J. Pharm. Pharmacol. 1982, 34: 51–53
Communicated February 11, 1981

0022–3573/010051–03 $02.50/0

The non-steroidal anti-inflammatory agent parsalmide prevents aspirin-induced H^+ back diffusion from the gastric lumen of the rat

ALBERTO BIANCHETTI*, ANTONIO LAVEZZO, PAOLO CARMINATI, *Clin-Midy Research Laboratories—Midy S.p.A. Via Piranesi 38—Milano, Italy*

Parsalmide is a non steroidal anti-inflammatory agent (NSAIA) the pharmacological activity of which has been demonstrated (Capretti & Marinoni 1974; Ferrero et al 1976; Maffi et al 1976; Di Penta & Mastrangelo 1978). A special characteristic is its gastric tolerance (Carminati et al 1978, 1981). In the rat, it did not induce gastric erosions at doses much greater than those that inhibit oedema formation (Carminati et al 1978). Paradoxically, it has also been found to prevent the ulcerogenic effects of aspirin and other NSAIAs in the rat without interfering with their anti-inflammatory activities (Carminati et al 1978). Our aim has been to see if parsalmide is able to counteract the disruption of the gastric mucosal barrier induced in the rat by aspirin. Aspirin is believed to induce local gastric damage by promoting back diffusion of H^+ ions present in the gastric lumen into the gastric mucosa (Davenport 1967).

Female Sprague Dawley rats, 180 ± 5 g, fasted for 24 h, were used. Abdomens were opened under light ether anaesthesia, then stomachs exposed and the gastric lumena gently washed with 4 ml of warm 0·9% NaCl (saline). Each pylorus was then tied with a silk thread and 4 ml of an aqueous solution of 100 mM HCl was immediately introduced into the lumen. A second ligature was then made around the cardiac end of the oesophagus. Dual ligation was used to minimize spontaneous gastric secretion (Levine 1965) thus emphasizing the disappearance of H^+ ions from the gastric lumen. After the operation, which took about 3 min, the animals were returned to their cages and after 1 h were killed and the gastric juices collected for determination of volume and of residual H^+ (titration to pH 7 with 0·1 M NaOH) and Na^+ (titration with a Beckman Astra 8 potentiometer with a Na-selective electrode) ions. Net flux of ions through the gastric mucosa was calculated by subtracting the amount of ion present at 0 time (rats killed immediately after instillation of gastric solution) and from the amount recovered 1 h after pyloric ligature.

For the evaluation of the intrinsic effects of drugs on the ion fluxes, aspirin or parsalmide was directly introduced into the gastric lumen. To study the effect of parsalmide on the change of flux induced by aspirin, parsalmide was given either intragastrically (added to the solution introduced into the gastric lumen) or orally in a volume of 1 ml of 0·5% aqueous carboxymethylcellulose suspension, 2 h before dual ligation. Controls received the vehicle only. Parsalmide placed into the gastric lumen did not interfere to any significant extent with the titration of gastric solutions.

* Correspondence.

There was a slight disappearance of H^+ ions from the gastric lumen and a gain in Na^+ ions in the control rats during the 1 h period (Fig. 1). Parsalmide directly introduced into the gastric lumens of rats, in doses of 45 and 90 μmol/rat, did not significantly change the net transmucosal fluxes of H^+ and Na^+ ions. At the dose of 180 μmol/rat, parsalmide slightly reduced the apparent H^+ disappearance from the gastric lumen. In contrast, aspirin caused its expected dose-dependent disappearance of H^+ from the lumen, concomitant with Na^+ gain. The lack of effect of parsalmide on ion fluxes between lumen and mucosa is consistent with its slightly basic nature (pK = 4·6). Parsalmide is, in fact, largely ionized at the acidic pH of the gastric solution and therefore should not diffuse to any significant extent into the gastric mucosa.

