Pharmacology Biochemistry & Behavior, Vol 28, pp 419–422 © Pergamon Journals Ltd , 1987 Printed in the U S A 0091-3057/87 $3 00 + 00

BRIEF COMMUNICATION

Inhibitors of Histidine Decarboxylase Decrease Basal Gastric Acid Secretion in the Rat

VERNER S. WESTERBERG AND JONATHAN D. GEIGER[1]

Department of Pharmacology and Therapeutics, University of Manitoba, Faculty of Medicine 770 Bannatyne Avenue, Winnipeg, Manitoba R3E 0W3

Received 21 April 1987

WESTERBERG, V S AND J D GEIGER *Inhibitors of histidine decarboxylase decrease basal gastric acid secretion in the rat*. PHARMACOL BIOCHEM BEHAV **28**(3) 419–422, 1987 —We examined the ability of two specific inhibitors of histidine decarboxylase, (s)-α-fluoromethylhistidine (FMHd) and (s)-α-fluoromethylhistamine (FMHm), to inhibit basal gastric acid secretion The two highest doses of FMHd administered, 50 and 100 mg/kg, decreased basal gastric acid secretion and total secretion volume but did not affect intraluminal pH FMHm decreased gastric acid secretion, raised intraluminal pH, and to a lesser degree decreased total secretion volume Neither compound changed the severity of gastric ulcers produced by cold restraint stress

Histidine decarboxylase Inhibitors Gastric acid Gastric ulcers

HISTAMINE plays a prominant role in regulating many different physiological functions including perhaps a pivotal role in the regulation of gastric acid secretion [3] Histamine, which may mediate the actions of other secretagogues [3] of gastric acid from parietal cells, has been found to itself stimulate gastric acid secretion both *in vivo* [6] and in isolated dog [7] and pig [13] parietal cells In addition to the physiological roles of histamine, it may also play an important role in such pathological states as gastric acid hypersecretion and ulcerogenesis. Although the causes of peptic ulcer disease are still unclear, gastric hypersecretion is thought to be one important pathogenic factor both in human duodenal ulcers [16] and in gastric ulcer formation in animal models such as restraint stress [15]. Since peptic ulcer disease affects about 10% of the North American population [11] and is a life-threatening disease, pharmacological intervention in the gut remains a very active area of investigation. One such intervention is via histamine H_2 receptor antagonists in the stomach· Stimulated and basal gastric acid secretion are inhibited by these agents [2] It is, however, less clear whether the actions of histamine are mediated through increased levels of cAMP or other less well defined mechanisms.

Besides receptor blockade, another approach to regulating the actions of histamine may be through altering its availability at its receptors. Histamine is synthesized from L-histidine by L-histidine decarboxylase (HDC) in peripheral and central nervous system tissues [4,8]. Inhibition of HDC activity by the irreversible inhibitor α-fluoromethylhistidine has been found to significantly decrease histamine levels in stomach [12] and brain [4]. Therefore, one approach to decreasing gastric acid secretion and gastric ulcer formation may be through the use of specific irreversible inhibitors of HDC such as α-fluoromethylhistidine (FMHd) and α-fluoromethylhistamine (FMHm)

METHOD

Animals

Male Sprague Dawley rats (n=28) weighing 145–170 g at the time of surgery were anesthetized with sodium pentobarbital (45 mg/kg) and stainless steel gastric cannulae were chronically implanted in the forestomach as described previously [14]. Animals were allowed to recover from surgery for 14 days, at which time all cannulae were firmly anchored and there was no evidence of infection or obvious manifestations of abnormal behavior.

Procedure

Gastric secretion was collected in rats deprived of food, but not water for 14 hr When gastric secretion was to be collected, the cannula plug was removed and the stomach rinsed with 0.9% saline until the rinse was clear (usually

[1]Requests for reprints should be addressed to J D Geiger

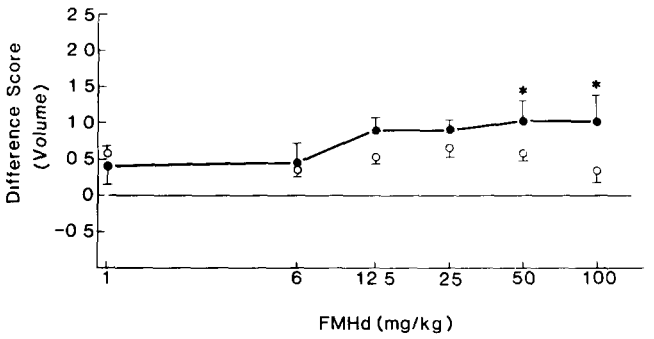

FIG 1 Dose related decreases of FMHd on acid secretion (●) as compared to VEH1 controls (○) Each point represents the mean±S E M of the combined difference scores as noted in the Method section *$p<0$ 05

