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Suprofen, A New Peripheral Analgesic[1]

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ABSTRACT

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The antinociceptive properties of suprofen [α-methyl-4-(thienylcarbonyl)benzene acetic acid] are described in a pathologically induced hyperalgesic model, the rat adjuvant arthritic flexion test. By using this assay, suprofen was characterized as an orally effective, non-narcotic analgesic with a rapid onset and 4-hr duration of activity. Suprofen is 50 times more potent than acetaminophen, five times more potent than codeine and equipotent to the new peripheral analgesics, zomepirac and diflunisal. In combination experiments, suprofen potentiates the analgesic effects of acetaminophen and, unlike morphine, the analgesic effect of suprofen is not blocked by naloxone. In other hyperalgesic assays, suprofen is an extremely potent inhibitor of arachidonate-induced writhing and is equipotent to morphine in the yeast-induced paw edema (Randall-Selitto) assay. Additionally, suprofen is inactive on the normal paw in the Randall-Selitto test, the mouse Eddy hot-plate test and the tail withdrawal reflex assay induced by warm water in rats, all sensitive tests capable of detecting central (narcotic) but not peripheral analgesics. Activity on prostaglandin biosynthesis from several species and tissues suggests that suprofen is a tissue selective inhibitor of prostaglandin synthesis. These experiments suggest that suprofen represents a new class of potent, orally effective, peripheral (non-narcotic) analgesics with potential usefulness in a variety of clinical pain situations formerly reserved for narcotics.

Analgesics can be broadly classified as acting centrally (narcotic) or peripherally (non-narcotic). The centrally acting analgesics are very effective in a variety of clinical situations but, in addition to relieving pain, cause an array of side effects that limit their clinical utility. The peripherally acting analgesics also have anti-inflammatory, antipyretic and antithrombotic properties. This class of agents has been developed in the past decade primarily for the purpose of improving anti-inflammatory activity and have generally been classified as nonsteroidal anti-inflammatory drugs (NSAID). With the greater clinical exposure of these newer anti-inflammatory compounds, more attention has been given to their analgesic action. Recent clinical testing has demonstrated these newer agents to be not only more potent than some of the narcotic analgesics, but, in certain clinical situations, more efficacious (Smith *et al.*, 1975; Bloomfield *et al.*, 1976; Cooper and Beaver, 1976; Cooper and Sullivan, 1978). This finding has stimulated a search to find new, safer and more effective peripherally acting agents capable of relieving pain.

This report will describe the analgesic profile of suprofen (Janssen, 1975; McGuire *et al.*, 1978), a new, potent, orally effective non-narcotic analgesic agent in a model of pathological pain and will discuss its differential activity on prostaglandin biosynthesis. The structure of suprofen is shown in figure 1.

Methods

Analgesic activity in the rat adjuvant arthritic flexion test. Polyarthritis was induced in male Wistar Lewis rats (150–200 g) by the injection (0.1 ml) of an autoclaved suspension of *Mycobacterium butyricum* (0.75 mg, Difco Laboratories, Detroit, MI) in Fruend's incomplete adjuvant. The injections were made in the subplantar tissue of the left hindpaw or into the distal third portion of the tail. Seventeen days later, the rats were tested for their tendency to vocalize after flexion of the tarso-tibial joint of the noninjected paw (Kuzuna and Kawai, 1975, Capetola *et al.*, 1978) or both paws in the case of the tail-injected animals. Only those rats that clearly vocalized five successive times at approximately 3-sec intervals were accepted into the test group. Rats with ankylosed tarso-tibial joints were rejected.

The rats were fasted for 18 to 24 hr. On day 18 (18 days after injection of adjuvant) test drugs were administered by gavage and at various times after administration of drug, usually 1, 2, 3, 4 and 5 hr) the number of vocalizations were recorded after five flexions of the tarso-tibial joint. The data are presented in either of two formats: mean number of vocalizations in the group or in all or none criterion such that if a rat vocalized three times or more it was considered nonanalgesic, two times or less, it was considered analgesic. Animals received the appropriate drug dose in 5 ml/kg.

Analgesic activity in the yeast-induced paw edema test. A modification of the method of Randall and Selitto (1957) was used. Male Sprague-Dawley rats (Charles River Laboratories, Inc., Wilmington, MA) weighing 200 to 300 g were fasted for 18 to 24 hr with water

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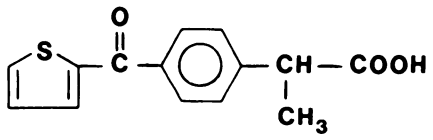

∝-Methyl-4-(2-thienylcarbonyl)

benzene acetic acid

Fig. 1. Chemical structure of suprofen.

ad libitum. Grams of pressure tolerated by both hindpaws were determined with a Ugo Basile analgesic meter (with one additional 70 g weight disc added). Rats tolerating 200 or more grams after three trials were eliminated from the test. One-tenth milliliter of a 20% suspension of brewer's yeast was injected into the subplantar tissue of the left hindpaw and 2 hr later compounds were administered (5 ml/kg) by appropriate routes. One hour after administration of compound or vehicle (0.5% methylcellulose), the pressure tolerated by both hindpaws was measured and the values recorded.

