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PROSTAGLANDIN ANTAGONISM BY SODIUM *p*-BENZYL-4-[1-OXO-2-(4-CHLOROBENZYL)-3-PHENYLPROPYL]PHENYL PHOSPHONATE (N-0164)

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1 The ability of sodium *p*-benzyl-4-[1-oxo-2-(4-chlorobenzyl)-3-phenylpropyl]phenyl phosphonate (N-0164) to antagonize contractions produced by prostaglandins E_2 and $F_{2\alpha}$ on isolated preparations of gerbil, rat and guinea-pig gastrointestinal muscle has been studied.

2 N-0164 was found to be a potent, partially selective prostaglandin antagonist in these isolated smooth muscle preparations. The blockade produced by N-0164 in the isolated stomach strip of the rat had some, but not all, the characteristics of a competitive antagonism.

3 N-0164 produced a dose-dependent decrease in tone in the rat stomach strip that was abolished by pretreatment of the preparation with indomethacin.

4 N-0164 prevented diarrhoea induced by prostaglandin E_2 in mice when given by intraperitoneal injection but was less effective when given orally.

5 N-0164 inhibited oedema induced with croton-oil and pyridine-ether in the mouse ear.

6 N-0164 delayed the onset of erythema following ultraviolet irradiation of guinea-pig skin only when an equimolar amount of pralidoxime chloride was added to the vehicle.

7 It is concluded that N-0164 is a potent, partially selective prostaglandin antagonist on several isolated smooth muscle preparations. N-0164 exhibits activity *in vivo* particularly following local application when problems associated with penetration and distribution are minimized.

Introduction

Prostaglandin antagonists are compounds which selectively interfere with the actions of prostaglandins as opposed to compounds such as aspirin and indomethacin which are known to interfere with the formation of prostaglandins by inhibition of the enzyme complex known as prostaglandin synthetase (Ferreira, Moncada & Vane, 1971; Smith & Willis, 1971; Vane, 1971).

The prostaglandin antagonists available at the present time belong to three chemically unrelated classes, 7-oxaprostaglandins (Fried, Santhanakrishnan, Himizo, Lim, Ford, Rubin & Grigas, 1969), dibenzoxazepine hydrazides (Sanner, 1969) and certain polymers of phloretin phosphate (PPP) (Eakins, Karim & Miller, 1970). The prostaglandin antagonist activity of PPP was found to reside in the low molecular weight material (Eakins, 1971) and a number of compounds related to the monomers and dimers of PPP were synthesized and shown to possess prostaglandin antagonist activity (Eakins, Fex, Fredholm, Hogberg & Veige, 1972). A further series of phenyl phosphonates was synthesized of which sodium *p*-benzyl-4-[1-oxo-2-(4-chlorobenzyl)-3-phenylpropyl]phenyl phosphonate (N-0164, Figure 1) is an example. In the present study we describe the activity and selectivity of N-0164 as a prostaglandin antagonist on some smooth muscle systems *in vitro*. In addition, we have investigated the activity of this compound *in vivo* against prostaglandin E_2-induced diarrhoea by different routes of administration and the effectiveness of N-0164 as a topical anti-inflammatory agent.

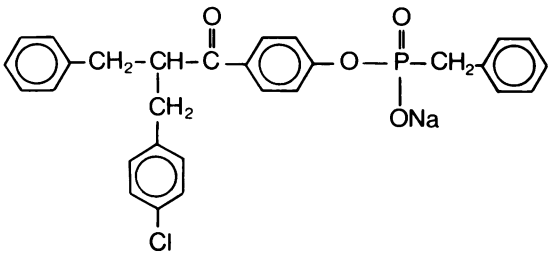

Figure 1 Chemical structure of N-0164.

Methods

In vitro *smooth muscle preparations*

Muscle contractions were measured isotonically with a Brush isotonic muscle transducer coupled to a Heath Servorecorder (Model EU20B).

Gerbil colon. Gerbils (*Meriones shawi*) weighing between 50 and 150 g were stunned and bled. The middle part of the ascending colon was removed and suspended in a 5 ml organ bath containing de Jalon's rat colon solution (Ambache, Kavanagh & Whiting, 1965) at 28°C gassed with O_2. A dose-cycle of 3 to 4 min and a contact time of 2 min was used for all the agonists studied.

