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The activity of non-steroidal anti-inflammatory drugs in the rat mesenteric vasculature

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Rat isolated, perfused mesenteric blood vessels have been used to determine whether non-steroidal anti-inflammatory drugs (NSAIDs) display selectivity for inhibition of vasoconstrictor responses to noradrenaline compared with those to calcium. The rank order of potency of the NSAIDs for inhibition of responses to noradrenaline was: meclofenamate > flufenamate = diclofenac > indomethacin > fenbufen > phenylbutazone > ibuprofen > ketoprofen > naproxen > paracetamol (included as an inhibitor of cyclo-oxygenase). All NSAIDs show selectivity for noradrenaline (mean selectivity molar ratio = 0·19; s.e.m. 0·15–0·23) but there was a positive correlation ($r = 0·98$, $P < 0·001$) between inhibition of responses to noradrenaline and those to calcium. The depressant effect of meclofenamate and of fenbufen, the most potent and most selective in the series, respectively, on responses to noradrenaline, was completely reversed to control values by prostaglandin E_2. The results support previous findings that inhibition of cyclo-oxygenase in rat mesenteric blood vessels leads to a loss of response to noradrenaline. Comparison of the present data for inhibition of responses to noradrenaline in the mesentery with published data indicates that the mesentery bears a greater similarity to other in-vitro rather than in-vivo models for screening NSAIDs.

Non-steroidal anti-inflammatory drugs (NSAIDs) have been used extensively as inhibitors of cyclo-oxygenase to investigate the role of prostaglandins in the reactivity of vascular smooth muscle to noradrenaline and other vasoconstrictors. Aspirin and indomethacin, for example, depress responses to noradrenaline which are then restored with prostaglandin E_2 (PGE_2) in rat isolated perfused mesenteric blood vessels (Horrobin et al 1974; Manku & Horrobin 1976; Coupar & McLennan 1978; Kondo et al 1980). The rat mesenteric vasculature releases PGE_2-like material at rest; the rate of release is increased by noradrenaline and inhibited by indomethacin (Coupar 1980; Desjardins-Giasson et al 1982, 1984; Soma et al 1985). This strongly implies that PGE_2 is essential for the vasoconstrictor effect of noradrenaline.

Indomethacin has been shown to inhibit responses to calcium (Northover 1968; Manku & Horrobin 1976). However, this effect does not appear to be related to inhibition of PGE_2 synthesis, since the responses to calcium, that are inhibited by indomethacin, are not restored by PGE_2, and calcium does not induce the release of any endogenous PGE_2-like substance (Coupar 1981).

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The aim of this study was to determine whether NSAIDs show selectivity in depressing vasoconstrictor responses to noradrenaline as opposed to calcium in rat mesenteric vascular tissue. In addition, the use of this tissue to predict NSAID activity was determined by comparison of the potency of these compounds in inhibiting vasoconstrictor responses to noradrenaline with their potencies in other screening tests.

METHODS

The perfused mesenteric vasculature of the rat

The mesenteric vasculature of male Hooded Wistar rats (200–355 g) was isolated according to McGregor (1965) following anaesthesia with pentobarbitone sodium (80 mg kg^{-1}, s.c.). The mesenteric blood vessels were perfused with a physiological solution at a constant rate of 2 ml min^{-1}, gassed with 5% CO_2 in O_2 and maintained at 37 °C. A modification to the method, as described by Coupar & McLennan (1978) was used, whereby the inlet tubing to the peristaltic pump (Cole-Palmer) had two equal lengths attached via a Y-piece to to allow for changes in the solution reservoirs without altering the baseline pressure. Changes in perfusion pressure were recorded via a Statham P23Db pressure transducer connected to a Grass polygraph. The experiments

