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Gastric Antisecretory Effect of 15(*R*)-15-Methyl PGE_2, Methyl Ester and of 15(*S*)-15-Methyl PGE_2, Methyl Ester (38707)

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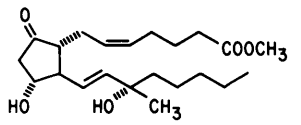
We have previously observed that 15(*S*)-15-methyl PGE_2, methyl ester, (a) inhibited gastric secretion in dogs when given intravenously, (b) was 30–50 times more potent than PGE_2, (c) was active when given orally and intrajejunally, and (d) exerted its activity for a longer interval than PGE_2 (1). Karim *et al.* reported that both 15(*S*)-15-methyl PGE_2, methyl ester, and 15(*R*)-15-methyl PGE_2, methyl ester, when given orally, inhibited gastric secretion in man, the 15(*S*) compound being about 10 times more potent than the 15(*R*) (2). The 15(*R*) compound was inactive intravenously at the dose tested. We also reported antisecretory activity after oral administration of the 15(*S*) compound in humans (3). Previously reported examples (4) have shown that several prostaglandins having the unnatural configuration (*4*) at C-15 are less potent than their natural (*S*) counterpart. The reason why the 15(*R*) compound was active orally was not readily apparent. The two studies reported here were performed to elucidate this point.

a. We compared the effect of 15(*R*)-15-methyl PGE_2, methyl ester, abbreviated 15(*R*), and 15(*S*)-15-methyl PGE_2, methyl ester, abbreviated 15(*S*), on gastric secretion in dogs. The compounds, dissolved first in ethanol, were diluted either in water or in acid.
b. Chemical analysis of the two analogs was performed after dilution in either water or acid.

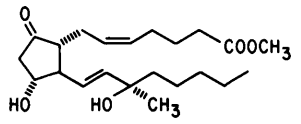
I. *Effect of 15(R) and 15(S) on gastric secretion. Methods.* Male mongrel dogs of 25–30 kg were used. A gastric denervated pouch (Heidenhain) was prepared surgically, and the animals were used 4 wk or more later. They were stimulated to secrete gastric juice by constant intravenous infusion of histamine dihydrochloride at the submaximal dose of 1 mg/hr. Gastric juice samples were collected from the pouch every 15 min. When a plateau of secretion was obtained (usually after 60–90 min), either the 15(*R*) or the 15(*S*) was administered. The structures of the two prostaglandin analogs are shown in Fig. 1. The synthesis of these analogs was previously described (5, 6). The route was either oral (in 10 ml), intrajejunal (in 5 ml) or intravenous (in 5 ml). Intrajejunal administration was made possible by the formation of a jejunostomy at the time the gastric pouch was made. The jejunostomy was kept closed at all times, except when a prostaglandin was introduced. The volume of gastric juice was measured to the nearest 0.1 ml, the acidity was determined by titration to pH 7 with 0.1 *N* NaOH (using a Copenhagen Radiometer), and expressed in mEq/L (concentration), and mEq/15 min (output). Pepsin was determined by the hemoglobin method (7), as modified for an autoanalyzer (8). The results were expressed in μEq of tyrosine/ml (concentration) and μEq/15 min (output).

Results. A. Oral administration. Both 15(*S*) and 15(*R*) inhibited gastric secretion. A linear dose response could not be established for either compound because of variability in the results and because both compounds provoked vomiting in most dogs. Because of this latter complication, we could not be certain of the amount of compound that was retained. The impression gathered from Table I was that (a) the 15(*R*) was less potent than the 15(*S*) when both are given in water; (b) the 15(*R*), when diluted in acid (HCl 0.15 *N*, incubated at 37° for 10 min prior to administration), was more potent than when diluted in water; (c) no such difference between aqueous and acid dilution was found for the 15(*S*); the doses given may have been too high to show such a difference (gastric secretion was totally inhibited in most cases).