Arachidonic acid writhing. Male Charles River CD_1 mice weighing 16 to 23 g were divided into groups ($N = 10$) and placed individually into Plexiglas observation arenas (2.5 × 5.5 × 3.5 inch). The mice (fasted at least 16 hr) were dosed orally with a suspension of drug or 0.5% methylcellulose vehicle. One hour later, 1 mg/kg of arachidonic acid (dissolved in 0.9% saline and titrated to pH 7.4 with 0.1 N NaOH and then peroxidized by aeration for 5 min) was administered i.p. The final product was termed arachidonic acid peroxide (AAP). Mice were observed from 2 to 15 min after administration of AAP for the presence of writhing.

Data were reported as all or none (number of mice writhing per number of mice treated) in vehicle and respective treatment groups. Treatment groups were compared with the control group by χ^2 analysis by using the Yates Correction. ID_{50} values for each drug treatment were estimated by using the linear portion of the dose-response curve (Finney, 1964).

Renal blood flow in the dog. Renal blood was monitored in the dog by a modification of the method described by Williamson *et al.*, 1978. Briefly, adult mongrel dogs of either sex (8–24 kg) were anesthetized with pentobarbital sodium (35 mg/kg i.v.) and surgically prepared for electromagnetic measurement of renal blood flow. The flow probe (Carolina Medical Electronics) was placed around the left renal artery at the site of branching from the abdominal aorta. The right femoral artery was cannulated to monitor arterial blood pressure and the right femoral vein was cannulated to administer drugs. Heart rate was continuously monitored by a cardiotachometer throughout the experiment. All recordings were made on a Beckman dynograph.

Arachidonic acid cascade. Cyclo-oxygenase activity was assayed in isolated perfused rabbit heart and kidney, prostacyclin synthetase activity from bovine aorta microsomes, thromboxane synthetase activity from human platelet plasma and anti-prostaglandin E_2 (PGE_2) or PGH_2 receptor activity on strips of rabbit aorta, bovine coronary artery, rat fundus and chicken rectum (P. Needleman, personal communication). Cyclo-oxygenase activity from bovine seminal vesicles was assayed according to the method of White and Glassman (1974).

Compounds used. The authors would like to thank the following organizations for their generous supply of compounds: acetaminophen and zomepirac Na (McNeil Laboratories, Inc., Fort Washington, PA); sulindac (Clinoril), diflunisal and codeine phosphate (Merck Sharp and Dohme, West Point, PA); proquazone (Sandoz Pharmaceuticals, Hanover; NJ); ibuprofen (Motrin; The Upjohn Company, Kalamazoo, MI); *d*-propoxyphene (Darvon) and morphine sulfate (Eli Lilly and Company, Indianapolis, IN); naloxone (Narcan; Endo Laboratories, Inc., Garden City, NY); pentazocine (Talwin; Sterling-Winthrop Research Institute, Rensselaer, NY); suprofen (Janssen Pharmaceutica, Beerse, Belgium); aspirin, indomethacin and phenylbutazone were purchased from Sigma Chemical Company, St. Louis, MO.

Suprofen was either suspended in 0.5% methocel or solubilized in water by the stoichiometric addition of 1 N NaOH. In all cases, the appropriate doses of compounds were calculated on the basis of their respective free acid or base.

Results

Analgesic activity in the adjuvant arthritic rat. The optimum time of vocalization subsequent to the initiation of adjuvant arthritis in unprovoked animals is 16 to 18 days (Pircio *et al.*, 1975). This time interval also coincides with the appearance of established polyarthritic signs, most notably prominent swelling of the hindpaws. Care was taken in these studies not to include animals with ankylosed joints since the objective was to measure the pain associated with flexion of an arthritic joint.

Table 1 presents the analgesic ED_{50} values and relative potency ranking of suprofen and nine compounds as determined by the vocalization response in adjuvant arthritic rats. For comparative purposes, codeine and morphine were chosen as representative narcotic analgesics. Sulindac and ibuprofen represented recently introduced NSAID. Proquazone (Gubler and Baggiolini, 1978), diflunisal (Steelman *et al.*, 1978) and zomepirac sodium were chosen as new non-narcotic analgesics not currently marketed in the U.S.A. The ED_{50} is defined as that dose which reduces the number of vocalizations by one-half relative to vehicle control animals. For the determination of relative potency values, suprofen was compared to each compound by using a common slope. With the exception of morphine, the regression lines for these drugs did not deviate significantly from parallelism (Finney, 1971).

As can be seen from the data in table 1, suprofen is 50 times more potent than acetaminophen and 5 times as potent as codeine, while being 20 and 1.56 times less potent than morphine (i.p.) and proquazone, respectively. Suprofen is statistically equipotent to sulindac, zomepirac, ibuprofen and diflunisal in this test. Interestingly, indomethacin was inactive at 3 mg/

TABLE 1

Analgesic activity of suprofen and nine standard compounds in the adjuvant-induced polyarthritic rat flexion test

Compound	N	Oral ED_{50}[a]	Relative Potencies (95% C.I.) at Peak Effect[b]
		mg/kg	
Suprofen	98[c]	4.62	1.00
Codeine	44	22.68	0.20 (0.2, 0.87)[d]
Morphine	40	0.23[e]	20.00 (2.85, 125)[d]
Sulindac	40	1.62	2.86 (0.36, 21.7)
Proquazone	39	2.96	1.56 (2.0, 500)[d]
Acetaminophen	50	232.34	0.02 (0.005, 0.06)[d]
Zomepirac	47	1.02	4.55 (0.25, 59)
Ibuprofen	58	11.34	0.41 (0.08, 1.74)
Diflunisal	42	4.44	1.04 (0.08, 6.8)
Indomethacin	10	>3.00	

[a] ED_{50}s and relative potencies were calculated by using a parallel line bioassay (Finney, 1964) with the common slopes of each compound's dose-response data compared with that of suprofen. A minimum of four doses was used to generate each dose-response curve.