involving vasoconstrictor responses to noradrenaline were carried out using a modified Krebs-Henseleit solution of the following composition (g litre^{-1}): NaCl 6·92, KCl 0·35, $MgSO_4.7H_2O$ 0·29, KH_2PO_4 0·16, $NaHCO_3$ 2·1, $CaCl_2$ 0·35 and glucose 2·0. In experiments involving responses to calcium a Ca^{2+}-free/K^+-rich depolarizing solution was used (K_2SO_4 16·0, $KHCO_3$ 1·0, $MgCl_2.6H_2O$ 0·215, $NaH_2PO_4.2H_2O$ 0·65 and glucose 1·02) (Northover 1968). In addition, phenoxybenzamine (3·3 μmol litre^{-1}) was perfused for the first 10 min to block possible interference with calcium responses caused by noradrenaline release. There was an equilibration time of 30 min and consistent responses to agonists occurred 60 to 90 min after commencement of perfusion. Noradrenaline was perfused for 10 s and again after 2 min. $CaCl_2$ was perfused for 90 s.

Effect of inhibitors on the responses to noradrenaline and calcium

Responses were initially established to noradrenaline (as duplicates 2 min apart) or calcium, and repeated every 30 min, then increasing concentrations of each inhibitor were perfused for 20 min. The potency of an inhibitor in depressing the response to noradrenaline or calcium was determined by measuring the IC50.

Restoration of responses to noradrenaline by PGE_2, following depression by meclofenamate, fenbufen or verapamil

Duplicate responses to noradrenaline with a 2 min interval, were repeated every 15 min, and once established meclofenamate, fenbufen or the calcium channel inhibitor verapamil were perfused to depress the response by approximately 75%. Increasing concentrations of PGE_2 were then introduced allowing a 10 min equilibration time and the ability of PGE_2 to restore the noradrenaline-induced responses was measured as a percentage of the difference between the depressed responses and the initial control responses to noradrenaline.

Statistical analysis

IC50 and 95% confidence interval values were calculated from a line of best-fit for a 'graded dose-response' (Tallarida & Murray 1981). A comparison of the 95% confidence intervals was used as an indication of the significances of difference between the IC50 values of an inhibitor against noradrenaline and calcium. The relation between the IC50 values was calculated using regression analysis (Tallarida & Murray 1981). The percentage restoration was calculated as $[(R - C_2)/(C_1 - C_2)] \times 100$ where C_1 = control response to noradrenaline alone, C_2 = response to noradrenaline in the presence of inhibitor and R = response to noradrenaline in the presence of inhibitor and various concentrations of PGE_2.

Drugs

Stock solutions of inhibitors were prepared in a minimum volume of 50 mM Na_2CO_3 and dissolved in the appropriate perfusing solution. Prostaglandin E_2 (PGE_2) was dissolved in ethanol to 28 mM (previous studies in this laboratory have shown that ethanol in the concentrations used for preparing PGE_2 solutions does not affect vasoconstrictor responses to noradrenaline or calcium), then diluted to 280 μM with 200 mM phosphate buffer, and used as a working solution. Drugs used were: diclofenac sodium and phenylbutazone (Ciba-Geigy), fenbufen (Cyanamid), flufenamic acid BP (Parke-Davis), ibuprofen (Boots), indomethacin (Merck, Sharp & Dohme), ketoprofen (May & Baker), meclofenamate sodium monohydrate (Warner-Lambert), naproxen (Syntex), noradrenaline bitartrate (Levophed, Winthrop), paracetamol (Riker), pentobarbitone sodium (Nembutal, Abbott Laboratories), phenoxybenzamine hydrochloride (Smith, Kline & French), prostaglandin E_2 (Upjohn) and verapamil hydrochloride (Isoptin, Schering).

RESULTS

Effects of NSAIDs on responses to noradrenaline and calcium

The baseline perfusion pressure of tissues perfused with modified Krebs-Henseleit solution was 11 ± 1 mmHg (n = 51) and that of tissues perfused with Ca^{2+}-free/K^+-rich depolarizing solution 25 ± 1·5 mmHg (n = 54). The approximate EC75 of noradrenaline was 50 μM and of calcium, 5 mM.