Figs 2 and 3 show the effect of parsalmide on the aspirin-induced changes in H^+ and Na^+ net fluxes. Parsalmide present with aspirin in the acidic solutions from gastric lumena of rats did not alter to any extent the effects of aspirin alone (Fig. 2). When administered orally 2 h before the test, parsalmide reversed the net H^+ flux observed in normal rats and prevented the action of aspirin, significantly inhibiting H^+ disappearance at doses of 45 and 90 μmol/rat (60 and 120 mg kg^{-1}). The Na^+ gain was also significantly antagonized (Fig. 3).

The fact that parsalmide counteracts aspirin only when given orally 2 h before dual ligation and not when placed directly in the pylorus-ligated stomach indicates that it probably has to be absorbed from the intestinal lumen to be active, suggesting that the protective effect is not topical. From these results it also appears that the effect of parsalmide is not due to a physicochemical interaction between aspirin and parsalmide, which, since it is basic, could have been trapped in the gastric lumen. It may still be argued that the antagonistic effect of parsalmide on aspirin-induced H^+ back diffusion might be more apparent than real. This would be true if pretreatment with parsalmide induced hypersecretion by parietal cells, which would compensate for the H^+ ions that disappeared from the gastric lumen. However, at the doses we used, parsalmide was found to decrease rather than to increase gastric secretion (Carminati et al 1981). Furthermore, in the present experiment the gastric mucosae of rats pretreated with parsalmide were normal, while those of rats treated with aspirin alone already exhibited diffuse reddening and superficial oedema when the gastric contents were collected. This last observation supports the argument that parsalmide effectively prevents the breakdown of the

