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EFFECTS OF ASPIRIN-LIKE DRUGS ON CANINE GASTRIC MUCOSAL BLOOD FLOW AND ACID SECRETION

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1 The effects of aspirin, paracetamol and benorylate were studied on gastric mucosal blood flow (MBF) and acid secretion in canine denervated gastric pouches.

2 Aspirin 20 mM in the unstimulated pouch had no effect; pentagastrin-stimulated acid output, but not MBF, was reduced. Aspirin buffered to pH 6 was ineffective.

3 Aspirin 3–50 mg/kg reaching the pentagastrin-stimulated pouch through the blood, increased acid secretion and MBF, but the MBF : secretion ratio was variably affected.

4 Paracetamol (10 or 20 mg/kg i.v., or 20 mM in the pouch) or benorylate (280 mg/kg orally) mainly had little effect.

5 Circular muscle strips from dog arteries were contracted by prostaglandins E_2, $F_{1\alpha}$ or $F_{2\alpha}$, and often slightly by indomethacin, but prostaglandin E_1 produced variable effects.

6 These results do not favour the view that aspirin causes gastric bleeding in dogs by breakdown of blood vessels due to ischaemia following mucosal vasoconstriction.

Introduction

Aspirin and other non-steroidal anti-inflammatory drugs cause gastric mucosal erosions and occasionally severe haemorrhage. They also inhibit prostaglandin synthetase, as first shown by Ferreira, Moncada & Vane (1971), Smith & Willis (1971), and Vane (1971). Virchow (1856) proposed a vascular aetiology of peptic ulceration, and it has been suggested that the vascular changes might be due to inhibition of prostaglandin synthesis (Bennett, Stamford & Unger, 1973; Main & Whittle, 1973). Thus, if prostaglandins maintain vasodilatation, removal of this effect by aspirin might cause ischaemia; breakdown of blood vessels might then occur, particularly in areas already damaged by other means. This suggestion was strengthened by the report that aspirin bathing the gastric mucosa of vagally denervated fundic pouches in unstimulated dogs substantially reduced mucosal blood flow (MBF) (O'Brien & Silen, 1973). However, Augur (1970) had previously found little effect, whereas Lin & Warrick (1974), using pentagastrin-stimulated dogs, found increased MBF with aspirin. The importance of this problem led us to re-investigate it, and to study the effect of prostaglandins and indomethacin (a more potent aspirin-like drug) on isolated mesenteric arteries of the dog.

Methods

Twelve mongrel bitches (12–23 kg) with well-established Heidenhain pouches were used not more than once weekly; they were not used when on heat. Food was withheld for 18 h before experiments, but water was allowed. Mucosal blood flow (MBF) (ml/min) was estimated by clearance of radioactive aniline (Curwain & Holton, 1973), and pouch acid secretion (μmol H^+/min) was measured by titration against 0.1 M NaOH with phenolphthalein as indicator. Where appropriate, acid output, MBF and changes in their ratio were calculated. In some experiments gastric acid secretion was stimulated to between 30 and 70% of maximum, by the infusion of pentagastrin (1–4 μg kg^{-1} h^{-1}) (Peptavlon, ICI), mixed with the aniline solution, into a leg vein at 1 ml/min throughout the experiment. The infusion (with or without pentagastrin) was begun 45–60 min

before observations were made, to allow stabilization of secretion and equilibration of aniline between the various body compartments. Gastric juice was then collected in 15-min periods, and normally 6 collections were made before administration of the test drug into the main stomach, intravenously or into the pouch. Three consecutive 15-min periods before drug administration were averaged (control value). The 15-min period after administration of a single bolus of drug into the main stomach or intravenously was ignored, to give time for drug effects to occur, and the average of the next three readings (test value) was expressed as percentage of control.

With drugs added to the pouch for 30 min in dogs stimulated with pentagastrin, the test values were the mean of three 15-min periods following the 30-min administration period. The pouch was connected to a reservoir and filled with 15–25 ml of the solution described later. Drug concentration in the pouch was kept fairly constant by replacing 5 ml of the contents with fresh solution every 10 min via a small tube which was also used to allow escape of air when the pouch was being filled. The intraluminal pressure was kept at about 2 cm water. In resting pouches, clearance measurements were made during the instillation period from the combined contents (removed each 30 min) and the washings.