Guinea-pig ileum. The animals were stunned and bled. Segments of ileum were suspended in a 5 ml organ bath containing Tyrode solution at 37°C gassed with 5% CO_2 in O_2. A dose-cycle of 3 min and a contact time of 1 min was used for all agonists.

Rat fundus. Strips of rat fundus were prepared according to Vane (1957). The preparations were suspended in Krebs solution at 37°C bubbled with 5% CO_2 in O_2.

Experimental procedure. The activity and selectivity of the prostaglandin-blockade produced by N-0164 was determined by measuring the effect of the antagonist on contractions produced by a constant dose of various agonists. Doses of each agonist were chosen to yield a contraction between 40–60% of maximum. Constant responses were then established and N-0164 added to the bathing fluid and left in contact for 2 min before the addition of each agonist. The concentration of N-0164 was then increased stepwise until the contractions produced by the prostaglandins were antagonized approximately 100%. In a separate series of experiments on the rat fundus preparation, three point dose-response curves were established for each agonist in the absence and presence of the antagonist following a 15 min contact period. Horizontal shifts of the dose-response curves were measured at the level of the ED_{50} and the dose-ratio calculated. The plot of log (dose-ratio − 1) against the negative log of the molar concentration of N-0164 was made according to Arunlakshana & Schild (1959) and the pA_2 value determined graphically.

In vivo *studies*

Inhibition of prostaglandin-induced diarrhoea. Unfasted Swiss Webster mice, weighing between 16–18 g were used in groups of 10 animals. Prostaglandin E_2 (50 μg/kg i.p.) produced diarrhoea within 30 min in 95% of the animals. Other groups of animals were pretreated with various doses of N-0164 dissolved in 0.2 ml 0.9% w/v NaCl solution (saline) administered either orally or by intraperitoneal injection. Control animals were pretreated with an equal volume of saline. The dose of N-0164 required to protect 50% of the animals from diarrhoea (ED_{50}) was calculated.

Croton oil-induced ear oedema. Male Sprague-Dawley rats (50–100 g) were divided into groups of 6 animals. N-0164 was dissolved in saline and added to a mixture containing 4 parts pyridine and 5 parts ether containing 4% croton oil. The mixture was then added to one ear of each animal as described by Tonelli, Thibault & Ringler (1965). Control ears received the mixture without N-0164. Six hours later the animals were killed and a uniform tissue sample (No. 8 biopsy punch) taken from each ear and weighed. The difference in weight between biopsies taken from treated and control ears was then determined.

Guinea-pig u.v. erythema. Hartley guinea-pigs (male) were depilated 18 h before the start of the test. Animals were divided into groups of four and given a standard 30 s burn with a 360 W mercury vapour arc tube filtered with a vycor filter placed 11 cm from the animals' backs. Prior to burning, each animal was covered with a rubber sheet with two standard holes (1–2 cm in diameter) for exposure of the skin to the light. Following the burn, each animal was treated topically with an appropriate solution over the entire shaved surface. In the initial experiments the animals were either treated with a control vehicle (90% polyethylene glycol in H_2O) or different concentrations of N-0164 prepared in the polyethylene glycol vehicle.

Each animal was scored every hour for 6 h following treatment with each spot being scored according to the following scale: 0 = no observed redness; 1 = reddish tint without distinct outline; 2 = definite outline of reddish tint; 3 = bright red; 4 = small blister development.

Results

The ability of N-0164 to reduce submaximal responses produced on the isolated gerbil colon and rat stomach strip by a variety of agonists was studied. The antagonist was added to the organ bath 2 min before each agonist and was present during the induced contraction. N-0164, 0.5 to 2 μg/ml (1 μM to 3.8 μM), antagonized responses to prostaglandins E_2 and $F_{2\alpha}$ of the gerbil isolated colon (Figure 2). At the higher concentrations of N-0164, contractions produced by angiotensin and bradykinin were depressed, although to a lesser extent than those produced by either prostaglandin. Contractions