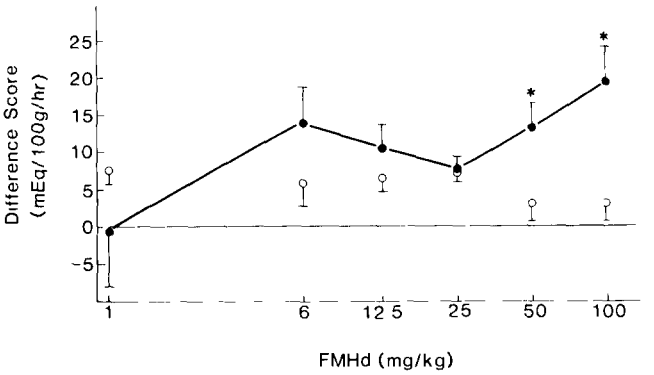

FIG 2 Dose related decreases of FMHd on total secretion volume (●) as compared to VEH1 controls (○) Each point represents the mean±S E M of the combined difference scores as noted in the Method section *$p<0$ 05

TABLE 1

EFFECT OF A SINGLE DOSE OF FMHm ON ACID SECRETION, TOTAL VOLUME, AND pH (DIFFERENCE SCORE)

		mEl/100 g Volume	Total pH	
VEH1	mean	9 40*	0 73*	0 12
	SEM	4 95	0 28	0 12
100 mg/kg	mean	38 87	1 99	−0 09
	SEM	9 40	0 31	0 12
VEH2	mean	12 32*	1 15	0 45*
	SEM	5 87	0 38	0 10

*Significantly different from 100 mg/kg, $p<0$ 05 by Newman-Keuls tests

20–30 ml) The cannula was then left open and allowed to drain for 30 min prior to three 1 hr gastric secretion collection periods The first hour was a preinjection baseline hour At the end of the first hour rats were injected IP with either vehicle (0.9% saline) or drug (FMHd or FMHm) and gastric secretions were again collected At the end of the second hour the vials were changed and a third hour of secretion was collected. The first and last three hr collection periods in all experiments were vehicle-only sessions (VEH1 and VEH2 respectively) Rats were allowed to recover for 96 hr before receiving the next higher dose in the regimen. The volume and pH of the secretions were recorded, and aliquots were titrated to pH 7.0 with 0.01 N NaOH Acid output was expressed as mEq/100 g body weight/hr.

Analyses of the data were done on the basis of difference scores. For all animals receiving either vehicle or drug, two difference scores (HR1 and HR2) were determined HR1 was the difference between the preinjection hour and the hour immediately following injection, and HR2, the difference between the first hr and the third hr. These data were then analyzed by multivariate analysis of variance (MANOVA) Significant MANOVAS were further analyzed by analysis of variance (ANOVA) and Newman-Keuls tests where appropriate The HR1 and HR2 scores for all three measures, mEq/100 g/hr, total volume, and pH, were not significantly different from one another Therefore, all HR1 and HR2 scores were combined

Restraint Ulcer

Male Sprague-Dawley rats (n=50) weighing 200±20 g were deprived of food, but not water, for 24 hr. Two hr after administration (IP) of vehicle (0 9% saline), rats were restrained in a supine position in a quiet ventilated 4°±2°C room for 3 hr [15]. After the restraint period, rats were killed by decapitation and their stomachs were examined by an observer unaware of experimental treatments. Gastric ulcer severity (cumulative length in mm) was recorded Blood from the cervical wound was collected and analyzed for corticosterone levels by the method of Van der Vies [18] All data were analyzed by ANOVA, followed by Newman-Keuls tests

RESULTS

In rats with chronically implanted gastric cannula, there was no evidence of blood in the gastric secretions, nor signs of gastric damage at autopsy indicating that mucosal integrity was maintained. We noticed no behavioral changes between vehicle-injected and FMHd or FMHm-injected rats

FMHd (Fig. 1), at doses of 50 and 100 mg/kg, significantly decreased gastric acid secretion, $F(2,49)=10$ 16, $p<0.0002$, $\eta^2=0$ 29 and $F(2,46)=11$ 73, $p<0.0001$, $\eta^2=0$ 34 No significant differences were found in gastric acid secretion between the VEH1 and VEH2 sessions associated with any dosage level indicating a high degree of reliability over the duration of all experiments (data not shown), and that the duration of the FMHd effect on gastric acid output was shorter than the 96 hr range that separated all successive drug or vehicle administrations

FMHd, at doses of 50 and 100 mg/kg of FMHd significantly decreased total gastric secretion (Fig. 2) volume compared to their respective VEH1 control groups, $F(2,49)=4$ 57, $p<0$ 015, $\eta^2=0$ 15, $F(2,46)=4$ 11, $p<0.023$, $\eta^2=0$ 15. No differences in pH were observed for any dose of FMHd tested (data not shown) There was, however, a slight rise in intraluminal pH at 100 mg/kg which was not statistically significant.