Drug solutions

Aspirin or paracetamol 20 mM were dissolved in distilled water 5 min before administration. The pH of the 20 mM aspirin solution was 2.9–3.1; 20 mM acetic acid was used as a control. Aspirin 20 mM in Sorensen's phosphate buffer 0.67 M, pH 6.0 formed buffered aspirin. When aspirin was studied without secretory stimulation 80 mM HCl was instilled into the pouch throughout to prevent back-diffusion of aniline (Curwain & Holton, 1973) and aspirin (20 mM) was instilled for 30 min as a solution usually in 80 mM HCl.

Small volumes were desirable for oral administration to the main stomach, and aspirin was therefore made up in a form similar to mixture of soluble aspirin BPC (aspirin : sodium carbonate : citric acid 13 : 5 : 3 by weight mixed with water in a pestle and mortar). Doses up to 50 mg/kg could therefore be given in less than 20 ml syringed into the dog's mouth while holding the head up. Control experiments showed that the vehicle had no effect on MBF, acid output or the subsequent titration of acid. Benorylate 40% w/v was administered as Benoral suspension (Sterling Winthrop).

Aspirin for intravenous administration was dissolved aseptically in sterile distilled water. Paracetamol was dissolved in 30 ml of the aniline/pentagastrin infusion and infused over 30 minutes. Benorylate is almost insoluble, and unsuitable for intravenous administration.

Studies on dog isolated arteries

Arteries were obtained from anaesthetized greyhounds and strips of circular muscle were prepared by cutting spirally. The preparations (gastric artery, 12 strips from 7 specimens, root mesenteric or splenic arteries, 1 specimen each) were set up under a load of 0.5 g in Krebs solution at 37°C bubbled with 5% CO_2 in O_2, and isotonic responses to prostaglandins E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ determined before and after indomethacin 1–2 μg/ml or (root mesenteric artery only) aspirin 70 μg/ml. The composition of the Krebs solution was as follows (g/l): NaCl 7.1, $CaCl_2.6H_2O$ 0.55, KCl 0.35, KH_2PO_4 0.16, $MgSO_4.7H_2O$ 0.29, $NaHCO_3$ 2.1 and dextrose 1.0.

Results are expressed as means ± s.e., and are analysed statistically by the *t* test for paired data where appropriate, unless stated otherwise. All probability values refer to 2-tailed tests.

Results

Effect of aspirin in the pouch on mucosal blood flow and acid output in unstimulated dogs

The acid recovered from the pouch after instillation of 80 mM HCl for 30 min was 2.7 ± 1.3% more than the blank control titres, indicating a low basal secretion (8 measurements in 4 dogs). Aspirin (20 mM in 80 mM HCl, instilled into the Heidenhain pouch for 30 min) did not significantly change MBF (4.5% ± 10.5), but only 94.3% of the instilled acid was recovered, indicating some back-diffusion of acid (4 experiments in 4 dogs followed for 90 min from the beginning of the instillation). In 2 of these dogs aspirin was also given in distilled water preceded and followed by 80 mM HCl (1.4 and 13% increase in MBF). In 3 further experiments with 3 dogs, aspirin (10 mg/kg i.v.) given during instillation of 80 mM HCl had a variable effect on MBF (−34 to 105% change); the large variations were probably because of the low and variable blood flow under basal conditions.