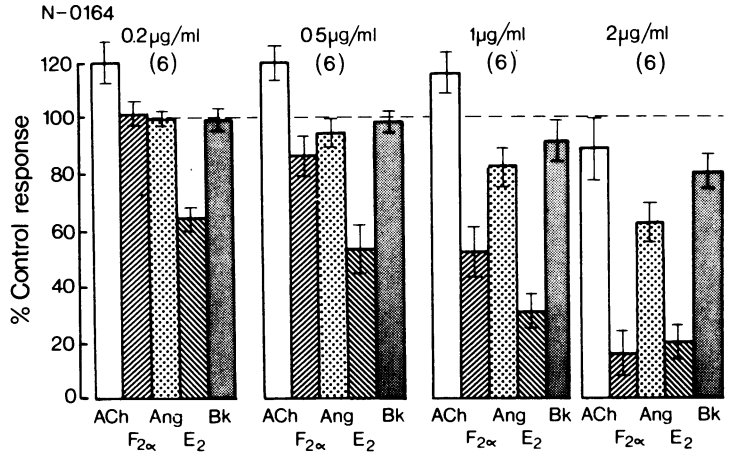

Figure 2 Effect of N-0164 on contractions produced by acetylcholine (ACh), prostaglandins E_2 and $F_{2\alpha}$ (E_2, $F_{2\alpha}$), angiotensin (Ang) and bradykinin (Bk) on the gerbil isolated colon. Each column represents the mean value, vertical bars the s.e. mean. Numbers of experiments in parentheses.

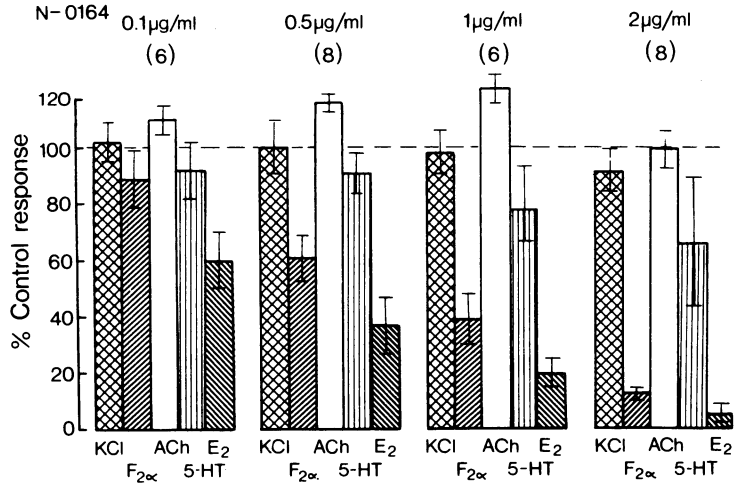

Figure 3 Effect of N-0164 on contractions produced by potassium chloride (KCl), prostaglandins E_2 and $F_{2\alpha}$ (E_2, $F_{2\alpha}$), acetylcholine (ACh) and 5-hydroxytryptamine (5-HT) on the stomach strip of the rat. Each column represents the mean value, vertical bars the s.e. mean. Numbers of experiments in parentheses.

produced by acetylcholine were not significantly reduced at any dose-level of N-0164. On the rat stomach strip preparation, contractions produced by prostaglandins E_2 and $F_{2\alpha}$ were markedly depressed by N-0164 at concentrations (0.19 μM to 3.8 μM) which did not consistently depress responses produced by acetylcholine. Some depression of responses produced by potassium chloride was noted at the higher dose-levels of N-0164 and these higher concentrations of N-0164 reduced responses produced by 5-hydroxytryptamine by some 30–40% (Figure 3). In all preparations the tone of the rat stomach strip preparation was reduced by N-0164 in a dose-dependent manner (Figure 4). In all experiments the responses to the agonists and in most experiments the baseline tone of each preparation returned to normal some 30 min after removal of N-0164 from the bathing fluid.