B. Intrajejunal administration: role of



15(S)-15-methyl PGE_2, methyl ester



15(R)-15-methyl PGE_2, methyl ester

FIG. 1. Structure of the prostaglandin analogs.

TABLE I. EFFECT OF 15(*R*)-15-METHYL PGE_2, METHYL ESTER AND 15(*S*)-15-METHYL PGE_2 METHYL ESTER, GIVEN ORALLY ON GASTRIC SECRETION.[a]

Dog No.	Dose (μg/kg)	15(*R*) In water	15(*R*) In acid	15(*S*) In water	15(*S*) In acid
138	30	36%	100%	100%	100%
130	40	36	58	100	100
129	100	40	87	95	100
136	100	80			
122	370	100			

[a] Values represent % inhibition of gastric secretion (volume for 15 min), in Heidenhain pouch dogs.

acid as a diluent. Since the 15(*R*) was active orally, it was then given intrajejunally to find out whether the oral activity was due to intestinal absorption of the compound after passage into the intestine. The 15(*R*), diluted in water, was inactive even when given at doses as high as 500 μg/kg. On the other hand, the 15(*S*), diluted in water, was very active when given intrajejunally. It was clear then that the 15(*R*) needed to be first in contact with the stomach to be active. There were three possibilities to explain why the 15(*R*) was active orally but not intrajejunally: (a) the 15(*R*) acted locally on the gastric mucosa by inactivating the parietal cells, responsible for acid secretion; (b) the 15(*R*) may be inactive as such, but, when in contact with either the gastric juice or the gastric mucosa, was transformed into an active compound; (c) the 15(*R*) was inactivated by the jejunum.

We decided to test the second possibility first, since it was the easiest. We incubated, at 37° for 10 min, 10 mg of 15(*R*) with 5 ml of gastric juice (pH 1.1) obtained from a dog stimulated with histamine. We then diluted the mixture with water to a total volume of 10 ml, and injected it intrajejunally to the same dog. The dose was 500 μg/kg, shown previously for that dog to be inactive by this route when simply diluted in water. The volume of gastric juice (per 15 min) fell from 15 ml to 7 ml after the first 15 min, and to zero after the second 15 min. We concluded that contact of the 15(*R*) with gastric juice "activated" the 15(*R*) so that it could then inhibit gastric secretion even after intrajejunal administration. Since gastric juice is a complex mixture, the "activation" could have been due to any chemical present in the juice, or simply to its low pH.

We tested the effect of low pH on the activity of 15(*R*). Five mg of 15(*R*) (200 μg/kg) was diluted in 2 ml of 0.15 *N* HCl, and incubated for 10 min at 37°. It was then diluted with water to a final volume of 10 ml, and injected intrajejunally. Again, total inhibition of gastric secretion was observed within 30 min. It was clear then that the 15(*R*), inactive when diluted in water, became active when diluted in acid of similar strength to that of gastric juice. We concluded that no chemical component of gastric juice other than hydrogen ion concentration was necessary to make the transformation.

We then proceeded to establish a dose response curve for both the 15(*R*) and 15(*S*) given intrajejunally, after dilution either in water or acid.

C. Intrajejunal administration: ED50. Figure 2 shows the results after intrajejunal administration at doses of 30 μg/kg (dog No. 138) and 40 μg/kg (dogs Nos. 130 and 110). The 15(*R*), diluted in water, was inactive as reported above, whereas when diluted and incubated in 0.15 *N* hydrochloric acid, it inhibited gastric secretion by 40–50%. The 15(*S*), diluted in water, was very potent, inhibiting gastric secretion by 80–90%. When the 15(*S*) was diluted and incubated in acid, it was less potent than when diluted in water, inhibiting gastric secretion by about 50%. The ED50s, i.e., dose inhibiting acid output by 50%, were