The effect of aspirin, paracetamol or benorylate on pentagastrin-stimulated acid secretion and mucosal blood flow

Aspirin instilled into the secreting pouch (20 mM for 30 min) reduced the acid secretory output by 22 ± 6% and the concentration by 7.4 ± 2.8% (both $P < 0.05$; 7 experiments in 5 dogs). However, MBF did not change and its ratio to acid output rose by 19 ± 8%. Aspirin buffered to pH 6.0 before instillation did not significantly affect secretory output (−13 ± 20%) or acid concentration (11 ± 7%, $P > 0.1$) (4 experiments in 4 dogs). No consistent changes in MBF, acid output or concentration occurred in control experiments with 20 mM acetic acid or Sorensen's pH 6 buffer (both 3

Table 1 Oral (into main stomach) or intravenous aspirin increased pentagastrin-stimulated acid output in conscious Heidenhain-pouch dogs but variably affected the ratio mucosal blood flow (MBF) : acid output (MBF/Acid)

Dog No.	*Dose (mg/kg orally)*	*% Increase in acid output*	*% Increase in MBF*	*% Change in MBF/Acid*	*Dog No.*	*Dose (mg/kg i.v.)*	*% Increase in acid output*	*% Increase in MBF*	*% Change in MBF/Acid*
1	20	18	18	0	5	3	22	17	−4.1
2	20	116	123	+3.2	3	10	19	15	−3.4
3	20	95	80	−7.7	2	10	3.3	3.5	+0.1
1	33	20	21	+0.8	2	15	70	94	+14.1
4	50	104	95	−4.5	3	20	68	90	+13.0
2	50	72	73	+0.5	4	20	25	27	+1.6
3	50	106	59	−22.8	2	20	52	62	+6.5
					6	20	34	35	+0.7
					4	44	55	56	+0.6
					2	50	113	103	−4.7

Six dogs were used: 4 received oral aspirin and 5 received intravenous aspirin.

experiments in 3 dogs). In all 17 experiments in 6 dogs, aspirin 3–50 mg/kg orally (i.e. into the main stomach) or intravenously increased acid output (3.3–116%) and MBF (3.5–123%) over a period of 120–135 min following the dose. The ratio of MBF to acid output was variably affected (Table 1). Paracetamol (20 mM) in the pouch had no significant effect on acid output ($9.4 \pm 5\%$ $0.1 > P > 0.05$), acid concentration ($4.8 \pm 3.3\%$ $P > 0.1$) or MBF ($15 \pm 10\%$ $P > 0.1$) (4 experiments in 4 dogs). Paracetamol (10 or 20 mg/kg i.v.; 3 experiments in 3 dogs) had no marked effect on acid output (range −1.7 to −11%) but MBF rose 7.5–47%. With benorylate 280 mg/kg orally acid output increased (by $57 \pm 34\%$) in each of 4 experiments 15–45 min after administration ($0.1 > P > 0.05$) and then declined to previous levels, but the MBF : acid secretion ratio did not alter significantly ($0.6 \pm 9\%$).

No bleeding from the pouch was visible with instilled drugs, but on two occasions dogs vomited some bloody gastric juice 1.5 h after oral administration of 50 mg/kg aspirin. The effects of aspirin on pouch secretion began within 15 min of administration.

Arterial strips

At the start of the experiment, each strip was made to contract to noradrenaline 150–600 ng/ml. The responses to prostaglandins E_2, $F_{1\alpha}$ and $F_{2\alpha}$ were similar before and after giving indomethacin (1–2 μg/ml). Prostaglandins E_2 and $F_{2\alpha}$ (50–300 ng/ml) always caused contraction, but lower concentrations were ineffective; prostaglandin $F_{1\alpha}$ (0.3–3 μg/ml) had no effect or caused contraction. Prostaglandin E_1 (0.03–1.5 μg/ml) had a variable effect, causing small relaxations, small contractions or no effect when given before indomethacin. Indomethacin 1–2 μg/ml often increased tone, but the effect was very slight. Prostaglandin E_1 given in the presence of indomethacin usually caused a relaxation, but this seemed unlikely to be due substantially to the raised tone (Figure 1). The mesenteric artery was initially unaffected by prostaglandin E_2 (25–