In six further experiments, preparations of the rat stomach strip were suspended in Krebs solution

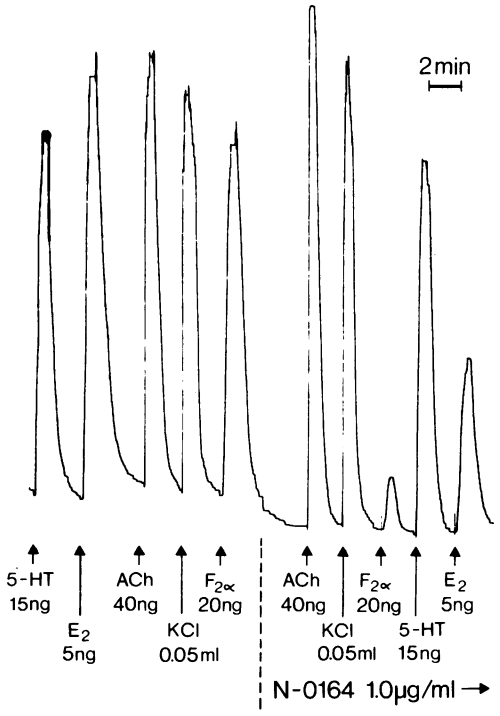

Figure 4 Rat isolated stomach strip. Effect of N-0164 (1 μg/ml) on contractions produced by potassium chloride (KCl), prostaglandins E_1 and $F_{2\alpha}$ (E_1, $F_{2\alpha}$), acetylcholine (ACh) and 5-hydroxytryptamine (5-HT). Note the fall in baseline tension following the introduction of N-0164 into the bathing medium.

containing indomethacin (1 μg/ml) and in these preparations, N-0164 failed to reduce the tone at any dose-level (up to 5 μg/ml N-0164), although addition of isoprenaline (2 μg/ml) decreased the tone, indicating that the tissue was not fully relaxed in the presence of this concentration of indomethacin.

Experiments carried out on the guinea-pig isolated ileum showed that N-0164 was a potent antagonist of prostaglandin E_2 with an IC_{50} value of 6.1 nM, as compared to values of 0.84 μM on the gerbil colon and 0.38 μM on the rat fundus preparation.

The antagonism by N-0164 of the responses of the rat stomach strip preparation to prostaglandins E_2 and $F_{2\alpha}$ appeared to be characteristic of a competitive antagonism, since in addition to the antagonism being reversible, parallel displacement of the dose-response curves to the right, without alteration of the maximum response produced by either prostaglandin E_2 or $F_{2\alpha}$, was observed over a wide range of antagonism. However, the shift of the dose-response curves to the right was not proportional to the increase in concentration of the antagonist. The dose-ratios for various agonists in this preparation at different concentrations of N-0164 are seen in Table 1. Dose-ratios of 138 and 153 were obtained for prostaglandins E_2 and $F_{2\alpha}$ respectively, in the presence of 9.5 μM N-0164, whereas the dose-ratio for 5-hydroxytryptamine was 10.95 and that for acetylcholine was essentially unaltered. The results from these experiments were plotted according to Arunlakshana & Schild (1959). This plot gave a pA_2 value of 6.7 for N-0164 against prostaglandin E_2 and 6.4 against prostaglandin $F_{2\alpha}$. The slope of the calculated regression line was −1.25 against prostaglandin E_2 and −1.45 against prostaglandin $F_{2\alpha}$.

Intraperitoneal injections of N-0164 dissolved in saline alone were found to prevent the diarrhoea produced in mice by the intraperitoneal injection of prostaglandin E_2. The ED_{50} for N-0164 given by this route of administration was found to be 55 mg/kg. N-0164 was less active when given orally, with an ED_{50} of 105 mg/kg.

N-0164 was found to inhibit chemically induced irritation (croton oil and pyridine-ether) in the mouse ear (Table 2). A minimum concentration of 1% N-0164 was required to produce a significant ($P < 0.025$) inhibition of oedema.