[b] C.I., confidence interval.

[c] Pooled from two experiments.

[d] Statistically different from suprofen.

[e] Morphine was administered i.p. The slope of the curve was significantly different from the suprofen slope. Therefore, the relative potency was not calculated by using the common slope.

kg, a dose which far exceeds its anti-inflammatory dose in adjuvant arthritic rats.

All the compounds tested, with the exception of acetaminophen, were capable of almost completely inhibiting the vocalization response. However, codeine at 30 and 100 mg/kg and morphine at 10 mg/kg had typical central nervous system effects, whereas proquazone caused sedation and lethargy at a dose of 30 mg/kg. Indomethacin was not tested at doses above 3 mg/kg.

Typical dose-response regression lines for suprofen and three reference compounds are plotted in figure 2. The four nonnarcotic analgesics have statistically common slopes probably indicating a common mechanism of action within this class of compounds.

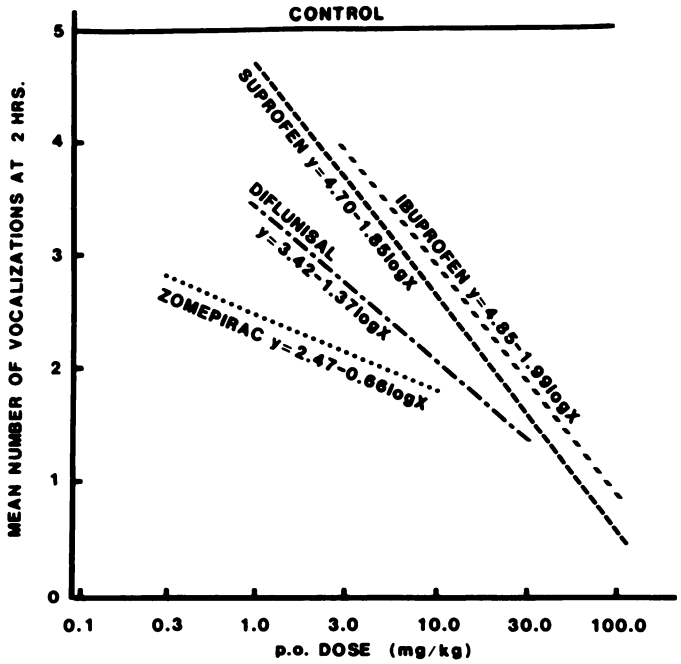

Fig. 2. Log dose response regression representing individual slopes of four compounds in the adjuvant-induced hyperalgesic flexion test for analgesic activity at 2 hr postdrug. Minimum of four doses used for each regression line and nine rats per dose. None of the regression lines deviated significantly from parallelism (Finney, 1971).

The onset of action of suprofen is rapid, progressively increasing from 0.5 to 2 hr. Peak activity of suprofen was recorded at 2 hr. Table 2 presents an additional experiment showing a comparison of suprofen and codeine with respect to their dose-response and duration of action relationship. The peak effect of codeine is seen at 1 hr, the response declines rapidly and is inactive at the end of 2 hr. In contrast, suprofen peaks at 2 hr and at the highest dose significant analgesia is still apparent at 4 hr.

Naloxone antagonism. The ability of naloxone to reverse the analgesic effect of morphine or suprofen was assessed in polyarthritic rats (fig. 3). Naloxone (0.5 mg/kg) administered s.c. in the nape of the neck had no effect when administered alone. Morphine (3 mg/kg i.p.) caused a 68% inhibition of the number of vocalizations measured at the time of peak activity and this effect was completely reversed by naloxone. In contrast, naloxone had no effect upon the analgesic action of suprofen measured at the time of peak activity.

Separation of the anti-inflammatory (antiedema) effect from analgesic activity. Table 3 presents simultaneous measurement of paw volume (by using a mercury plethysmograph) and the number of vocalizations in adjuvant arthritic rats after the oral administration of 10 mg/kg of suprofen. In the control animals, there is a slight but insignificant decrease in paw volume at 1 and 2 hr, but there is no decrease in the number of vocalizations. Suprofen caused a significant and progressive decline in the number of vocalizations, 1 and 2 hr postdrug, while causing no significant decrease in paw volume.

Interaction of suprofen and acetaminophen in the adjuvant arthritic rat. Numerous clinical studies point out the many possible interactions that occur between the NSAID and the peripheral analgesics (Bulletin on the Rheumatic Diseases, 1977-78). For example, an investigation of indomethacin efficacy in rheumatoid arthritis failed to find a drug-placebo dif-

TABLE 2

Onset and duration of analgesic activity in the adjuvant arthritic rat after oral administration (mean number of vocalizations ± S.E.M.)