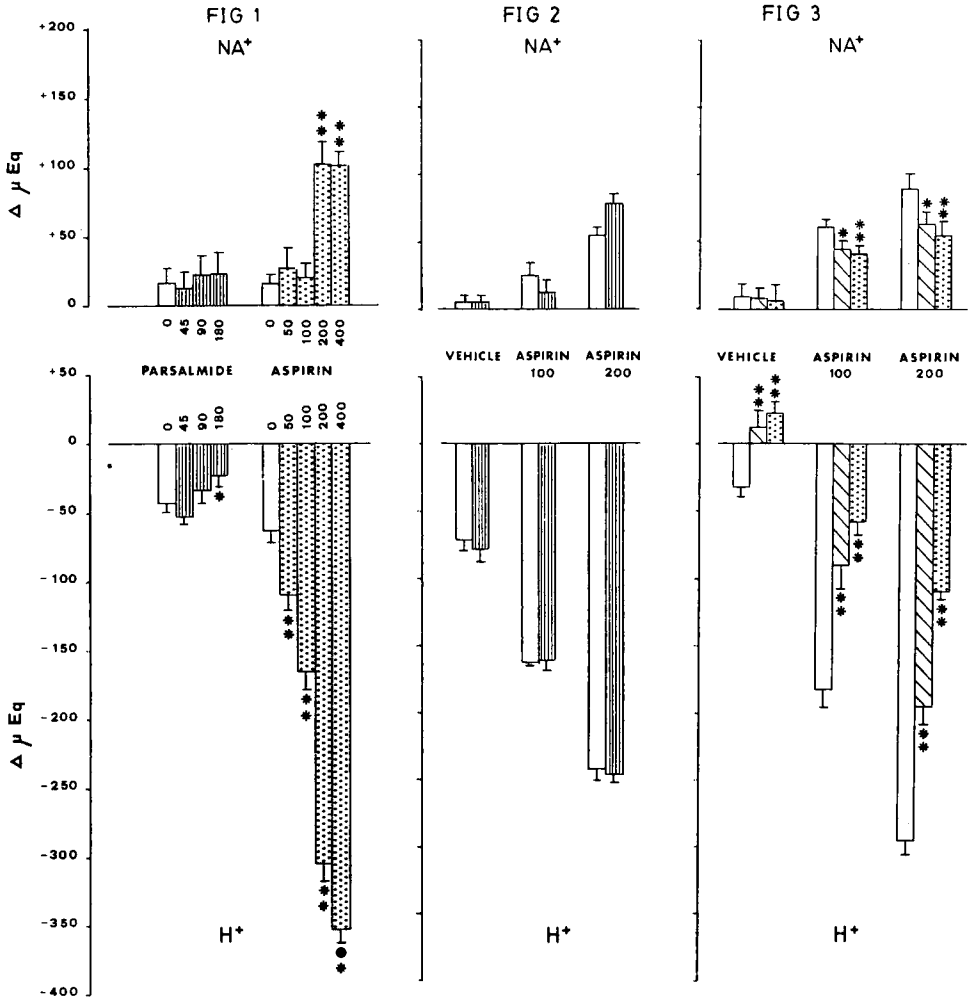

FIG. 1. Effects of parsalmide and aspirin on the H^+ and Na^+ net fluxes through the gastric mucosa of pyloric ligated rats. Columns represent the mean net fluxes of ions in the presence of vehicle (open), parsalmide (hatched) or aspirin (dotted). Net fluxes are the differences between the ions recovered from the gastric lumen 1 h after ligature and those present at 0 time. A negative sign indicates disappearance from the gastric lumen. Each column is the average of at least 8 animals. Bars represent standard errors about the means. Figures at the base of the columns represent the dose of drug in μmol/rat introduced into the gastric lumen. Significance of difference vs vehicle (Dunnett's test): * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$.

FIG. 2. Effects of parsalmide in the gastric lumen on the aspirin-induced alteration of H^+ and Na^+ net fluxes through the gastric mucosa of pyloric ligated rats. Columns represent the mean net fluxes of ions in the absence (open) or in the presence of parsalmide (hatched) 45 μmol/rat. Parsalmide was added to the solution of aspirin introduced into the gastric lumen. Figures at the base of the columns represent the amount of aspirin administered in μmol/rat. For other details see Fig. 1.

FIG. 3. Effects of orally administered parsalmide on the aspirin-induced alteration of H^+ and Na^+ net fluxes through the gastric mucosa of pylroic ligated rats. Columns represent the mean net fluxes of ions in rats orally treated with vehicle (open) or parsalmide (hatched) 45 μmol/rat, (dotted) 90 μmol/rat, 2 h before the pyloric ligation. Figures at the base of the columns represent the amount of aspirin administered in μmol/rat. Significance of difference vs vehicle (Dunnett's test): * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$. For other details see Fig. 1.

gastric mucosal barrier, which is considered of major importance in the gastric damage induced by aspirin.

Parsalmide also prevented gastric damage induced by other NSAIAs (Carminati et al 1981). Antagonism of experimentally induced ulcers has already been reported for anti-inflammatory drugs other than parsalmide (Robert et al 1977; Seegers et al 1978). The mechanism of action of this protection is not well understood. On the basis of our experiments, we can only speculate on the mechanisms by which parsalmide may prevent the effects of aspirin. It is likely that the strengthening of the mucosal barrier depends on some intrinsic property of parsalmide functioning at the level of the gastric mucosa. Recent results suggest that parsalmide is able to increase the production of gastric mucus in the rat (Bertaccini et al 1979) and gastric mucus is known to be essential for the protection of gastric mucosa (Allen & Garner 1980). Therefore, this property of parsalmide could at least partially account for its beneficial effects.

REFERENCES

Allen, A., Garner, A. (1980) Gut 21: 249–262

Bertaccini, G., Molina, E., Coruzzi, G., Chiavarini, M. (1979) Il Farmaco (Ed. Pr.) 34: 482–491

Capretti, G., Marinoni, C. (1974) Clinica Ter. 68: 233–245

Carminati, P., Lavezzo, A., Manzoni, L., (1978) 7th International Congress of Pharmacology, Paris, July 16–21, Abstr. 529: 202

Carminati, P., Lavezzo, A., Manzoni, L., Giudice, A., Aureggi, G., Bianchetti, A. (1981) Il Farmaco (Ed. Pr.) 36: 58–72

Davenport, H. W. (1967) New Engl. J. Med. 276: 1307–1312

Di Penta, A., Mastrangelo, R. (1978) Minerva Ortop. 29: 383–388

Ferrero, E., Giudice, A., Guzzon, V., Pedrazzoli, A. (1976) Boll. Chim. Farm. 115: 145–156

