcine liver, Sigma Chemicals) was converted to the salt by brief treatment with alcoholic KOH and was dissolved in saline to 2 mg/ml. It was stored deep frozen under nitrogen for periods of up to one week and, for *in vivo* experiments, it was mixed immediately before use with an equal volume of 4% bovine serum albumin, according to the method of Larsson & Änggård (1974).

### *Statistical analyses*

In vivo *experiments* Results within each group are expressed as the mean ± s.e. mean (absolute values and % change) of the difference between the fifth (S5) and ninth (S9) samples collected from the onset of pentagastrin stimulation. Statistical significance of absolute values was calculated by Student's $t$ test for paired variates. For group comparisons, mean % differences (S5-S9) were compared by Student's unpaired $t$ test.

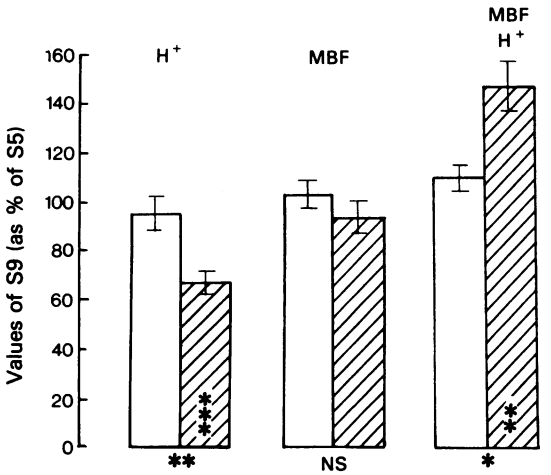


**Figure 2** Pentagastrin-stimulated acid ($H^+$) secretion, mucosal blood flow (MBF) and MBF/$H^+$ in anaesthetized control rats (open columns; $n = 5$) and rats treated with arachidonate 80 μg $kg^{-1}$ $min^{-1}$ (hatched columns). Values at S9 expressed as percentage of S5. Significances within groups (paired $t$ test) are shown within columns and significances between groups (unpaired $t$ test) below the columns.

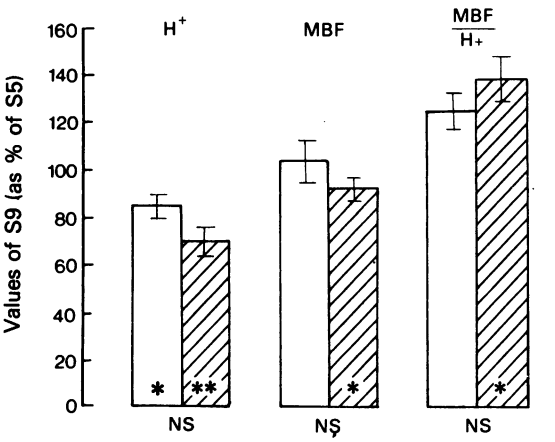


**Figure 3** Pentagastrin-stimulated acid ($H^+$) secretion, mucosal blood flow (MBF) and MBF/$H^+$ in anaesthetized rats treated with indomethacin (open columns; $n = 7$) or with indomethacin and arachidonate (hatched columns; $n = 7$). Values at S9 expressed as percentage of S5. Significances are indicated as for Figure 2.

In vitro *experiments* Similar statistics were performed on absolute secretory responses (paired $t$ test) and % changes between groups of responses (unpaired $t$ test).

$P < 0.05$ was taken as significant*; $0.001 < P < 0.02$**, $P < 0.001$***.

## Results

In vivo *experiments*

(a) *Control pentagastrin* During infusion of a submaximal dose of pentagastrin, 0.33 μg $kg^{-1}$ $min^{-1}$, a progressive rise in acid secretion closely followed by an increase in [$^{14}$C]-aniline clearance was observed. Acid ($H^+$) secretion reached a plateau prior to collection of sample 5 (S5) whilst aniline clearance continued to rise slightly throughout the experiment. The ratio of MBF/$H^+$ fell at the onset of stimulation with pentagastrin and then remained constant throughout the experiment, rising only slightly towards the end (Figure 1). None of these parameters was significantly altered between collection of samples 5 and 9 in five experiments (Figure 2).