COMPOUND	n	1 Hr.	2 Hr.	3 Hr.	4 Hr.	5 Hr.
CONTROL	16	5.0 ±0.0	5.0 ±0.0	4.7 ±0.2	4.9 ±0.1	5.0 ±0.0
CODEINE-SO_4						
0.3 mg/kg	9	4.4 ±0.4	4.4 ±0.6	4.4 ±0.6	5.0 ±0.0	5.0 ±0.0
1.0 mg/kg	9	5.0 ±0.0	4.9 ±0.1	4.7 ±0.3	4.9 ±0.1	5.0 ±0.0
3.0 mg/kg	8	5.0 ±0.0	4.4 ±0.4	4.6 ±0.3	4.6 ±0.4	5.0 ±0.0
10.0 mg/kg	9	2.8 ±0.6	4.3 ±0.3	4.7 ±0.3	5.0 ±0.0	5.0 ±0.0
30.0 mg/kg	9	1.0 ±0.4	2.2 ±0.6	4.6 ±0.3	4.8 ±0.2	5.0 ±0.0
SUPROFEN						
1 mg/kg	8	4.6 ±0.4	4.3 ±0.4	4.8 ±0.2	5.0 ±0.0	4.9 ±0.1
3 mg/kg	9	3.1 ±0.7	2.1 ±0.8	2.9 ±0.7	3.9 ±0.6	5.0 ±0.0
10 mg/kg	9	2.6 ±0.6	1.0 ±0.6	2.2 ±0.7	3.6 ±0.6	4.6 ±0.3
30 mg/kg	9	2.3 ±0.7	1.0 ±0.6	2.9 ±0.7	3.4 ±0.7	5.0 ±0.0
100 mg/kg	9	1.9 ±0.6	1.0 ±0.4	2.0 ±0.6	2.6 ±0.7	4.6 ±0.2

⊞ $P < .05$ compared to control value (modified t test, Cochran and Cox, 1960).

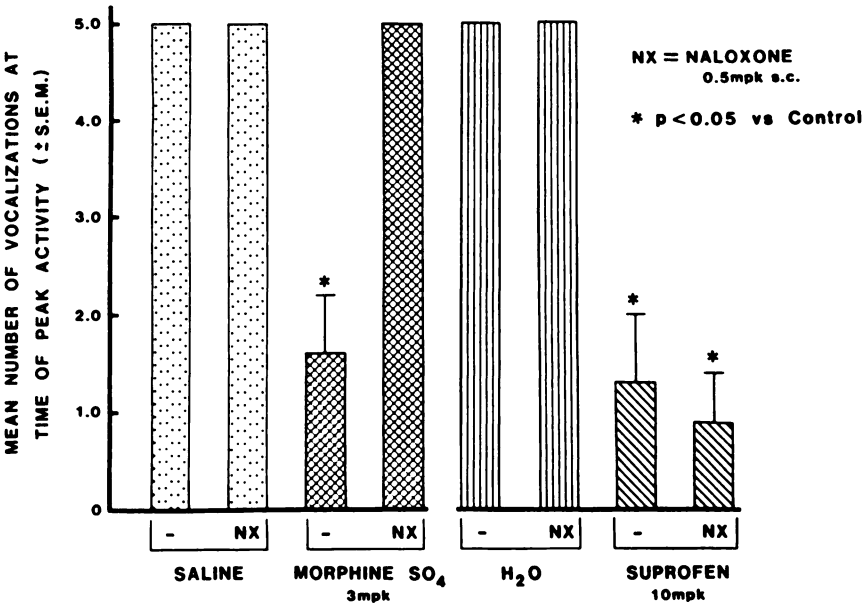

Fig. 3 Failure of naloxone (NX) to block the analgesic activity of suprofen in the rat adjuvant arthritic flexion test. Each treatment group represents a minimum of 10 rats. The saline treatment represents the controls for the i.p. administration of morphine SO_4 and the distilled water treatment for suprofen (p.o.). Morphine was tested 1 hr postdrug, suprofen 2 hr postdrug. In all groups, naloxone HCl was administered s.c. 15 min before measurement of vocalization.

TABLE 3
Dissociation of the analgesic from the antiedema activity of suprofen

Treatment[a]	Dose	Mean Decrease in Paw Volume		Mean No. of Vocalizations		
		1 hr	2 hr	0.5 hr	1 hr	2 hr
	mg/kg	*ml*				
Distilled H_2O	5	0.05 ± 0.05	0.15 ± 0.06	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0
Suprofen	10	0.06 ± 0.04	0.14 ± 0.07	4.58 ± 0.28	3.42 ± 0.58*	2.41 ± 0.54*

* $P < .05$ (modified *t* test, Cochran and Cox, 1960) compared to the distilled water control group).
[a] Suprofen was administered orally in distilled water as the sodium salt. The number of vocalizations was determined immediately after measuring the paw volume by mercury plethysmography. Minimum of 12 rats per group.

ference when aspirin was allowed *ad libitum* during the course of this study (Cooperating Clinics of the American Rheumatism Association, 1967). Similar data have been demonstrated in the adjuvant arthritic rat (Van Arman *et al.*, 1972). Accordingly, the interaction of suprofen and acetaminophen was tested in the adjuvant arthritic rat with an experimental design capable of detecting additivity, antagonism or potentiation.

A total of thirty groups (8–10 rats per group) were used for the entire experiment. Randomization was accomplished by assigning each cage a number drawn from a box and the experiment was done in a blind manner. Each dose-response curve for acetaminophen (30, 60, 120 and 240 mg/kg p.o.) was repeated in the presence of an increasing concentration of suprofen (0.0, 0.3, 1, 3, 10 and 30 mg/kg p.o.) and ED_{50} values were calculated for each dose-response curve interaction. In these experiments, the criteria used for an effect were that if an animal squeaked three or more times it was considered nonanalgesic, two times or less, it was analgesic. The doses of acetaminophen and suprofen were plotted as rectangular coordinates and an isobole (using ED_{50} values) was constructed (fig. 4) with acetaminophen along the *x*-axis and suprofen values along the *y*-axis. Most of the points were found lying below the theoretical straight line joining the two points with the coordinates. The straight line is defined as the line of additivity and the hyperbola as potentiation. Statistical analysis (Scaf, 1974) showed the experimental hyperbola to be significantly different from linearity ($P < .01$). Thus, the data indicates that suprofen and acetaminophen potentiate each other in this model.