Increasing concentrations of each NSAID were tested for their inhibitory effect on the EC75 values of noradrenaline and calcium. Fig. 1 shows the inhibitory effect of meclofenamate on responses to noradrenaline (NA) and calcium (Ca^{2+}). The IC50 values were used to analyse the inhibitory potencies and selectivities of the drugs shown in Table 1. For fenbufen, no inhibition of responses to calcium could be detected up to a concentration of 1 mM. Paracetamol, although not an NSAID, was included for comparison. The ratios of IC50 NA/IC50 Ca^{2+} are all less than one, indicating selectivity for noradrenaline. Verapamil was included as a control drug of known calcium antagonistic action. The data

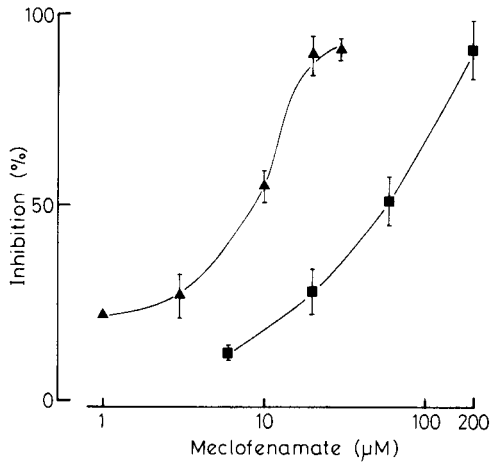

FIG. 1. Percentage inhibition of vasoconstrictor responses to noradrenaline (n = 4) (▲), and calcium (n = 3) (■) by meclofenamate in the rat mesenteric vasculature. The bars indicate the standard error of the mean for this and all following figures.

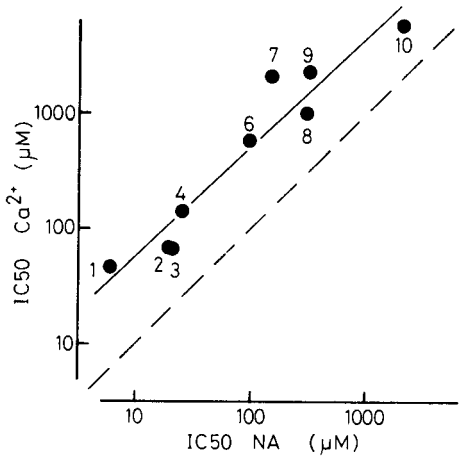

FIG. 2. Correlation between the inhibition of vasoconstrictor responses to noradrenaline and calcium by NSAIDs and paracetamol. The numbers refer to those used in Table 1. Points above, and to the left of the broken line have selectivity for noradrenaline.

from Table 1 are plotted in Fig. 2 to show the significant correlation between inhibition of responses to noradrenaline and inhibition of responses to calcium.

Restorative effect of PGE_2 on responses to noradrenaline inhibited by meclofenamate, fenbufen or verapamil

Prostaglandin E_2 restored responses to noradrenaline depressed by meclofenamate to a maximum of 140% of control levels. In the presence of fenbufen, responses to noradrenaline were restored to approximately 100% of control levels, however, verapamil-depressed responses were only restored to approximately 25% by PGE_2 (Fig. 3).

Table 1. Potencies of NSAIDs, paracetamol and verapamil as inhibitors of vasoconstrictor responses to noradrenaline and calcium, together with selectivity ratios in the rat perfused mesenteric vasculature.

		Concentration (μM)		IC50 (NA)
No	Drug	IC50 (NA)	IC50 (Ca^{2+})	IC50 (Ca^{2+})
1	Meclofenamate	5·93*	45·7	0·13
2	Flufenamate	19·1*	67·6	0·28
3	Diclofenac	20·8*	66·5	0·31
4	Indomethacin	25·0	140	0·18
5	Fenbufen	44·6	†	<0·05
6	Phenylbutazone	95·9*	579	0·17
7	Ibuprofen	142*	2127	0·067
8	Ketoprofen	292*	1004	0·29
9	Naproxen	316*	2280	0·14
10	Paracetamol	2027*	5960	0·34
	Verapamil	7·14*	0·049	143

† No inhibition up to 1 mM (limit of solubility).
* $P < 0·05$ comparison of IC50(95% confidence intervals). The mean selectivity ratio of drugs 1–10 (excluding 5) was 0·19 (s.e.m. 0·15–0·23).