(b) *Effect of sodium arachidonate on pentagastrin-stimulated $H^+$ secretion and mucosal blood flow* Infusion of sodium arachidonate (A), 80 μg $kg^{-1}$ $min^{-1}$ during a plateau of stimulated secretion, from the beginning of S6 until the end of S9, caused a significant decrease in acid secretion (S5-S9) of $-0.25 \pm 0.5$ μmol/min ($-32.6 \pm 4.4\%$, where S5 = 100%) $n = 10$, $P < 0.001$ using the paired $t$ test (Figures 1 and 2). MBF was not significantly altered but the ratio MBF/$H^+$ was significantly increased by $44.1 \pm 10\%$ $n = 9$, $P < 0.02$ using the paired $t$ test (Figure 2). When compared with the control group using the unpaired $t$ test, these experiments showed a significant reduction (S5-S9) in $H^+$ secretion, $P < 0.01$, but no significant difference in MBF. MBF/$H^+$ was significantly increased, $P < 0.05$ (Figures 1 and 2). Both $H^+$ secretion and MBF returned to previous levels when the infusion of A was stopped.

(c) *Effect of indomethacin on pentagastrin-stimulated $H^+$ secretion and mucosal blood flow* These experiments were performed as controls for comparison with A plus indomethacin (A Indo)-treated animals. A dose of 30 mg/kg Indo was injected intravenously at the beginning of pentagastrin stimulation (S1) and this was followed by a maintenance infusion of 4 mg $kg^{-1}$ $h^{-1}$.

$H^+$ secretion fell significantly by $0.21 \pm 0.04$ μmol/min ($-15.8 \pm 5.11\%$), $n = 7$, $P < 0.05$, using the paired $t$ test, between S5 and S9, but MBF and MBF/$H^+$ were not significantly affected (Figure 3). However, mean differences (S5-S9) in $H^+$ secretion, MBF and MBF/$H^+$ were not significantly different from those in control experiments (a) according to the unpaired $t$-test.

(d) *Effect of sodium arachidonate administered in the presence of indomethacin on pentagastrin-stimulated $H^+$ secretion and mucosal blood flow* Infusion of A, 80 μg $kg^{-1}$ $min^{-1}$ as in (b), in the presence of Indo infused as in (c), produced a significant reduction in $H^+$ secretion of 0.53 ± 0.14 μmol/min (−30.6 ± 5.9%), $n = 7$, $P < 0.01$ and in MBF of −11.8 ± 4.0%. $n = 7$, $P < 0.05$. The ratio of MBF/$H^+$ was not significantly affected. However, when compared with the Indo-treated group (c) by the unpaired $t$ test, none of the three parameters was found to be significantly different (Figure 3). Indomethacin abolished the vasodepressor action of A.

(e) *Mucosal erosions* In the five control animals stimulated with pentagastrin only, no erosions were observed in mucosae at the end of the experiments. Five out of seven of the animals which received Indo during pentagastrin stimulation exhibited erosion formation. Of the ten A-treated rats, none exhibited erosion formation whilst five out of seven A Indo-treated rats had erosions.

(f) *Effect of sodium arachidonate on $H^+$ secretion stimulated by dibutyryl cyclic AMP* In four control experiments, acid secretory responses were elicited by intravenous injection of 30 mg/kg dibutyryl cyclic AMP (db cyclic AMP) at times equivalent to S1 and S9. The mean first response was 0.38 ± 0.09 μmol/min. Second responses were consistently lower than first responses, reaching a mean peak of only 0.15 ± 0.06 μmol/min. This difference between the responses was significant, $P < 0.05$, $n = 4$, giving a mean change of −62.5 ± 9.3%.

In a further four experiments, A was infused intravenously from the beginning of S7 until the end of S10 (80 min) i.e. for 40 min before and 40 min after the second injection of db cyclic AMP. The mean first response was 0.31 ± 0.07 μmol/min and it fell to 0.13 ± 0.03 μmol/min (i.e. by 49.4 ± 13.1%). This fall was not significant according to the paired $t$ test. The mean change in acid secretion between responses did not differ significantly from that in the control group, according to the unpaired $t$ test.

## In vitro *experiments*

(a) *Control pentagastrin-stimulated $H^+$ secretion* Responses to pentagastrin were obtained by addition of the drug to the serosal solution ($3.6 \times 10^{-8}$ M, in contact for 30 min) 120, 300 and 390 min after setting up the preparation. The initial 2 h period was allowed for the preparation to settle to a steady basal secretion. The normal 90 min dose cycle was increased to 180 min between the first and second responses to permit incubation with A and/or Indo in appropriate experiments. Results are expressed as absolute secretory responses or % change between first and second or first and third responses.