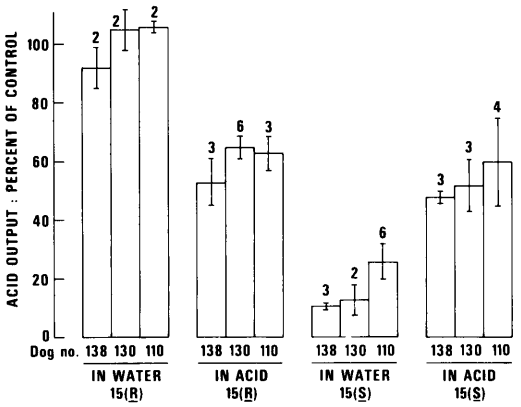


FIG. 2. Effect of 15(*R*)-15-methyl PGE_2, methyl ester and 15(*S*)-15-methyl PGE_2, methyl ester on gastric secretion in Heidenhain pouch dogs, *intrajejunally*. The values represent percent change of acid output per 15 min, obtained from a gastric pouch, after intrajejunal administration of the prostaglandin analogs. The doses were 30 μg/kg for dog No. 138, and 40 μg/kg for dogs No. 130 and 110. The numerals on top of the columns represent the number of studies for each dog at each dose level. The bars show the standard error of the mean. The 15(*R*) and the 15(*S*) were diluted either in water or in 0.15 *N* HCl as indicated.

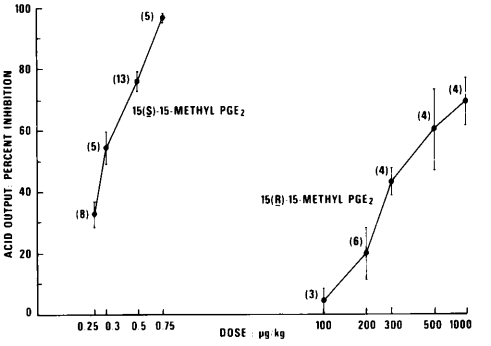


FIG. 3. Effect of 15(*R*)-15-methyl PGE_2, methyl ester and 15(*S*)-15-methyl PGE_2, methyl ester on gastric secretion in a Heidenhain pouch dog, *intravenously*. The prostaglandin analogs were administered as a single intravenous injection. The values represent the percent inhibition of acid output per 15 min. The studies were performed in three dogs, and the numerals in parentheses represent the number of studies for each dose level.

as follows: 15(*R*) in water, inactive; 15(*R*) in acid, 50 μg/kg; 15(*S*) in water, 15–20 μg/kg; 15(*S*) in acid, 40 μg/kg. Note that when diluted in acid, the 15(*R*) and the 15(*S*) were about equally potent by intrajejunal administration.

D. Intravenous administration. The results are shown in Fig. 3. By this route, the ED50 for the 15(*S*) and the 15(*R*) were 0.3 μg/kg and 300 μg/kg, respectively. Thus the 15(*S*) is 1000 times more potent than the 15(*R*) when given intravenously.

II. *Chemical analysis of the two prostaglandin analogs Methods.* Five mg of the 15(*R*) and the 15(*S*) were dissolved in 0.5 ml of 95% ethanol. To these solutions, either 0.15 *N* hydrochloric acid, dog gastric juice, or pH 3 buffer was added to a final volume of 10 ml. These solutions were further diluted with 25 ml of water and then extracted well with ether. The ether extracts were combined, washed sequentially with water (until the aqueous wash was pH 5–7) and brine, dried (sodium sulfate), and evaporated *in vacuo* at 40°. The oily residue was then dissolved in 5 ml of ether and 5 ml of methanol. To this solution was added ethereal diazomethane. The resulting solution was evaporated *in vacuo* at 40°. The residue was dissolved in methylene chloride to make 0.2 ml. Analyses for chemical integrity were accomplished by thin-layer chromatography (TLC), twice up on 2″ × 7″ Anatech (Uniplate) glass plates precoated with 250 μm of Silica Gel GF using 20% (v/v) acetone in methylene chloride. The completed chromatograms were visualized (charred) with vanillin–phosphoric acid spray and heat. Under these conditions pure samples of 15(*R*) and 15(*S*) showed R_f values of 0.17 and 0.14, respectively. Each of the epimers, 15(*R*) and 15(*S*), was stable to the isolation conditions.