Levine, R. J., (1965) Life Sci. 4: 959–964

Maffi, G., Dall'Asta, L., Pedrazzoli, A. (1976) Boll. Chim. Farm. 115: 135–144

Robert, A., Hanchar, A. J., Lancaster, C., Nezamis, J. E. (1977) Gastroenterology 72: 1120

Seegers, A. J. M., Jager, L. P., Van Noordwijk, J. (1978) J. Pharm. Pharmacol. 30: 84–87

J. Pharm. Pharmacol. 1982, 34: 53–55
Communicated May 28, 1981

0022–3573/82/010053–03 $02.50/0

Modulation of central noradrenaline release by postsynaptic receptors

MICHAEL G. WYLLIE*, MARTYN D. WOOD, JUDI PHEYSEY, JULIAN FOX, *Biochemical Pharmacology, Wyeth Institute of Medical Research, Huntercombe Lane South, Taplow, Maidenhead, Berkshire, U.K.*

The release of noradrenaline (NA) from nerve terminals, in addition to being dependent on nerve impulses, is modified by α-adrenoceptors (Langer 1977; Starke 1979; Doxey & Roach 1980). In comparison with studies in the autonomic nervous system, studies in the c.n.s. are more difficult to interpret as there is no easily measurable equivalent to 'end-organ response'. The relative contributions of pre- and post synaptic components of drug action, therefore, are difficult to assess. By use of selective α-adrenoceptor agonists and antagonists, it has become apparent that there is some degree of α-adrenoceptor modulation of NA release in the c.n.s. (Anden et al 1967; Haggendal 1973; Meek & Neff 1973; Farnebo & Hamberger 1973; Braestrup & Nielsen 1976; Starke 1979). In contrast to the autonomic system, however, where the α-adrenoceptors modulating release are generally considered to be located presynaptically, the location of these receptors in the c.n.s. is more equivocal. In both systems the results obtained using selective antagonists at α_2-adrenoceptors such as yohimbine and rauwolscine, are generally considered as evidence indicative of a presynaptic locus (Langer 1977; Starke 1979; Doxey & Roach 1980). This has been substantiated by examining regulation of release from cell cultures containing no postsynaptic material and from synapses with no well-defined postsynaptic α-adrenoceptors (for review see Langer 1979). Certain anomalies (Kalsner & Chan 1979; Chan & Kalsner 1979) led (Kalsner et al 1980) to suggest that this hypothesis is too simplistic. It may be necessary, therefore, to re-examine the original suggestion that postsynaptic α-adrenoceptors may also modulate NA release (Haggendal 1970; Hedqvist 1970; Farnebo & Hamberger 1971; Farnebo & Malmfors 1971). This is of particular relevance in the c.n.s. where the location of receptors is less well defined than in the autonomic system. Using two selective postsynaptic α-adrenoceptor antagonists; prazosin (Doxey & Everitt 1977; Drew 1977) and indoramin (Rhodes & Waterfall 1978), we have attempted to examine the α-adrenoceptor mediated control of NA release in the c.n.s.

Synaptosomes were isolated from rat cerebral cortex using differential and density-gradient centrifugation (Gilbert & Wyllie 1976). Uptake was measured in a medium of the following composition (mM): NaCl 136; KCl 5; $MgCl_2$ 1·2; $CaCl_2$ 2·5; Tris 20; ascorbate 1; and glucose 10. The medium was gassed with pure oxygen and then adjusted to pH 7·4 with HCl. Uptake was initiated by addition of (−)-[^{3}H]NA[10^{-7} M], and terminated by filtration (Sugden 1974) on cellulose acetate 0·45 μm filters. All other analyses were as described elsewhere (Gilbert & Wyllie 1980; Wyllie & Gilbert 1980).

Treatment of animals with indoramin (5, 25 mg kg^{-1} orally) or prazosin (12·5, 62·5 mg kg^{-1} orally) reduced NA levels in 4 brain areas 2 h after dosing (Table 1).

* Correspondence.