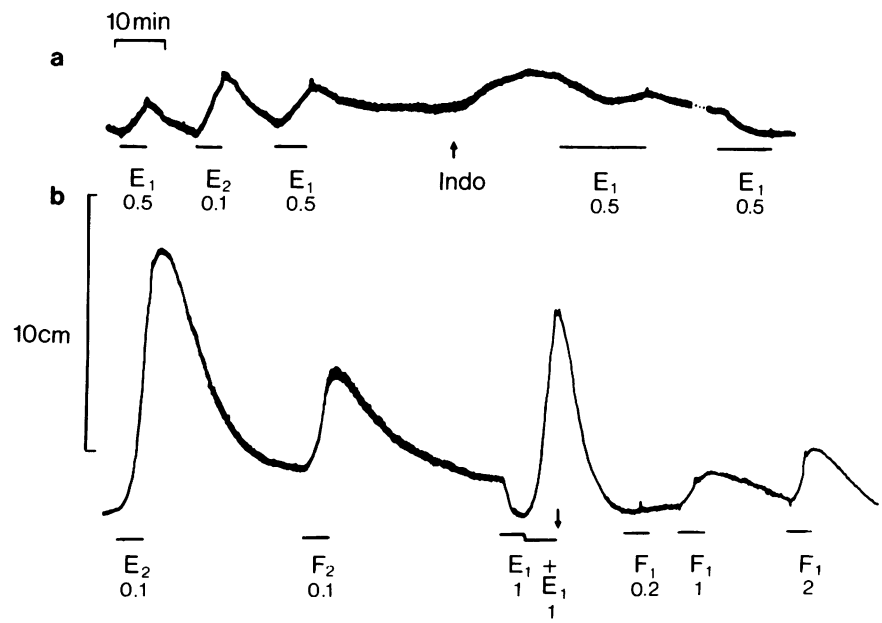

Figure 1 Dog spiral artery strip. (a) Prostaglandin E_1 (E_1) or prostaglandin E_2 (E_2) initially evoked contractions. Indomethacin 2 μg/ml (Indo) at the arrow (and re-added with every change of bath fluid) temporarily increased the tone. Subsequent doses of prostaglandin E_1 were inhibitory, even when the tone was at a pre-indomethacin level. (b) Follows immediately from (a), with indomethacin still present. Prostaglandins E_2 or $F_{2\alpha}$ (F_2) caused contraction. Prostaglandin E_1 in double the dose used previously was inhibitory, but quadruple the dose was excitatory. Indomethacin was washed out at the arrow. Prostaglandin $F_{1\alpha}$ (F_1) caused dose-dependent contractions. Contact times of prostaglandin indicated by solid lines. Numbers below trace indicate μg/ml. The minute lines on the trace were due to air bubbles.

50 ng/ml). After aspirin 70 μg/ml, which slightly increased tone, prostaglandin E_2 or $F_{2\alpha}$ (50–100 ng/ml caused contraction but prostaglandin E_1 (100 ng/ml) caused relaxation.

Discussion

We were unable to produce substantial changes in MBF with aspirin instilled into the non-secreting Heidenhain pouch. This is in contrast to the marked reduction obtained by O'Brien & Silen (1973), but similar to the findings of Augur (1970). During stimulation of secretion with pentagastrin, administration of 20 mM aspirin into the pouch had no effect on mucosal blood flow, whereas Lin & Warrick (1974), using 28 mM aspirin in 0.1 M HCl, found an increase of 25–30%. They and others (e.g. Chvasta & Cooke, 1972) obtained gastric mucosal bleeding which was absent in our studies. Nevertheless, like Lin & Warrick (1974) we found that aspirin reduced acid output from the secreting pouches. We confirm the findings of numerous investigators that aspirin causes back-diffusion of H^+ through the gastric mucosa (e.g. Davenport, 1964; O'Brien & Silen, 1973; Lin & Warrick, 1974; 1975). Buffering the aspirin to pH 6 prevented this effect in our experiments, presumably because absorption of acetylsalicylic acid was inhibited and there was little acid to diffuse back. Aspirin given intravenously or into the main stomach during pentagastrin infusion increased acid output from the pouch. This is consistent with an inhibitory role of prostaglandins in gastric secretion (Robert, Nezamis & Phillips, 1967); removal of the inhibition by aspirin would increase acid secretion, and the rise in output would be only partly offset by loss of acid by back-diffusion following mucosal damage. Aspirin also increased MBF, presumably secondarily to increased secretion or to back-diffusion of H^+ (see later), although the ratio of increased MBF to secretion was variable.