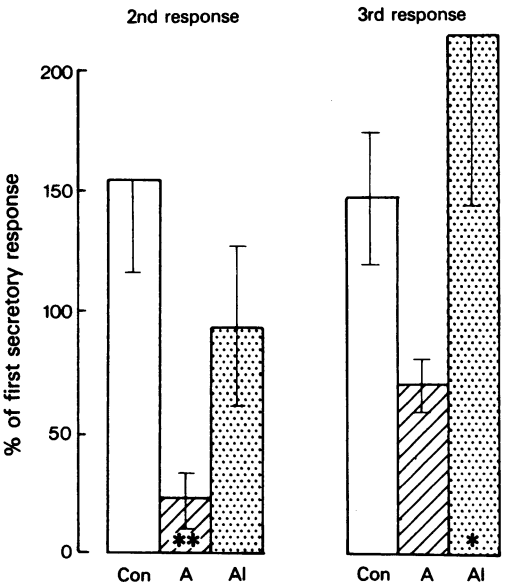


**Figure 4** Secretory responses of the rat isolated gastric mucosa to pentagastrin $3.6 \times 10^{-8}$ M alone (open columns, $n = 5$), and in the presence of arachidonate $3.2 \times 10^{-5}$ M (hatched columns, $n = 5$), or arachidonate and indomethacin $3 \times 10^{-5}$ M (stippled columns, $n = 5$). Results are expressed as percentage of the first secretory response. Significance within groups (paired $t$ test) is indicated within the columns.

The mean first response was 0.29 ± 0.04 μmol $cm^{-2}$ 15 $min^{-1}$ increasing to 0.43 ± 0.12 (+54.9 ± 38.9%) and 0.40 ± 0.07 (+47.4 ± 27.9%) in the second and third responses respectively, $n = 5$ (Figure 4).

(b) *Effect of sodium arachidonate on pentagastrin-stimulated $H^+$ secretion* A ($3.2 \times 10^{-5}$ M) was added to the serosal solution 1 h after washing out the first dose of pentagastrin (210 min) and left in contact for 90 min before and during the second pentagastrin administration. In these experiments, the first response was 0.31 ± 0.09 μmol $cm^{-2}$ 15 $min^{-1}$ and the second and third responses were reduced to 0.11 ± 0.05 (−76.5 ± 9.9%) and 0.22 ± 0.08 (−30.3 ± 11.4%) respectively, $n = 5$. Thus A significantly decreased $H^+$ secretion ($P < 0.01$) during the second response, an effect which was only partially reversed after washout of A (Figure 4).

(c) *Effect of sodium arachidonate administered in the presence of indomethacin on $H^+$ secretion* In two preliminary experiments, Indo 3 and $6 \times 10^{-5}$ M was added to the serosal solution 30 min after washout of

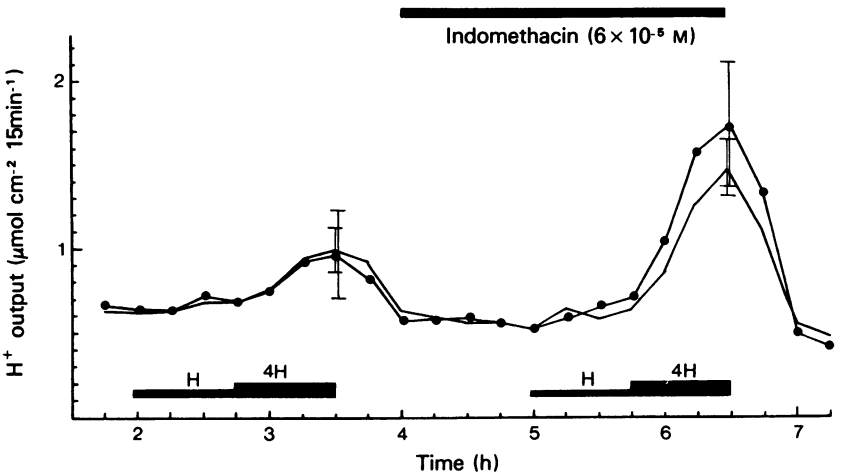


**Figure 5** Acid secretory responses of the rat isolated gastric mucosa to two cumulative doses of histamine (H and 4H, 0.64 and 2.6 × $10^{-5}$ M respectively), alone (——) or in the presence of indomethacin (6 × $10^{-5}$ M) (—●—). Standard errors of the mean ($n$ = 4) are indicated at the response peaks.

the first dose of pentagastrin (at 180 min) and left in contact with the mucosa until the end of the second response. These concentrations of Indo did not appear to alter the pattern of responses from those in paired controls.