In an attempt to determine whether peripheral as opposed to central HDC inhibition has a greater effect on gastric acid levels we tested the ability of FMHm, which is less able to enter into the CNS than is FMHd, to affect gastric acid se-

cretion. As shown in Table 1, FHMm significantly reduced gastric acid secretion relative to both VEH1 and VEH2 sessions, $F(2,24)=4.89$, $p<0.017$, $\eta^2=0.28$, and the vehicle conditions were not different from one another FHMm significantly reduced total gastric secretion volume relative to VEH1, $F(2,24)=3.47$, $p<0.047$, $\eta^2=0.22$, and there was a significant rise in interluminal pH as compared to VEH2, $F(2,24)=5.27$, $p<0\ 013$, $\eta^2=0.30$

When rats were subjected to cold restraint stress, neither 50 nor 100 mg/kg FMHd affected the severity (cumulative length in mm) of gastric ulceration. The vehicle-injected control rats developed 33±4 mm of ulcer compared to the rats treated with 50 and 100 mg/kg of FMHd that developed 27±3 and 33±6 mm of ulcer, respectively Similarly, FMHm at 50 mg/kg did not affect ulcer formation with vehicle-injected control rats developing 34±3 mm of ulcer compared to 24±3 mm of ulcer in the FMHm group Neither FMHd nor FMHm changed plasma corticosterone levels which were 52±2 μg/dl in restrained control rats

DISCUSSION

Previously, it has been shown that FMHd can effectively inhibit HDC activity by up to 90% in *in vitro* preparations [1,4] and in tissues from animals given a single injection of FMHd [1,12] Daily administration or continuous infusion of FMHd produced long-lasting declines of HDC activity in rodents [9, 10, 17]. One consequence of the reduced activity of HDC is a marked reduction in the histamine levels of certain tissues [4,10] Given the importance of histamine in governing acid secretion and in the formation of duodenal ulcers, decreasing the functional pool of histamine by inhibiting its formation could represent a therapeutic alternative to the use of histamine receptor antagonists In this regard, it may be significant that FMHd reduced the number of duodenal ulcers in the rodent *Mastomys* into which histamine-rich gastric carcinoids had been implanted [9].

In this study we found that FMHd at the two highest doses, and FMHm, reduced basal gastric acid secretion in rats For both of these drugs, the decrease in gastric acid secretion was evident within the first hour post-injection and continued into the second hour This suggests that newly synthesized histamine is necessary for maintenance of basal levels of gastric secretion The FMHd-induced decrease in acid secretion appears to have been due, in some measure, to a decreased volume of gastric secretion and not due to a rise in pH of the secretions

Previously, it was found that doses of FMHd up to 100 mg/kg had no effect on basal gastric acid secretion in the 48-hour food-deprived Shay rat preparation [1] differences between our animal model and theirs may explain the apparent discrepancies between our results and those reported in their study We found that FMHm, an HDC inhibitor that reportedly does not easily cross the blood brain barrier, was also able to reduce levels of basal gastric acid secretion However, this decrease appears to have been due to both a decrease in total secretion and a small rise in intraluminal pH. Thus the different actions of these two HDC inhibitors on gastric secretions may be due to the greater ability of FMHd, as compared to FMHm, to enter into the CNS

Neither HDC inhibitor was effective in attenuating cold restraint stress-induced gastric ulceration or increases in plasma corticosterone levels. At first, this may seem at odds with the ability of FMHd and FMHm to decrease gastric acid secretion However, it has been reported that gastric ulcer formation resulting from cold restraint stress has been found in animals in which gastric acid levels were unchanged [5] and that acute shock stress *decreased* acid secretion [14] Therefore, the inability of FMHd or FMHm to protect animals against stress-induced gastric ulceration may indicate that a greater degree of gastric acid reduction was needed to effect such protection or that factors other than acid secretion are involved in this pathological response to stress.

ACKNOWLEDGEMENTS

We wish to thank Dr G B Glavin for his advice, Dr J Kollonitsch (Merck Sharp and Dohme, Rahway, NJ) for generous gifts of FMHd and FMHm, Ms K Kiernan for technical assistance and Ms B Hunt for typing the manuscript This work was supported by grants from the Medical Research Council of Canada (MRCC) and Manitoba Health Research Council VSW is a post-doctoral Fellow of the St Boniface General Hospital Research Foundation and JDG is a Scholar of MRCC

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