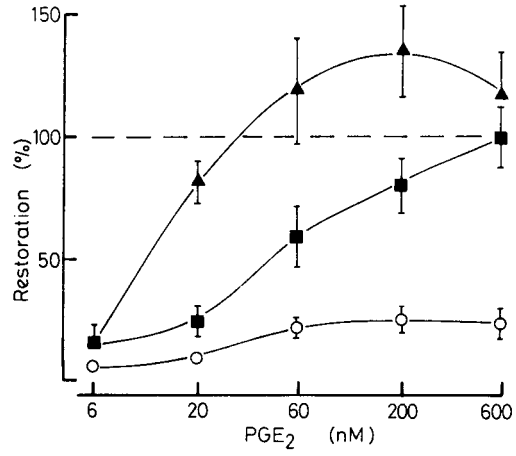

FIG. 3. Restoration by PGE_2 of responses to noradrenaline depressed by meclofenamate 8 μM (n = 3) (▲), fenbufen 300 μM (n = 4) (■) and verapamil 30 μM (n = 6) (○). The broken line indicates the level of complete (100%) restoration.

Comparison of results with other published data

The results were compared with published data using other animal models for screening NSAIDs. It can be seen from Table 2 that the relation between inhibition of noradrenaline-induced responses in the mesentery and in-vivo models is poor. Other models display a better relation, with inhibition of prostaglandin synthesis in the dog spleen reaching statistical significance ($P = 0·02$) (see Fig. 4).

Table 2. Correlation between inhibition of responses to noradrenaline in the rat mesenteric vasculature and models used for screening NSAIDs.

Test	n	Correlation coefficient	Reference
Carageenan paw oedema	9	0·043	Dearden & Nicholson (1984)
Gastric mucosal damage	8	0·055	Dearden & Nicholson (1984)
Castor oil diarrhoea delay	9	0·33	Awouters et al (1978)
Sheep seminal vesicles (prostaglandin E_2 synthesis)	5	0·51	Flower (1974)
Bovine seminal vesicles (total prostaglandin synthesis)	5	0·71	Flower (1974)
Dog spleen (prostaglandin E_2 synthesis)	4	0·98*	Flower et al (1972)

*P = 0·02; all other comparisons were not statistically significant.

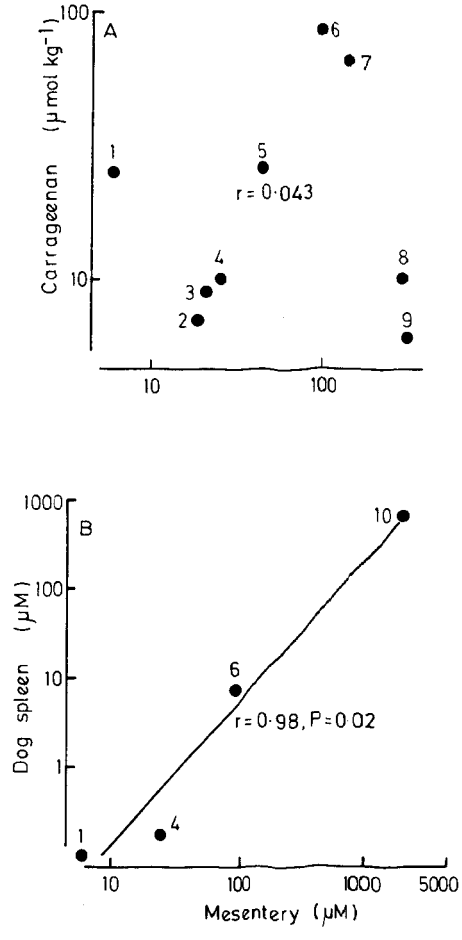

FIG. 4. (A) Comparison of the potency of NSAIDs at inhibiting responses to noradrenaline in the rat mesenteric vasculature and anti-inflammatory potency in the rat carageenan-induced paw oedema (Dearden & Nicholson 1984). (B) Relation between potency of inhibition of noradrenaline and that of inhibition of dog spleen cyclo-oxygenase (Flower et al 1972).