The reason why the results of Augur (1970) and our results differ from those of O'Brien & Silen (1973) is not clear. The latter authors drew attention to their antrectomy procedure, and suggested that circulating gastrin might affect the vascular responses in the pouch mucosa. In both Augur's and our experiments the dogs were not antrectomized, so that our experiments were more 'physiological'. Another difference is that only O'Brien & Silen instilled polyethylene glycol into the pouch. Neither Augur nor O'Brien & Silen stated the sex of animals used; ours were female. Another possible explanation is that aspirin might affect processes which have different effects on MBF. Perhaps prostaglandin within the canine gastric mucosa normally inhibits acid secretion (Robert *et al.*, 1967) but the tendency for aspirin to cause vasoconstriction by inhibiting prostaglandin synthesis in blood vessels is offset by stimulation of blood flow secondary to increased acid secretion, or by vasodilatation caused by back-diffusion of H^+ (Ritchie, 1975; Whittle, 1976). This argument seems plausible with pentagastrin stimulation since aspirin increased acid output. It seems less likely in the resting pouch where aspirin did not change the zero output, but perhaps secretion occurred which was lost by back-diffusion. Benorylate or paracetamol, in amounts respectively four times higher and the same as used in man, mainly had little effect on the stomach, and it might be relevant that in man these drugs produce little or no gastric bleeding. However, it is not known if they inhibit prostaglandin synthesis in dog stomach; prostaglandin synthetases vary in their sensitivity to drugs.

Formation of prostaglandin-like material by dog mesenteric arteries is inhibited by indomethacin (A. Robert, personal communication). Like aspirin, this drug too causes gastric bleeding and back-diffusion of H^+ in the dog (Lin & Warrick, 1975). It also produces vasoconstriction in rat gastric mucosa (Main & Whittle, 1975) and at other sites (human cerebral and conjunctival vessels, Sicuteri, Michelacci & Anselmi, 1965; Vecchio & Fontana, 1965). Similarly, we obtained a slight contraction with indomethacin in canine arterial strips, and it seems likely that a similar effect occurs in the dog gastric microvasculature. However, *in vivo* changes in other factors such as acid secretion might offset the vasoconstriction. Of the prostaglandins tested, only prostaglandin E_1 caused relaxation, in agreement with the findings (Shehadeh, Price & Jacobson, 1969) that infusion of prostaglandin E_1 0.05–1 μg kg^{-1} h^{-1} intra-arterially increased mesenteric artery blood flow in anaesthetized dogs, whereas prostaglandin $F_{2\alpha}$ 0.1–1 μg kg^{-1} h^{-1} usually reduced flow. Thus, if the weak vasoconstrictor effect of indomethacin which occurred in some strips was due to inhibition of locally produced prostaglandins, perhaps the formation and effect of prostaglandin E_1 predominated in those arteries. Alternatively, other prostaglandins or thromboxanes might be produced, or indomethacin might act on another pathway such as inhibition of cyclic adenosine 3′,5′-monophosphate phosphodiesterase (Flower, 1974).

It is possible that in rats, aspirin causes gastric bleeding by inhibiting prostaglandin synthetase and causing vasoconstriction (Main & Whittle, 1973). Our results suggest that this is not so in the dog, although a tendency to cause vasoconstriction might be offset by secretory stimulation and by back-diffusion of H^+. It might be relevant that we did not observe bleeding from the unstimulated pouch with locally administered aspirin, but the dose was sufficient to produce secretory changes when given by other routes during pentagastrin stimulation. What happens in man is not

known, but since indomethacin (administered rectally) did not increase human gastric acid secretion during submaximal stimulation with pentagastrin (Bennett *et al.*, 1973) there seems unlikely to be a secretory stimulus to offset any direct indomethacin-induced vasoconstriction that might occur. The answer must await clinical studies on gastric mucosal blood flow.

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