Other analgesic assays. In the Randall-Selitto paw pressure test in rats, suprofen raised the threshold of only the inflamed paw (fig. 5) but had no effect on the normal paw. The data shown are from 1 hr postdrug administration.

The centrally acting analgesic agent, morphine, was able to raise the thresholds of both the normal and inflamed paws to about 100% above control values. The other peripherally acting analgesics, indomethacin, aspirin and phenylbutazone, raised the threshold of only the inflamed paws. In the hyperalgesic (inflamed) paw, suprofen was 7 times more potent than indomethacin, 142 times more potent than phenylbutazone, 238 times more potent than aspirin and equipotent to morphine, *i.e.*, within the 95% confidence interval.

AAP injected i.p. in mice produces a writhing syndrome which is inhibited by narcotic analgesics and antagonists and the peripherally acting analgesics (Collier *et al.*, 1968, 1973; Helfner and Jaques, 1968, 1970; James and Church, 1978). Recent evidence suggests that prostaglandins, formed from exogenously administered AAP, are the causative agents in this model of algesia (Collier *et al.*, 1973; Moncada and Vane, 1977). Inhibitors of prostaglandin biosynthesis would therefore be expected to attenuate the writhing induced by AAP adminis-

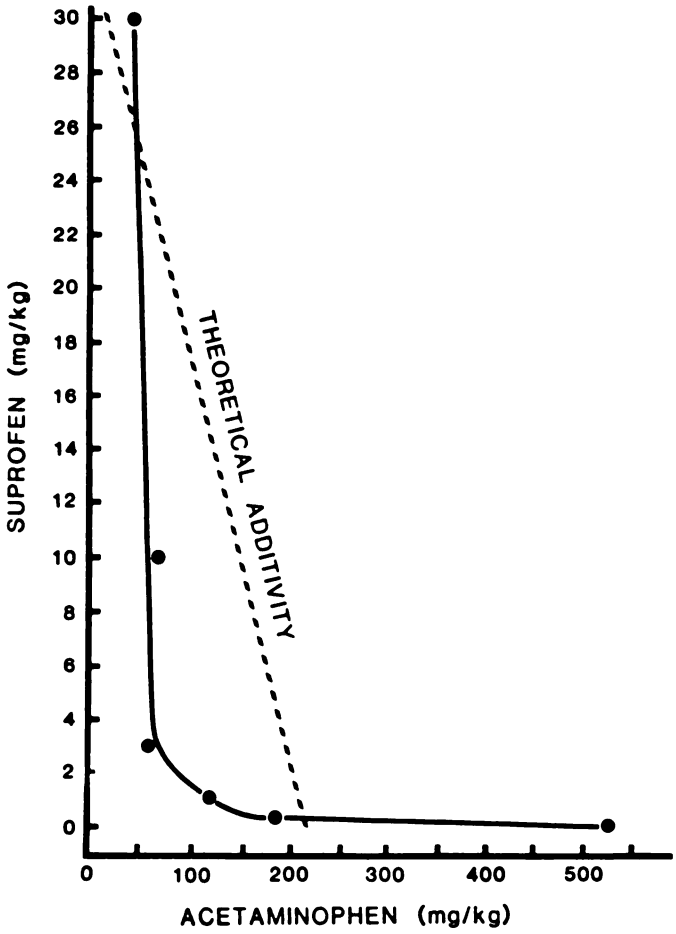

Fig. 4. Isobolograph of the analgesic interaction of suprofen and acetaminophen in the adjuvant-induced hyperalgesic flexion test. Each ● represents an ED_{50} determination of the various combination of doses and shows syngergy between acetaminophen and suprofen; - - -, represents theoretical additivity.

tration. Table 4 shows the oral ID_{50} values of suprofen and four standard peripheral analgesics on AAP-induced writhing in mice. The approximate order of potency is indomethacin > suprofen > aspirin > phenylbutazone > acetaminophen.

Mydriatic activity in mice and the Eddy hot-plate assay (as modified by Janssen and Jageneau, 1957) as well as the warm water-induced tail withdrawal reflex in rats (Janssen *et al.*, 1963) are assays capable of detecting narcotic analgesics. Oral doses of suprofen up to 160 mg/kg were without effect on increasing the hot-plate reaction time, pupil diameter in mice or prolonging the tail reaction time in rats to warm water (C. J. E. Niemegeers, personal communication).

Arachidonic acid cascade. The ability of suprofen to inhibit prostacyclin synthetase was determined by bioassay on bovine coronary artery strips. The control response generated by 200 ng of PGH_2 and blood vessel microsomes was 6.2 ± 0.2 cm relaxation. Concentrations of suprofen ranging from 200 to 1000 μg/ml failed to inhibit prostacyclin formation (6.3 cm). As a positive control, 0.3 to 1.0 μg/ml of 15-hydroperoxy-arachidonic acid was used and caused complete inhibition. At very high doses of suprofen, (1500 μg/ml or higher) slight inhibition was detectable, but was probably due to nonspecific effects.

Thromboxanes produce a rapid and profound contraction of the isolated rabbit thoracic aorta strip. The control response produced by the incubation of 200 ng of PGH_2 plus the platelet microsomes was 6.3 ± 0.8 cm contraction. Suprofen, at doses of 150 to 500 μg did not change the response (5.9 ± 1 cm, $n = 5$), whereas 1000 and 1500 μg produced a 60% inhibition. Imidazole, a known inhibitor of thromboxane synthetase, inhibited the platelet enzyme at concentrations of 50 to 200 μg/ml.