In other experiments, N-0164 was observed to delay the onset of erythema following ultraviolet

Table 1 Antagonism of different agonists on the rat isolated stomach strip by N-0164

	*Dose-ratio (A/a)**			
Concentration† of N-0164 (μM)	*Acetylcholine*	*PGE_2*	*$PGF_{2\alpha}$*	*5-Hydroxytryptamine*
0.95	0.8 ± 0.1	5.0 ± 1.5	3.2 ± 0.2	2.0 ± 0.5
1.9	1.07 ± 0.07	17.5 ± 0.6	14.6 ± 2.6	2.1 ± 0.2
5.7	1.6 ± 0.2	50.75 ± 5.3	46.1 ± 4.5	11 ± 2.6
9.5	1.47 ± 0.02	138 ± 11	153 ± 8.4	10.95 ± 1.3

Results shown are the mean of four experiments ± s.e. mean. * A/a is the dose-ratio for each agonist calculated at the level of the ED_{50}. † The antagonist was allowed to remain in contact with the tissue for 15 min before determination of the dose-ratio using 3-point dose-response curves.

irradiation of guinea-pig skin. These experiments also demonstrated that the *in vivo* potency of N-0164 depends on the vehicle used since in the normal polyethylene glycol vehicle routinely used in this test, N-0164 was inactive at the three concentrations tested (1, 2 and 3%). However, when pralidoxime chloride (2-PAM) was added to the vehicle in equimolar amounts to N-0164, the ability of the antagonist to suppress erythema was observed (Table 3).

Discussion

N-0164 has been found to be a potent, reversible, reasonably selective antagonist of contractile responses produced by both E and F prostaglandins on isolated preparations of the gerbil colon, rat stomach strip and guinea-pig ileum. The antagonism seen with N-0164 on the rat fundus had some of the characteristics of competitive blockade; the antagonism was reversible and the dose-reponse curves of both prostaglandins E_2 and $F_{2\alpha}$ were displaced to the right in parallel to a similar extent without any diminution of the maximum response over a 10-fold range of antagonist concentration. However, the shifts to the right of the dose-response curves were not proportional to the increase in concentration of the antagonist. Furthermore, the plots of log (dose-ratio − 1) against the negative log of the molar concentration of N-0164 yielded regression lines with slopes significantly greater than the theoretical value of 1.0 that would be expected from a simple bimolecular competitive antagonism. Further studies are required to resolve these discrepancies. However, the similarities between the dose-ratios obtained at the 50% maximal response for both prostaglandins at each concentration of N-0164 would suggest that prostaglandins E_2 and $F_{2\alpha}$ share a common site of action on the rat fundus preparation.

N-0164 was found to produce a dose-dependent decrease in tone in the rat stomach strip. This observation is similar to that obtained previously with another prostaglandin antagonist, SC 19220, on this

Table 2 Topical anti-inflammatory activity of N-0164 in the croton oil mouse ear oedema test

Concentration of N-0164 (%)	*% increase in ear weight*
0	93.5 ± 5.6 (17)
0.3	87.5 ± 8 (15)
1.0	72.4 ± 6.3 (16)*
3.0	49.1 ± 4.6 (16)**

Results expressed as mean with s.e. where *n* is number of experiments.
* $P < 0.025$; ** $P < 0.005$ (unpaired data, Students' *t* test).

Table 3 Topical anti-inflammatory activity of N-0164 in ultraviolet erythema in guinea-pigs

Treatment after burn	*Intensity of burn**					
	1 h	*2 h*	*3 h*	*4 h*	*5 h*	*6 h*
Vehicle† + 6% 2-PAM	1 ± 0 (8)	1.75 ± 0.16 (8)	2.75 ± 0.16 (8)	3.0 ± 0 (8)	3.0 ± 0 (8)	3.0 ± 0 (8)
Vehicle + 2% 2-PAM + 1% N-0164	1 ± 0 (8)	1.75 ± 0.16 (8)	2.75 ± 0.16 (8)	2.9 ± 0.13 (8)	3.0 ± 0 (8)	3.0 ± 0 (8)
Vehicle + 5% 2-PAM + 2% N-0164	0.63 ± 0.18 (8)	1.5 ± 0.19 (8)	2.5 ± 0.19 (8)	2.75 ± 0.16 (8)	3.0 ± 0 (8)	3.0 ± 0 (8)
Vehicle + 6% 2-PAM + 3% N-0164	0.25 ± 0.16 (8)	0.63 ± 0.18 (8)	1.4 ± 0.18 (8)	2.25 ± 0.16 (8)	2.5 ± 0.19 (8)	2.88 ± 0.13 (8)