Results. Incubation at 37° of either 15(*R*) or 15(*S*) in solutions of either gastric juice or 0.15 *N* hydrochloric acid containing 5% ethanol at prostaglandin concentrations of 0.5 mg/ml resulted in complete epimerization of each prostaglandin within 30 min. As analyzed by TLC after isolation (see Methods above), an apparently equal mixture of 15(*R*) and 15(*S*) was obtained starting from either prostaglandin in each medium. Less than 10% of any products other than 15(*R*) and 15(*S*) were seen at 30 min. Examination of the incubation solutions at 3 hr showed that only 25–50%

of the products were 15(*R*) and 15(*S*), with the remainder being a mixture of materials less polar than 15(*R*) and 15(*S*). In contrast, incubation of the 15(*R*) in pH 3 buffer at 37° resulted in only a trace (less than 5%) of formation of the 15(*S*) after 30 min, with no other products formed.

Discussion. In an acid medium, each prostaglandin analog epimerizes at carbon 15 to give approximately a 1:1 ratio, so that half of the 15(*R*) is transformed into the 15(*S*), and half of the 15(*S*) is transformed into the 15(*R*). This epimerization explains why the 15(*R*) is very active orally and not intrajejunally. When it enters the stomach, it is mixed with gastric juice which is acid (pH around one). It is then quickly epimerized to the (*S*) form, which is known to be very active by any route, even when administered intrajejunally in an aqueous solution. Therefore, the 15(*R*), given orally, appears to be highly antisecretory because much of it is transformed into the 15(*S*). Although this appears to be the most likely explanation, we cannot exclude the possibility that the 15(*R*) itself could also inhibit gastric secretion by a local action on parietal cells. The lack of epimerization in the pH 3 buffer, however, suggests that the degree of acidity of gastric contents may determine whether the 15(*R*) will exert an antisecretory effect.

Our results also explain why the 15(*R*) is inactive when given intrajejunally in water, but active when given in acid. In the latter case, inhibition of gastric secretion is primarily due to formation of the 15(*S*), which is then absorbed.

Finally, the 15(*R*) showed very weak activity when given intravenously. This is probably because the pH of blood (7.4) does not allow epimerization to take place. There is, however, some activity [1/1000th of the 15(*S*)] after intravenous administration. This could be due to some intrinsic activity of the 15(*R*), or, possibly, to the excretion into the stomach of a fraction of the administered 15(*R*); this fraction would thus be in contact with acid gastric juice, and transformed into the 15(*S*), which is very active. Another possibility might be that the 15(*S*) was present as an impurity in the 15(*R*) to the extent of 0.1%.

Summary. Gastric juice was collected from gastric pouches in dogs stimulated with histamine. 15(*R*)-15-methyl PGE_2, methyl ester inhibited gastric secretion in dogs when given orally, but was almost inactive when given intravenously, whereas 15(*S*)-15-methyl PGE_2 methyl ester was active by both routes. When given directly into the small intestine (intrajejunally), the 15(*S*) was active and the 15(*R*) was inactive. The 15(*R*), diluted in acid and administered intrajejunally, became active in inhibiting gastric secretion. When the 15(*S*) was diluted in acid and administered intrajejunally, it lost half of its activity. When each analog was incubated in an acid medium, each was epimerized to give approximately a 1:1 mixture of both 15(*R*) and 15(*S*). Incubation of the 15(*R*) in pH 3 buffer resulted in only a trace of formation of 15(*S*). These results explain why the 15(*R*) is active orally but not intrajejunally. When given orally, the low pH of gastric secretion epimerizes much of the 15(*R*) into the 15(*S*), which is active by any route. The degree of acidity of gastric contents may determine whether the 15(*R*) will exert an antisecretory effect.

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