The antiprostaglandin effects were determined on strips of rabbit aorta, bovine coronary artery, rat fundus and chicken rectum by infusing suprofen at a rate of 0.1 ml/min into the Krebs-Henseleit media before bathing the tissues. Final concentrations of suprofen at 3 to 50 μg/ml did not alter the contractile response to directly applied PGE_2 or PGH_2 tested at 50, 100 and 200 μg.

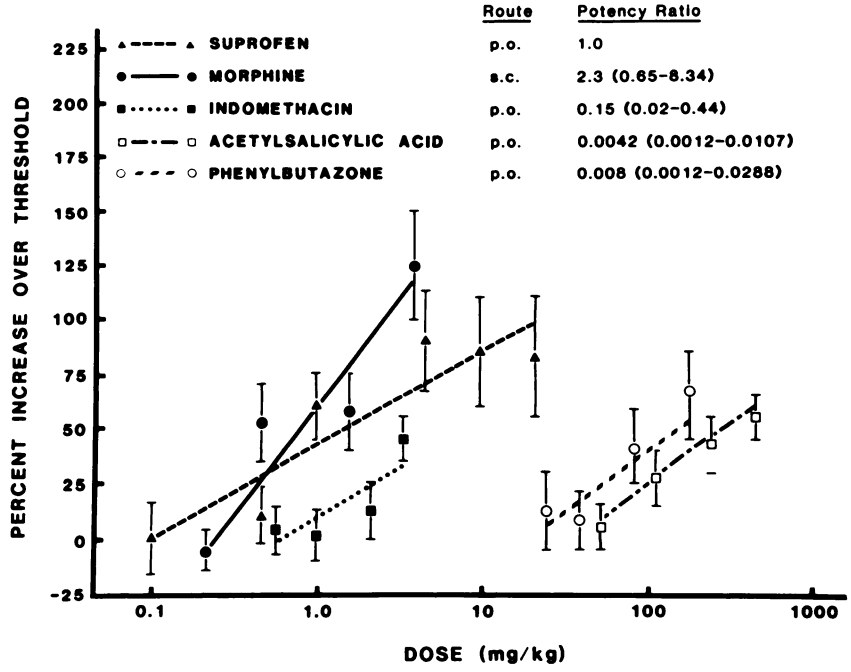

Fig. 5. Dose-response curves on the inflamed paw of suprofen and four standards for analgesic activity 1 hr postdrug in the Randall-Selitto paw pressure test in the rat. The potency ratios (95% confidence interval) are shown in the upper right-hand corner. Minimum 10 rats per dose.

TABLE 4

The oral inhibitory potency of suprofen and four other peripherally acting analgesics on arachidonic acid-induced writhing[a]

Treatment[b]	Dose	N/N[c]	% Inhibition	ID_{50} (95% F.L.)
	mg/kg			mg/kg
Vehicle control		22/30[d]		
Suprofen	0.01	7/10	4.5	
	0.032	4/10	45.5	0.072
	0.10	3/10*	59.1	(0.03, 0.14)
	0.32	2/10*	72.7	
	1.0	0/10*	100.0	
	3.2	0/10*	100.0	
Acetaminophen	50	6/10	18.2	
	100	2/10	72.7	91.5
	200	2/10	72.7	(80.8, 509)
	300	1/10*	86.4	
Acetysalicylic Acid	6.25	6/10	18.2	
	12.5	4/10	45.5	15.0
	25.0	2/10*	72.7	(10.0, 21.1)
	50.0	1/10*	86.4	
Indomethacin	0.003	7/10	4.5	
	0.01	4/10	45.5	
	0.03	2/10*	72.7	0.014
	0.1	0/10*	100.0	(0.0092, 0.021)
	0.3	0/10*	100.0	
	1.0	0/10*	100.0	
	3.3	0/10*	100.0	
Phenylbutazone	20.0	5/10	31.8	
	40.0	2/10*	72.7	28.3
	80.0	1/10*	86.4	(15.1, 131)

[a] Arachidonic acid (1 mg/kg) was dissolved in 0.9% NaCl containing a minimal amount of 0.1 N NaOH (final pH = 7.4), perioxided by aeration for 5 min and administered i.p.

[b] Drugs in 0.5% methylcellulose were administered orally 60 min before arachidonic acid challenge.

[c] Number of mice writhing per number of mice tested. Mice were observed from 2 to 15 min after challenge.

[d] Data pooled from three experiments.

* Significantly different from control ($P < .5$, Fisher exact test, 1956).