DISCUSSION

The non-steroidal anti-inflammatory drugs (NSAIDs) tested are representative of different chemical classes: meclofenamate and flufenamate are anthranilic acids, diclofenac and indomethacin are acetic acids, fenbufen, ibuprofen, ketoprofen and naproxen are propionic acids and phenylbutazone is a pyrazolone. Paracetamol, although not an NSAID, has been shown to inhibit cyclo-oxygenase (Flower et al 1972) and was therefore included. All drugs depressed vasoconstrictor responses to noradrenaline and calcium, except fenbufen which did not depress responses to calcium in concentrations up to 1 mM (the limit of its solubility). The relation between their ability to depress vasoconstrictor responses to noradrenaline and calcium is statistically significant which could indicate a common mechanism of inhibition of the two agonists but this is unlikely because responses to noradrenaline that have been depressed by aspirin (Horrobin et al 1974), indomethacin (Coupar & McLennan 1978), or meclofenamate and fenbufen (present study) are restored by PGE_2, while responses to calcium that have been depressed by indomethacin are not affected (Coupar 1981). Also, noradrenaline stimulates the release of PGE_2-like material which is inhibited by indomethacin, whereas calcium does not promote the release of PGE_2 (Coupar 1980, 1981). This strongly implies that the response to noradrenaline is inhibited by the NSAIDs by virtue of their inhibitory effect on the cyclo-oxygenase pathway. On the other hand, the inhibition of the response to calcium is by a mechanism which appears to be independent of cyclo-oxygenase but may have similar structure-activity requirements considering the high correlation for the inhibition of both agonists. Additionally, fenbufen which has been assumed to be a pro-drug (Martindale 1982), depresses responses to noradrenaline but not those to calcium. The remaining NSAIDs have selectivity for noradrenaline with a mean selectivity ratio of 0·19 (s.e. mean 0·15–0·23). This demonstrates that inhibition of calcium is not a prerequisite for inhibition of noradrenaline, thus supporting the hypothesis that the two mechanisms of inhibition are not directly related.

It is likely that the NSAIDs inhibit the effect of noradrenaline at a point distal to the α-adrenoceptor, since PGE_2 does not affect the affinity of phentolamine for this receptor (Coupar & McLennan 1978). Noradrenaline acts on α_1-adrenoceptors of rat mesenteric vascular smooth muscle cells to release internal calcium in a clonic response, indi-

cated by a 60–100% response to noradrenaline in the absence of extracellular calcium (Adeagbo & Okpako 1980; Cauvin & Malik 1984; Skarby et al 1984). Therefore, it is likely that the PG-dependent action of NSAIDs involves inhibition of intracellular calcium release rather than inhibition of calcium action once it has been released. The work of Gorog & Kovacs (1970) lends support to this hypothesis. They concluded that NSAIDs may affect the ability of the sarcoplasmic reticulum to accumulate and release calcium and that they inhibit the contraction of actomyosin (using super-precipitation of actomyosin). However, the concentrations required to produce this effect were much greater than those required to inhibit responses to noradrenaline and comparable to those required to inhibit responses to calcium in the present study. This may explain the inhibitory effect of NSAIDs on responses to calcium, a conclusion strengthened by the fact that indomethacin and flufenamate have no effect on ^{45}calcium entry into rat mesenteric arterial smooth muscle (Northover 1968).

The IC50 values of the NSAIDs for noradrenaline were compared with other well recognized models for screening these drugs (Table 2). Relations with in-vivo models, i.e. the carageenan paw oedema, delay of castor oil diarrhoea and gastric lesion are poor, but with in-vitro models such as dog spleen and bovine or sheep seminal vesicular cyclo-oxygenase systems, there is closer agreement. This may be explained on the basis that in-vivo potency is determined by many pharmacokinetic as well as pharmacodynamic factors. The mesenteric vasculature model may, therefore, be of limited value in the preliminary screening of NSAIDs but the comparative data of this and other studies need to be treated with caution.

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