In a further five experiments, Indo (3 × $10^{-5}$ M) was added as above and A was added 30 min later (as in (b) above). The mean first response was 0.40 ± 0.1 μmol $cm^{-2}$ 15 $min^{-1}$ which was reduced to 0.28 ± 0.07 (−5.4 ± 33.8%) in the second response and increased to 0.65 ± 0.15 (+117.1 ± 71.9%) in the third response. Thus Indo reduced the inhibitory effect of A considerably, such that the second response was not significantly different from the first response. The third response shows complete recovery and potentiation, although there was considerable variation in this response, as reflected in the large s.e. mean (Figure 4).

(d) *Effect of sodium arachidonate and indomethacin on histamine-stimulated $H^+$ secretion* Comparisons between control and treated groups were performed on unpaired mucosae, randomizing according to litter and sequence of experiment. Cumulative responses to two concentrations of histamine (0.64 and 2.6 × $10^{-5}$ M, each 45 min contact) were obtained starting at 120 min. The serosal solution was changed four times during the next 30 min and then drugs (A and Indo) were added and left in contact for 1 h before and during construction of a second dose-response curve. In control experiments (i.e. no A or Indo), the second secretory response to histamine was greater than the first.

Indo (6 × $10^{-5}$ M) had no significant effect on basal or stimulated $H^+$ secretion (Figure 5).

A caused a dose-related inhibition of the responses to histamine. This inhibitory effect was greatly reduced by Indo. In the experiments shown in Figure 6, the response in the presence of A (1.6 × $10^{-5}$ M), $n$ = 4, was significantly lower than that in the presence of both A (1.6 × $10^{-5}$ M) and Indo (3 × $10^{-5}$ M), $n$ = 4, $P < 0.01$.

(e) *Effect of sodium arachidonate on db cyclic AMP-induced $H^+$ secretion* The experimental design was the same as that used for histamine, a single concentration of db cyclic AMP ($10^{-4}$ M) being present during the 90 min stimulation periods. The second response to db cyclic AMP did not differ significantly from the first response either in the absence or presence of A (3.2 × $10^{-5}$ M).

## Discussion

Our results, *in vivo*, demonstrate that A inhibits pentagastrin-induced $H^+$ secretion from the rat stomach. This effect might be due either to a direct or an indirect action of A or its metabolites on secretory cells and the formation of metabolites might occur locally or elsewhere in the body. The inhibition of $H^+$ secretion was associated with a rise in the ratio of MBF/$H^+$, showing that A does not act by reducing MBF. Bieck, Oates & Adkins (1971) observed similar inhibition when A was tested on histamine-stimulated secretion in the Heidenhain-pouch dog. They attributed the effect of A to prostaglandin formation, since the saturated derivative, arachidic acid, had no effect

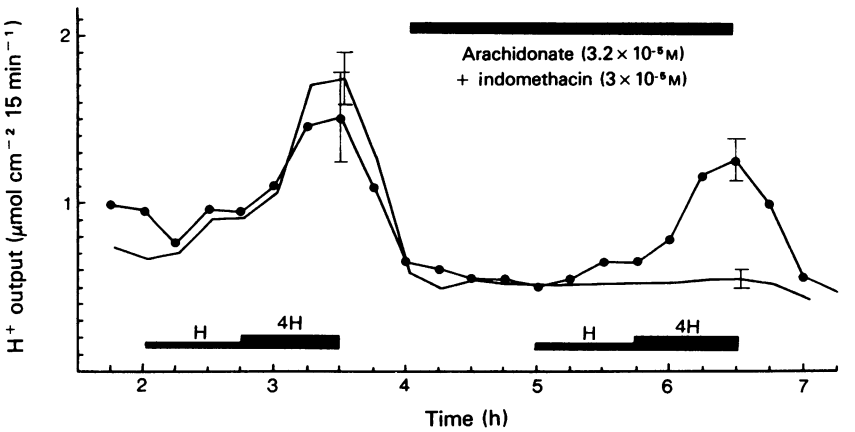


**Figure 6** Acid secretory responses of the rat isolated gastric mucosa to two cumulative doses of histamine (H and 4H, 0.64 and 2.6 × $10^{-5}$ M respectively) in the presence of arachidonate (3.2 × $10^{-5}$ M) (——) or arachidonate and indomethacin (3 × $10^{-5}$ M) (—●—). Standard errors of the mean ($n$ = 4) are indicated at the response peaks.

on secretion. Konturek, Mikos, Pawlik & Walus (1979) also observed an inhibitory effect of A on histamine-stimulated secretion from canine mucosa using a gastric chamber technique.