The ability of suprofen to inhibit cyclo-oxygenase activity in rabbit heart and kidney was determined on the basis that an intracardiac or intrarenal injection of bradykinin (1 μg) caused the release into the cardiac and renal venous effluents of prostaglandin-like spasmogenic substances that contract rat stomach and chick rectum strips. Bradykinin (1 μg) elicited a release equivalent to 60 ± 20 ng of PGE_2 from the heart and kidney, whereas, exogenous arachidonate (10 μg) released 35 ± 15 ng of PGE_2 equivalents. Suprofen infused into the perfused heart or kidney at 3, 5, or 10 μg/ml final concentration failed to alter the amount of PG released by bradykinin or arachidonic acid (n = 6). However, at 50 μg/ml suprofen caused a 55% inhibition of the conversion of exogenous arachidonate into PGE_2, whereas the blockade of bradykinin-induced prostaglandin release was only 15% (n = 4). Bradykinin works by releasing endogenous arachidonate which is more efficiently coupled to the cyclo-oxygenase system than is exogenous arachidonate and would therefore be expected to be more resistant to blockade. Indomethacin was used as a positive control and caused a 100% inhibition of arachidonate- or bradykinin-induced PG release by heart and kidney at 0.1 to .3 μg/ml final concentrations. This minimal effect of suprofen on cyclo-oxygenase activity in these two tissues was surprising in view of previous data that suggested its mechanism of action was due to inhibition of prostaglandin biosynthesis (Janssen, 1975; Van Nueten *et al.*, 1976). Therefore, a direct assessment of the ability of suprofen to inhibit prostaglandin biosynthesis was determined from cyclo-oxygenase activity in bovine seminal vesicles (table 5). The approximate rank order of potency is suprofen > indomethacin > naproxen > ibuprofen > phenylbutazone > sodium salicylate.

PGE is capable of dilating the renal vasculature and inhibitors of PG synthesis, *e.g.*, phenylbutazone has been reported to decrease blood flow to the kidneys (Williamson *et al.*, 1978). Studies were therefore conducted to determine the effect of suprofen on renal artery blood flow in the anesthetized dog. For comparative purposes, phenylbutazone was also studied. Table 6 shows that an i.v. infusion of suprofen (2 mg/kg) did not significantly alter renal artery blood flow. Even at a higher i.v. dose (10 mg/kg), suprofen had no significant effect on renal blood flow (data not shown). In contrast, however, 2 mg/kg of phenylbutazone reduced kidney blood flow by 21.4 ± 9.2 ml/min. These results are in agreement with the relative lack of effect of suprofen on cyclo-oxygenase activity in isolated rabbit kidney. These *in vivo* data, taken together with the *in vitro* data presented above, suggest that suprofen may be a tissue selective inhibitor cyclo-oxygenase activity.

Discussion

Animal models commonly employed to assay narcotic and non-narcotic analgesics require the use of artificial provocations such as heat, mechanical or chemical insult. The phenylquinone writhing test in mice and the Randall-Selitto test in rats are used extensively for the experimental evaluation of analgesic agents and provide reasonable correlations to clinical efficacy and potency. However, a variety of centrally and peripherally acting nonanalgesic pharmacological agents are active in this test and the Randall-Selitto test demonstrates a high degree of variability. A major complaint of rheumatoid arthritic patients is the pain associated with an affected joint. Adjuvant arthritis in rats resembles the human condition with respect to both inflammation and hyperalgesia upon joint movement. In this regard, the adjuvant rat flexion test represents a unique model of pathologically induced pain.

A variety of narcotic and non-narcotic analgesics are active in the adjuvant rat flexion test (table 1) (Kuzuna and Kawai, 1975). There also seems to be a good correlation of potency in this model with clinical potency as is also true with the model of Pircio *et al.* (1975) who used unprovoked adjuvant arthritic rats in comparative potency assays. By using the flexion test described here, suprofen was shown to be more potent than codeine and acetaminophen, equipotent to sulindac, diflunisal, zomepirac and ibuprofen and less potent than morphine and proquazone. Suprofen is characterized as having a rapid onset of action, *i.e.*, progressively increasing from 0.5 to 2 hr with analgesic activity still evident at 4 hr after oral administration. The analgesic potency of suprofen was also demonstrated *vs.*

TABLE 5

Activity of suprofen and five standards on prostaglandin biosynthesis from bovine seminal vesicles

Compound	Mean IC_{50} ± S.E.M.[a]
	μM
Suprofen	1.82 ± 0.18
Indomethacin	3.16 ± 0.42
Naproxen	14.42 ± 2.03
Ibuprofen	45.20 ± 2.44
Phenylbutazone	169.00 ± 13.64
Sodium salicylate[b]	3130 ± 328.50

[a] Mean IC_{50} value from five replicate experiments.

[b] Acetylsalicylic acid was solubilized by the stoichimetric addition of 1 N NaOH. The product was assumed to be sodium salicylate.

TABLE 6

Effect of suprofen or phenylbutazone on renal artery blood flow in the anesthetized dog

Treatment	Dose	Mean Change in Renal Artery Blood Flow		Mean of Difference ± S.E.
		Saline-NaOH vehicle control	Posttreatment	
	mg/kg i.v.	*ml/min*		*ml/min*
Phenylbutazone	2	−10.2 ± 6.4	−31.6 ± 12.9	−21.4 ± 9.2[a]
Suprofen	2	−18.0 ± 9.5	−23.6 ± 4.3	− 5.6 ± 9.6[b]

[a] Statistically different from vehicle control ($P < .05$) $N = 5$.
[b] Not significantly different from vehicle control value ($P > .05$) $N = 5$.

arachidonate-induced writhing and in the Randall-Selitto test where it was equipotent to parenteral morphine (table 4; fig. 5).

Many of the interactions which have major clinical significance in medicine occur with drugs that are used in the treatment of rheumatic diseases (Bulletin on the Rheumatic Diseases, 1977–1978). Experimentally, Van Arman *et al.* (1972) found antagonism at some doses of the anti-inflammatory effects of indomethacin and of aspirin in the adjuvant arthritic rat. Therefore, the data in the present report concerning the interaction potential of suprofen and acetaminophen are important because not only was antagonism absent but potentiation occurred. The mechanism of this interaction remains unclear at the present time. Clinical trials with these combinations will determine their overall importance.