* Intensity of the burn was scored as described in Methods section. † Control vehicle contained the highest concentration of pralidoxime chloride (2-PAM) used. N-0164 was without effect at the three concentrations tested in the absence of 2-PAM.

preparation (Bennett & Posner, 1971) and supports the concept (Bennett & Posner, 1971; Ferreira, Herman & Vane, 1972; Eckenfels & Vane, 1972) that prostaglandins contribute to the maintenance of tone in this and other isolated smooth muscle preparations. It is of interest that this action of N-0164 differs qualitatively from its antecedent, PPP, which produced dose-dependent contractions of the rat stomach strip (Bennett & Posner, 1971). The observation that pretreatment with indomethacin abolished the decreased tonus produced by N-0164 in the present experiments, together with the observation that 50 μM N-0164 did not inhibit prostaglandin synthetase derived from bovine seminal vesicles (R. Schroer, unpublished observations), is further evidence that this effect of N-0164 is mediated by blockade of the actions of endogenous prostaglandins.

Contractions produced by potassium chloride but not acetylcholine were somewhat reduced by the higher concentrations of N-0164 on the rat stomach strip. A reduction in contractions produced by potassium chloride by other prostaglandin antagonists has been described before (Bennett & Posner, 1971). The reason for this is not immediately apparent. The reduction of contractions produced by 5-hydroxytryptamine on the rat stomach strip and angiotensin and bradykinin on the gerbil colon by N-0164 in the present experiments may reflect a degree of involvement of prostaglandins in contractions produced by these substances (Chong & Downing, 1974; Frankhuijzen & Bonta, 1975; Crocker & Willavoys, 1976) but a definitive conclusion must await further investigation. However, it is interesting to note that although the dose-ratios for prostaglandin E_2 and $F_{2\alpha}$ increased with increasing concentrations of N-0164, the dose-ratio for 5-hydroxytryptamine increased a certain amount and then remained unaltered when the concentration of N-0164 was approximately doubled. Under the same experimental conditions the dose-ratio for acetylcholine remained unaffected. These experiments illustrate the importance of studying alterations in dose-ratios rather than changes in the effect of a single dose of an agonist when investigating a pharmacological antagonist.

N-0164 exhibited prostaglandin blocking activity *in vivo*. High doses were required to prevent diarrhoea induced by prostaglandin E_2 in mice, the compound being less effective orally (ED_{50}, 105 mg/kg) than when given by intraperitoneal injection (ED_{50}, 55 mg/kg). It should be noted that the LD_{50} at 24 h in mice was 408 mg/kg intraperitonealy and 715 mg/kg orally (R. Schroer, unpublished observations).

N-0164 was more effective *in vivo* when problems associated with penetration and distribution were minimized, as in the croton oil-induced mouse ear oedema system. Further support for this proposal came from experiments on ultraviolet light-induced erythema in guinea-pig skin. N-0164 failed to influence either the intensity or duration of the erythema when applied topically in the normal polyethylene glycol vehicle. In contrast, when equimolar amounts of 2-PAM were added to the vehicle, N-0164 clearly suppressed the onset of the erythema. 2-PAM is thought to enhance penetration by a mechanism involving ion-pairing (F. Dea, personal communication). Prostaglandins have been implicated in the reaction of the skin to sunburn and ultraviolet irradiation (Greaves & Sondergaard, 1970; Mathur & Ghandi, 1972). Furthermore, a single topical application of indomethacin in a vehicle containing ethanol, propylene glycol and dimethylacetamide decreased the effects of sunburn in man for 24 h or more (Snyder & Eaglstein, 1974a). On the basis of their proposed mechanisms of action, N-0164 would be expected to have a shorter duration of action than indomethacin since the latter is an irreversible inhibitor of prostaglandin synthetase (Smith & Lands, 1971) and prostaglandins are thought to be synthesized continuously for some time after the exposure to ultraviolet irradiation (Snyder & Eaglstein, 1974b).

In conclusion, N-0164 was found to be a potent, partially selective prostaglandin antagonist *in vitro* in several isolated smooth muscle preparations. N-0164 exhibited activity *in vivo* particularly following local application when problems associated with penetration and distribution were minimized.

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