Inhibition of both pentagastrin and histamine-stimulated $H^+$ secretion by A in our *in vitro* experiments is consistent with these results and indicates that A or metabolites formed by the mucosa can act directly. In contrast to our findings, Ramwell & Crane (1976) found that A potentiated histamine-stimulated $H^+$ secretion in the frog isolated mucosa. However, this effect was not blocked by aspirin suggesting that products of cyclo-oxygenase were not involved.

Inhibitory effects of A on pentagastrin or histamine-induced secretion *in vivo* and *in vitro* were partially or wholly reversed by indomethacin in concentrations which themselves did not increase basal or stimulated secretion.

Indo abolished the effect of A on canine gastric acid and pepsin secretion although injection of Indo alone did not significantly affect mean acid or pepsin output (Konturek *et al.*, 1979). Conolly, Bieck, Payne, Adkins & Oates (1977) observed inhibition of the antisecretory effect of A by eicosatetraynoic acid, an analogue of A which blocks prostaglandin synthesis, although it alone did not increase the secretory response to histamine. In our *in vivo* studies, Indo also blocked the effect of A on blood pressure. Similar inhibition by Indo of the vasodepressor action of A was observed by Larsson & Änggård (1973) in the rabbit. Our results suggest that products of cyclo-oxygenase are responsible for most if not all of the observed effects of A.

The lack of effect of A on $H^+$ secretion induced by db cyclic AMP *in vivo* and *in vitro*, suggests an action prior to production of cyclic AMP. The observation that db cyclic AMP responses are not inhibited by $PGE_2$ *in vivo* (Main & Whittle, 1974) or *in vitro* (Main & Pearce, 1978) is compatible with the suggestion that A acts via prostaglandin-like products.

Since Indo did not increase basal or stimulated acid secretion, either *in vivo* or *in vitro*, our results do not support an inhibitory role for endogenous products of cyclo-oxygenase in $H^+$ secretion under these conditions. This is consistent with previous studies with Indo on $H^+$ secretion and MBF in the dog (Nicoloff, 1968; Bennett & Curwain, 1977; Konturek *et al.*, 1979)) and $H^+$ secretion in man (Winship & Bernhard, 1970; Bennett, Stamford & Unger, 1973). In contrast, Main & Whittle (1975) reported studies in the rat where Indo (40 mg/kg) significantly increased pentagastrin and db cyclic AMP-stimulated $H^+$ secretion and reduced the ratio of MBF/$H^+$, while Gerkins, Shand, Flexner, Nies, Oates & Data (1977) observed that Indo potentiated pentagastrin-stimulated $H^+$ secretion and reduced total gastric blood flow in the anaesthetized dog. Recent studies on the rat isolated mucosa have shown that Indo greatly potentiates the secretory response to db cyclic AMP, an effect which is not blocked by A or $PGE_2$ (Main & Melarange, 1978). Thus the marked effect of Indo on db cyclic AMP-induced $H^+$ secretion *in vivo* may be due to a mechanism other than inhibition of cyclo-oxygenase, such as inhibition of phosphodiesterase (Karppanen & Puurunen, 1974).

The hypothesis that a reduction in MBF and increase in $H^+$ secretion following blockade of endogenous postaglandin formation may under certain conditions contribute to erosion formation (Main & Whittle, 1975; Whittle, 1976) remains unproven. Endogenous prostaglandins may, however, have other

important roles, for example in the control of sodium transport (Chaudhury & Jacobson, 1978) or bicarbonate secretion (Garner & Heylings, 1978), which are essential to the maintenance of mucosal integrity. Various prostaglandins have been shown to be cytoprotective against mucosal lesions caused by Indo and various other damaging agents (Robert, Nezamis, Lancaster & Hanchar, 1978). Although in the present studies, A, administered 1.6 h after Indo, did not prevent erosion formation, a possible protective effect of A administered before Indo cannot be excluded.

In summary, our results demonstrate that the functional rat gastric mucosa can synthesise active products from exogenous A, which may reflect endogenous conversion of the prostaglandin precursor.

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