Several lines of evidence support the conclusion that suprofen is not a narcotic analgesic. Firstly, its analgesic effects in the adjuvant rat flexion test were not blocked by naloxone at doses which completely block the analgesic effects of morphine. Secondly, in the Randall-Selitto test, suprofen raised the threshold of the inflamed paw but had no effect on the threshold of the normal paw, as did morphine. Thirdly, suprofen was without effect on the warm water tail-withdrawal reflex, a sensitive assay capable of detecting narcotic analgesic drugs. Lastly, at doses of 160 mg/kg, suprofen was inactive in the hot-plate test and failed to increase pupil diameter in mice. Clearly, suprofen evokes an analgesic effect at some site and by a different mechanism than that of the narcotics.

Vane (1973) was the first to propose that tissue selective inhibition of prostaglandin biosynthesis may exist within the family of aspirin-like compounds and that this could be responsible for the observed variations in their ability to generate various metabolites of arachidonic acid. Thus, tissue selective inhibition of prostaglandin synthetases may be related not only to differential drug distribution or to specificity toward different multiple molecular forms of the prostaglandin generating system but possibly to different metabolites as well. Several lines of evidence, both direct and indirect, suggest that these selective effects may also explain the dissociation of analgesic and anti-inflammatory properties reported for a variety of NSAID and shown in the present study for suprofen.

Previous data indicated that suprofen was a potent inhibitor of arachidonic acid hydroperoxide-induced contraction of the guinea-pig ileum and rat fundic strips (Van Nueten *et al.*, 1976). Human, guinea pig and dog platelet aggregation induced by various agents both *in vivo* and *in vitro* was also inhibited by very low doses of suprofen (De Clerck *et al.*, 1975). In addition, delay of castor oil diarrhea in rats is another method used to evaluate inhibitors of prostaglandin biosynthesis (Awouters *et al.*, 1978). In this assay, the activity pattern of suprofen was consistent with that of a very potent, short-acting inhibitor of prostaglandin biosynthesis. Data in the present report show suprofen to be a potent inhibitor when tested against both AAP-induced writhing in mice and prostaglandin biosynthesis from bovine seminal vesicles (tables 4 and 5). In contrast, suprofen was shown to be a weak inhibitor of cyclo-oxygenase activity measured from rabbit heart and kidney (P. Needleman, personal communication). *In vivo* confirmation of these data in another species, the dog, was demonstrated by the lack of effect of suprofen on renal blood flow (table 6). Decreased renal blood flow, induced by inhibitors of prostaglandin biosynthesis such as meclofenamate and phenylbutazone, has been demonstrated in several species (Williamson *et al.*, 1978; Chrysant *et al.*, 1978; Aiken and Vane, 1973). Suprofen thus appears to have differential effects on the synthesis of prostaglandins in a variety of tissues. Acetaminophen may be another example of a compound with specificity toward prostaglandin production. Vane (1973) interpreted the experiments of Lim *et al.* (1964) on the analgesic effects of acetaminophen on bradykinin-induced vocalization in the dog to mean that prostaglandin production in nervous tissue has a greater sensitivity to acetaminophen.

Hyperalgesia, a state in which a previously inadequate mechanical or chemical stimulus can result in a pain response, is a unique phenomenon produced by prostaglandins. Although there is abundant evidence that prostaglandins contribute to and may modulate the inflammatory process, analgesic doses of aspirin and related compounds given in the Randall-Selitto assay are not accompanied by a significant antiedema action (Gilfoil *et al.*, 1963). Suprofen exhibits a similar profile of activity in that a dose of more than twice the analgesic ED_{50} causes significant pain relief in adjuvant arthritic rats with no simultaneous effect on attendant paw volume. Conversely, prednisone, an anti-inflammatory steroid, caused only a minimal analgesic effect at doses up to 20 mg/kg in this model (Kuzuna and Kawai, 1975). Latranyi and Taber (1979), using the carrageenan-inflamed paw test, showed that parachlorophenylalanine, an inhibitor of serotonin biosynthesis, antagonized the analgesic but not the anti-inflammatory action of clonixin, a non-narcotic agent, in the same animals. Their data suggest that after exposure to a phlogistic stimulus, activation of the arachidonic acid cascade can occur both in the cellular and neural compartments, and it is reasonable, therefore, to suggest that compounds can inhibit one or the other, or both. Additionally, the aminophenaquine derivatives, glafenine and floctafenine, are potent analgesics and inhibitors of prostaglandin biosynthesis but are relatively weak anti-inflammatory agents (Deraedt *et al.*, 1976).

The prostaglandins producing hyperalgesia in the adjuvant arthritic rat must be relatively short-lived because suprofen causes significant pain relief within 1 hr and has no capacity to block prostaglandin receptors. Ferreira (1978b) showed kinetic

differences between the hyperalgesic effects of prostacyclin and PGE_2 in the rat paw and dog knee joint. As an alternative explanation, part of the analgesic effects of the peripheral analgesics may occur through a central non-narcotic action as suggested by Ferriera, 1978a. Although the total mechanism of action of suprofen cannot be determined on the basis of the present experiments, the data are consistent with the hypothesis that suprofen and other peripheral analgesics have analgesic activity independent from their anti-inflammatory effects.

In summary, in a series of tests designed to demonstrate antinociceptive properties of peripheral analgesics, suprofen was a potent, orally effective, non-narcotic analgesic demonstrating some specificity on prostaglandin biosynthetic pathways. Clinical studies are now in progress.

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